Comparison of the shear modulus of mature and immature rabbit lungs

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Tepper, Robert S., Barry Wiggs, Susan J. Gunst, and Peter D. Pare. Comparison of the shear modulus of mature and immature rabbit lungs. J. Appl. Physiol. 87(2): 711–714, 1999.—Maximal airway narrowing during bronchoconstriction is greater in immature than in mature rabbits. At a given transpulmonary pressure (Pl), the lung parenchyma surrounding the airways resists local deformation and provides a load that opposes airway smooth muscle shortening. We hypothesized that the force required to produce lung parenchymal deformation, quantified by the shear modulus, is lower in immature rabbit lungs. The shear modulus and the bulk modulus were measured in isolated mature (n = 8; 6 mo) and immature (n = 9; 3 wk) rabbit lungs at Pl of 2, 4, 6, 8, and 10 cmH2O. The bulk modulus increased with increasing Pl for mature and immature lungs; however, there was no significant difference between the groups. The shear modulus was lower for the immature than the mature lungs (P < 0.025), progressively increasing with increasing Pl (P < 0.001) for both groups, and there was no difference between the slopes for shear modulus vs. Pl for the mature and the immature lungs. The mean value of the shear modulus for mature and immature rabbit lungs at Pl = 6 cmH2O was 4.5 vs. 3.8 cmH2O. We conclude that the shear modulus is less in immature than mature rabbit lungs. This small maturational difference in the shear modulus probably does not account for the greater airway narrowing in the immature lung, unless its effect is coupled with a relatively thicker and more compliant airway wall in the immature animal.

shear modulus; lung parenchyma; maturation; rabbits

INFANTS AND YOUNG CHILDREN have more frequent episodes of wheezing and lower airway obstruction compared with adults. It is unclear whether this maturational effect is due to differences in the structure or function of the airway or lung parenchyma. There is a more than twofold greater increase in maximal airway narrowing in immature compared with mature rabbits when pulmonary resistance is used as the measure of narrowing (12, 17, 19). The mechanisms accounting for this maturational difference in airway responsiveness have not been identified. One of the important determinants of the degree of airway narrowing during bronchoconstriction is the elastic load that the airway smooth muscle (ASM) must shorten against (6). Studies in vivo and in vitro have demonstrated that ASM shortening and airway narrowing increase as the preload decreases (1, 2). At a constant transpulmonary pressure (Pl), the lung parenchyma provides not only the elastic load related to lung elastic recoil but also an additional elastic load secondary to the forces of interdependence between the airways and the surrounding lung parenchyma (4, 7). The lung parenchyma surrounding the airway will resist the local deformation produced by ASM shortening and airway narrowing. The elastic load produced by local tissue deformation can be estimated from measurements of the shear modulus (µ) for the lung. In mature animals of different species, µ increases with increasing Pl (4, 16). The potential importance of the elastic load provided by lung parenchymal distortion has recently been emphasized in computational models of airway narrowing (5). In a model for human airway narrowing, decreasing the ratio µ/Pl from 0.7 to 0.5 produced a twofold greater maximal airway response at Pl = 4 cmH2O.

Mansell and co-workers (8) have reported the only data assessing maturational differences in µ of the lung. These investigators found that at Pl = 5 cmH2O, µ of the lung increased more than twofold in pigs between 5 and 85 days of age. We hypothesized that µ of the immature rabbit lung is lower than that of the mature rabbit lung and that the resultant decrease in ASM elastic load contributed to the greater airway narrowing in the immature lung.

METHODOLOGY

Mature (6 mo; 2.5–3.0 kg) and immature (3–4 wk; 0.26–0.36 kg) New Zealand White rabbits were killed by an overdose of pentobarbital sodium, and the lungs were excised. After several inflations to an airway pressure of 20–25 cmH2O, Pl was set at 10 cmH2O by connecting the tracheal cannula to a bias flow with an underwater seal used to adjust the Pl.

Bulk modulus (κ). By using the bias-flow system to set the lung at Pl = 10 cmH2O, the lung was then closed to the bias flow. With a syringe attached at the airway opening, small-volume oscillations were applied at ≤0.25 Hz, and airflow opening pressure was measured by using a piezoresistive pressure transducer. Oscillation volumes of 5 and 1 ml were used for the mature and the immature lungs, respectively. These measurements were repeated at Pl = 8, 6, 4, and 2 cmH2O. Before each change in Pl, the lung was inflated twice to Pl = 20 cmH2O. In addition, while the lung was closed to the bias flow, the volume between each Pl was measured by withdrawing volume from the lung with a syringe. The volume in the lung at Pl = 0 cmH2O was measured by water displacement.

