HIGHLIGHTED TOPIC | Biomechanics and Mechanotransduction in Cells and Tissues

Chronic strain alters the passive and contractile properties of rabbit airways

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Tepper, R. S., R. Ramchandani, E. Argay, L. Zhang, Z. Xue, Y. Liu, and S. J. Gunst. Chronic strain alters the passive and contractile properties of rabbit airways. J Appl Physiol 98: 1949–1954, 2005. First published January 27, 2005; doi:10.1152/japplphysiol.00952.2004.—Pathophysiological conditions of the lung may shift the balance of forces so as to chronically alter the amount of strain imposed on the airways. This chronic strain could result in changes in the structure and/or function of the airways that affect its physiological properties. We evaluated the effects of imposing physiological levels of chronic mechanical strain on the passive and active physiological properties of intraparenchymal rabbit airways. Isolated bronchial segments were cultured for 48 h at transmural pressures of 0 cmH2O (No Strain) or 7 cmH2O (Strain). Effects of strain on small parenchymal airways were evaluated in lung tissue slices cultured under conditions of No Strain or ~50% increased in diameter (Strain). Chronic strain resulted in a higher passive compliance of the bronchial segments and larger airway lumen size. In addition, bronchi not subjected to chronic Strain were more responsive to ACh than bronchi subjected to chronic Strain, and airways in lung slices subjected to No Strain narrowed more in response to ACh than airways in lung slices subjected to Strain. The greatest effects of chronic strain occurred in the smallest sized airways. Our results suggest that chronic distension of the airways has physiologically important effects on their passive and active properties, which are most prominent in the smaller, more peripheral airways.

Peripheral airways; mechanical stretch

Inflammatory conditions of the lung that result in adventitial edema, changes in the elasticity of the airways and the lung parenchyma, as well as airway closure and atelectasis, may shift the balance of forces within the lung tissue so as to chronically alter the amount of strain imposed on the airways. This chronic strain could result in changes in the structure and/or function of the airways that affect its physiological properties.

Studies of tissues and cells isolated from the airways suggest that chronic strain can induce changes in their physiological properties and responses. The imposition of several hours of mechanical stress on cultured pulmonary fibroblasts can initiate collagen synthesis in these cells (2). Similarly, imposing mechanical stress on cultured bronchial epithelial cells for several hours increases the secretion of several growth factors (early growth response-1, endothelin-1, transforming growth factor-β1) that can affect airway smooth muscle responsiveness and proliferation and that elicit fibrotic protein synthesis in fibroblasts (12, 19, 25, 29, 30). Rabbit and canine tracheal smooth muscle strips subjected to constant levels of strain for 1–2 days exhibit strain-induced alterations in passive stiffness and changes in contractility (14, 18, 31). The effects of chronic strain of intact airways should reflect the combined outcome of chronic strain on all of these separate components.

In the present study, we evaluated the effects of chronic mechanical strain on the passive and active physiological properties of isolated rabbit airways. Intact intraparenchymal bronchial segments were used to determine the effects of chronic distension on airway compliance and airway responses to methacholine. We used lung tissue explants to evaluate the effect of chronic strain on smaller sized airways that were too small to be isolated for physiological measurements of pressure and volume.

Our results suggest that chronic distension of the airways has physiologically important effects on their passive and active properties and that the effects of chronic strain are most prominent in the smaller, more peripheral airways.

METHODS

Isolated Bronchial Segment Preparation and Experimental Apparatus

Mature New Zealand White rabbits (2.5–2.7 kg) were anesthetized with thiotentoral sodium (30 mg/kg) and exsanguinated by severing the abdominal artery. The left lobe of the lung was excised and placed in a physiological saline solution (PPS; which contains in mM 110 NaCl, 3.4 KCl, 2.4 CaCl2, 0.82 MgCl2, 25.8 NaHCO3, 1.2 KH2PO4, and 5.6 glucose) and aerated with 95% O2 and 5% CO2. With the use of a stereomicroscope, the parenchymal tissue was dissected away from the airways along the main axial pathway. This protocol was approved by the Institutional Animal Care and Use Committee.