Indentation test. The excised lung was kept inflated at a constant Pl (2, 4, 6, 8, 10 cmH2O) by using the bias flow of air connected to an underwater seal. The lung was positioned on a precision digital balance so that the relatively flat costal surfaces of the right and left lower lobes were facing upward.

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and remained horizontal. The vertical movement of the 2.1-mm probe was measured by using an attached digital micrometer. The probe was lowered to the lung surface until contact was achieved. The probe was then lowered in increments of 0.25 mm to a maximum of 2.0 mm. With each incremental lowering of the probe, the increase in the force was measured. At each PL, measurements were obtained on both the right and the left lower lobes, and the results from the two lobes were averaged. The indentation test was repeated at PL of 8, 6, 4, and 2 cmH2O. Before each change in PL, the lung was inflated twice to PL = 20 cmH2O.

Analysis: calculation of the shear moduli. The force (F) developed as a probe of diameter (D) produced a displacement (w) and is related to µ and the Poisson ratio (ν) as follows

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

(1)

The Poisson ratio is also related to µ as follows

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

(2)

κ was calculated at each PL as the product of the elastance (EL; measured from the volume and pressure oscillations) and the lung volume (V) at that PL

\[ \kappa = V \times EL \]  

(3)

By using the values for κ and the relationship between force and displacement, µ was calculated at each PL.

RESULTS

κ. The group mean (±SD) values for κ at PL = 2, 4, 6, 8, and 10 cmH2O are illustrated in Fig. 1 for the mature (n = 7) and the immature (n = 8) rabbit lungs. In one mature and one immature lung, there were leaks that prevented the measurements. There was a significant increase in κ with increasing PL in both groups; however, there was not a significant difference between the mature and the immature groups.

µ. The relationship between force and displacement was linear (r² ≥ 0.90) for the mature and the immature lungs at all PL, as illustrated in Fig. 2. µ at each PL was calculated by using Eqs. 1–3. The mean values for κ for the immature and the mature rabbit lungs were used because two of the animals did not have individual values for κ. The mean (±SD) values for µ are illustrated in Fig. 3. When analyzed by ANOVA for repeated measures, µ was significantly lower (P < 0.025) for the immature lungs than for the mature rabbit lungs. There was a significant increase in µ with increasing PL for both immature and mature animals (P < 0.001); however, there was no difference between the slopes of

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Fig. 1. Bulk modulus (κ; cmH2O) vs. transpulmonary pressure (PL; cmH2O) for group of mature and immature rabbit lungs. Values are means ± SD. n, No. of lungs. There was no significant difference in κ between mature and immature rabbit lungs.

Fig. 2. Force (g) vs. displacement (mm) at PL of 2, 4, 6, 8, and 10 cmH2O for representative mature rabbit lung. Force increased linearly with displacement at each PL, and slope increased with increasing PL.

Fig. 3. Shear modulus (µ; cmH2O) vs. PL for group of mature and immature rabbit lungs. Values are means ± SD. n, No. of lungs. With increasing PL, µ increased, and immature lungs had significantly lower values of µ compared with mature lungs (P < 0.025).
μ vs. PL. The mean values of μ were significantly lower for the immature group than for the mature group at PL = 6 and 10 cmH₂O, when assessed by t-test with correction for multiple comparisons. The results were similar when μ was assessed from the individual values for k from each lung instead of the group mean; however, the group effect had a slightly higher P value (P < 0.06) because of a smaller sample size and greater variability.

**DISCUSSION**

Our results indicate that μ of the lung was lower for the immature rabbit compared with the mature rabbit. We also found that μ increased with increasing PL in both the mature and the immature rabbit lung. In addition, k values for the mature and the immature rabbit lungs were not different. This finding suggests that the forces that resist local deformation of the lung parenchyma during airway narrowing are lower in the immature than in the mature rabbit lung.

The indentation test was used to assess the stress-strain relationship of the lung parenchyma to local deformation. The methodology assumes that the size of the probe used for the indentation is small relative to the size of the surface area of the lobe and the local radius of curvature of the surface. In previous studies in which μ was measured in mature rabbits, probe diameters between 7 and 10 mm were used (16). As the probe size can affect the values measured, it was necessary to use the same-sized probe to compare the mature and the immature lungs. A 7- to 10-mm probe would be much too large for the size of the immature lung, and therefore we chose a probe with a diameter of 2.1 mm. A probe diameter that is too small can increase the edge effects of the probe relative to the effects of the applied surface of the probe on the deformation of the lung parenchyma. However, we found a linear increase in force with displacement and a linear increase in μ with increased PL. These results were similar to previous studies using the indentation test with a larger-sized probe. In addition, our values for μ for the mature rabbit lung are similar to those previously reported using a larger sized probe (15, 16). If there is a significant pleural membrane tension, then the greater μ that we measured for the mature lung could have resulted from a greater pleural membrane tension in the mature lung. We were not able to assess pleural membrane tension, as the small size of the immature lung would not enable us to vary punch diameter over a wide range. However, in the pig lung, pleural membrane tensions were detected only at PL > 15 cmH₂O, and the maturational differences in μ that we observed were at a much lower PL. Therefore, we believe that the values we obtained for μ with the smaller sized probe accurately reflect the stress-strain characteristics of the lung parenchyma to local deformation and our comparison of mature and immature lungs.