A bronchial segment that included generations 6 and 7 was isolated, and all branches from the segment were tied off with silk sutures to produce a leak-free bronchial segment. Each end of the bronchial segment was mounted and securely tied to a stainless steel cannula with a diameter comparable to that of the end of the bronchial segment. The bronchial segment with the inserted cannula was mounted in a 20-ml tissue bath, a Plexiglas chamber filled with PSS solution at 37°C. The bronchial segment was flushed and filled with PSS using a stopcock and syringe connected to the cannula on the distal, smaller end of the segment. The cannula on the proximal end of the bronchial segment was connected to a differential pressure transducer (model 156PC05GW, Honeywell Sensing), which measured transmural pressure (Ptm), and a microsyringe, which measured changes in bronchial volume. The syringe was attached to a servo-controlled motor that could adjust bronchial volume and

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pressure. The pressure and volume signals were amplified, digitized (Data Translation DT 2801-A, Marlborough, MA), and recorded on a personal computer for real-time visualization of signals, as well as for subsequent data analysis using commercial software (RHT Infodat, Montreal, PQ, Canada).

*Passive pressure-volume curves.* Each bronchial segment was slowly cycled over a 2-min period between Ptm values of −25 and +10 cmH2O until a constant pressure-volume curve was obtained; this usually required three to five cycles. Airway volume at a pressure of −25 cmH2O was defined as zero volume, and the volume of the segment at a Ptm of +10 cmH2O was defined as V10. The next inflation and deflation cycle was then used for pressure-volume measurements.

*Maximal isovolumetric contraction.* Maximal pressure generation to a single dose of acetylcholine (ACh; 10⁻⁵ M) was evaluated at a bronchial segment volume of 50% V10. ACh as added to the tissue bath and the pressure was continuously recorded while the bronchial segment was maintained at a constant volume.

*Protocol.* Bronchial segments were incubated in DMEM culture media for 48 h at 37°C under conditions of Strain (Ptm = 7 cmH2O) or No Strain (Ptm = 0 cmH2O). Postincubation, measurements of the passive pressure volume curve and the maximal isovolumetric pressure generation to 10⁻⁵ ACh were measured.

*Statistical analysis.* Differences between the measurements obtained from strained and unstrained airway segments were compared using unpaired t-tests; P ≤ 0.05 was considered statistically significant.

**Lung Tissue Explant Preparation and Experimental Apparatus**

Eight mature male New Zealand White rabbits (2.5–2.9 kg) were anesthetized (50 mg/kg pentothal sodium), tracheotomized, and mechanically ventilated. A cannula was inserted into the jugular vein, and the abdominal cavity was opened. The animal was administered 1,000 units of heparin intravenously. After inspiriting 100% O₂ for 1 to 2 min, ventilation was discontinued with the airway occluded, which resulted in degassing of the lung by oxygen consumption. The rabbit was exsanguinated by severing the abdominal vein. The in situ lung was lifted to a Ptm of 3 cmH2O with a 2% agarose solution (Type XI, Sigma) at 37°C; this required ~20 ml agarose. The lung was cooled and the agarose solidified by infusing 300 ml of cold DMEM through the venous cannula. The cold, agarose-filled lungs were then removed from the chest and maintained in the refrigerator for 1 h. The right and left lobes were then cut into three coronal sections. With the use of a 10-mm-diameter coring tool, cylinders of tissue were obtained from each section along the main axial airway pathway. From each tissue core, which contained an airway surrounded by lung parenchyma, 300-μm-thick circular tissue slices were cut (Brendel Vitron, Tucson, AZ). Slicing the tissue removes the agarose within the airway; however, the agarose remains within the parenchyma. Tissue slices were collected consecutively and grouped into pairs within each tissue core, which ensured that both slices in a pair were from the same location in the airway, thus having similar airway size and structure (Fig. 1).

*Straining of lung tissue explants.* Tissue slices were strained radially using a Flexcell system (Flex Cell International, Hillsborough NC). All tissue slices were glued to the center of a BioFlex membrane plate by applying tissue glue (Nexaband SC) only to the perimeter of the circular tissue slice; 5.0 ml of DMEM was added to each BioFlex well. The BioFlex membrane was stretched radially by application of a negative vacuum pressure below the membrane, which rests on a supporting post. The supporting posts were modified from their original diameter of 25 mm to a smaller diameter of 15 mm, which enabled us to achieve a desired strain of approximately a 50% increase in diameter. Paired slices were randomly assigned to strain or no-strain treatment groups, which were maintained in an incubator at 37°C with 5% CO₂ for 48 h. The culture media (5.0 ml DMEM) was changed at 24 h.