Our findings of a greater μ in the mature rabbit lung compared with the immature rabbit lung is consistent with the results of Mansell and co-workers (8), who reported a greater μ in mature than immature pig lungs. However, the difference between the mature and the immature groups was much smaller in our rabbit lungs than in the pig lungs. In the pigs, μ measured at PL = 5 cmH₂O increased from 5 cmH₂O at 3–5 days, to 7 cmH₂O at 25–30 days, to 13 cmH₂O at age 80–85 days. In the oldest pigs, the high value of μ at PL = 5 cmH₂O was also much higher than the values of μ measured at higher PL in this same age group. The unusually high value of μ at PL = 5 cmH₂O made the relationship between μ and PL very nonlinear in the pig lungs. In the pig lungs the nonlinear behavior in the infant compared with the mature airway. The impact of the smaller μ would influence of μ are based on a model of human adult airway narrowing for adult humans. For values of k > 0.7, decreases in k had a very small effect on resistance during bronchoconstriction. However, for k < 0.7, decreases in k can result in two- to threefold greater increases in resistance, particularly at PL = 5 cmH₂O. In our present study of rabbits, the values of k are >0.6 at PL = 6 cmH₂O, and the k values differed by 0.1–0.2 for mature and immature lungs (Table 1). This would suggest that differences in μ do not account for the markedly greater airway narrowing in the immature than the mature rabbit lung. However, there are strong interactions among μ, airway wall thickness, and airway wall compliance as determinants of airway narrowing, particularly at low PL (5, 7). The above estimates of the influence of μ are based on a model of human adult airway narrowing. The impact of the smaller μ would be magnified if the immature airway wall were both thicker relative to lumen diameter and more compliant than the mature airway.

The lung parenchyma has been modeled as open-cell foam in attempts to describe its mechanical properties; however, the structural components that resist deformation and determine μ have not been adequately identified. We can speculate on structural differences be-

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| PL, transpulmonary pressure; μ, shear modulus; k, μ-to-PL ratio (μ/PL). |
between the lung parenchyma of mature and immature animals that might contribute to a lower $\mu$ in the immature lung. Lung growth early in life is associated with increases in alveolar number and alveolar size, a thinning of the alveolar wall thickness, and potentially an increase in the number of alveolar attachments to the airways. We did not find maturational differences in $k$ of the lung. However, microstructural differences in the relative amounts or types of elastin and collagen at important structural bearing points in the alveolar ducts, walls, and airway attachments could produce differences in the ability of the lung parenchyma to resist local deformation.

Parenchymal resistance to local deformation could be altered under conditions of bronchoconstriction. Okazawa and colleagues (10) did not find that bronchoconstriction increased $\mu$ of isolated mature rabbit lungs; however, Salerno and Ludwig (11) recently reported that bronchoconstriction increased $\mu$ in isolated rat lungs. The increase in $\mu$ in rats but not in rabbits could have resulted from species differences, such as a greater degree and heterogeneity of airway narrowing in the rats because air trapping has been demonstrated to increase $\mu$. As immature compared with mature rabbits have greater airway narrowing, heterogeneity of the airway response, and hyperinflation, bronchoconstriction could potentially produce an increase in $\mu$ of the immature but not the mature rabbit lung (14, 18).

In conclusion, we have found that $\mu$ of the immature rabbit lung is less than that of the mature rabbit lung. Our findings suggest that a lower elastic load from local tissue deformation could contribute to greater airway narrowing in the immature than the mature rabbit lung. The relative magnitude of this maturational difference in $\mu$ as a determinant of greater airway narrowing in the immature lung will be better estimated when a computational model of airway narrowing is developed by using physiological and morphometric measurements for mature and immature rabbit airways.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-48522 and HL-29289; a Fogarty International Senior Fellowship (F66TW02271); and Medical Research Council Grant 40145.

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Received 2 December 1998; accepted in final form 26 April 1999.

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