*Assessment of airway lumen area and airway narrowing with ACh stimulation.* After 48 h of culture, the tissue slices were cut from the BioFlex membrane leaving only the airway surrounded by a thin rim of lung parenchyma. Each airway was placed with 0.5 ml of fresh DMEM into a well of a six-well plate. The airway was visualized with an inverted microscope (Nikon), and images were recorded with the attached video camera (Panasonic, GP-KR222) using imaging software (Metamorph, Universal Imaging, Downingtown, PA). Airways were stimulated by dropping 10 μl of 10⁻⁵ M ACh directly onto the airway using a micropipette. The initial airway size and the contractile response to ACh were recorded for 90 s at three frames per second. From the recorded images, the airway lumen area before ACh stimulation was measured. In addition, the airway lumen area after constriction with ACh was measured and expressed as a percentage of the prestimulation area.

*Statistical analysis.* On the basis of the airway lumen area of the tissue slice in the pair that was not strained, the airways were divided into three groups (large, medium, small), which were estimated to be generations 6–7, 8–9, and ≥10, respectively. The paired tissue slices were analyzed using a linear model that included treatment (Strain vs. No Strain), airway group (large, medium, small), and the treatment-by-group interaction. To account for the pairing of slices and by slices coming from the same rabbit, the model included the random effects for slices nested in rabbits. A P value ≤0.05 was considered statistically significant.

**RESULTS**

**Effect of Chronic Strain on Isolated Rabbit Bronchial Segments**

The effect of chronic strain on bronchial compliance and distensibility was evaluated in isolated rabbit bronchial segments. Pressure-volume curves obtained on isolated bronchial segments after 2 days incubation with No Strain (Ptm = 0 cmH₂O) and with Strain (Ptm = 7 cmH₂O) were normalized to airway volume at a pressure of 10 cmH₂O (V₁₀) to compare the elastic properties of the segments (Fig. 2A). The airway segment that was maintained with No Strain during incubation was shifted to the right relative to the bronchial segment that was maintained with Strain during incubation. Ptm at an airway volume of 50% V₁₀ was significantly greater in segments that had been subjected to No Strain (5.1 ± 0.6 cmH₂O)
than in segments that had not been subjected to Strain (1.6 ± 0.3 cmH₂O; \( P < 0.001 \)). In addition, the specific compliance, the slope at 50%\( V_{10} \) divided by \( V_{10} \), was significantly lower in the No Strain group (0.10 ± 0.01 cmH₂O\(^{-1}\)) than in the Strain group (0.36 ± 0.06 cmH₂O\(^{-1}\)) of bronchial segments (\( P < 0.002 \); Fig. 2B). Airway segments incubated at Ptm of 0 cmH₂O (No Strain) also tended to have smaller volumes than the airway segments subjected to Strain (51 ± 6 vs. 63 ± 12 μl; mean ± SE; \( P = 0.25 \)).

The effect of chronic strain on pressure generation in response to ACh was also determined in the intact bronchial segments, which were incubated with or without strain for 2 days and then stimulated with ACh at 50% \( V_{10} \). The mean transmural pressure generated with ACh stimulation was greater for the segments that had not been subjected to strain compared with segments that had been subjected to strain, although the difference did not quite achieve statistical significance (29.6 ± 3.0 vs. 19.3 ± 3.9 cmH₂O; \( P < 0.07; n = 6 \); Fig. 3).

**Effect of Chronic Strain on Rabbit Lung Tissue Explants**

To extend our evaluation of the effect of chronic strain to smaller sized intraparenchymal airways, we analyzed 81 pairs of lung tissue slices. Airways in the slices were divided into three groups based on their diameters: large (0.92–1.45 mm), medium (0.63–0.92 mm), and small (<0.63 mm). There were 26, 41, and 14 pairs of tissue slices in the large, middle, and small airway groups, respectively. During culture, constant strain was maintained on one tissue slice from each pair to increase its diameter by ~50%. The other slice from each pair was maintained without strain. After subjecting tissue slices to strain or no strain for 48 h, they were removed from the Flexcell for evaluation of airway size. Airway size in all slices was evaluated without strain under the same conditions. After removal from the Flexcell, tissue slices that had been subjected to strain had significantly larger airway lumens compared with the partner slice not subjected to strain (\( P = 0.001 \); Fig. 4A). When the airway lumen area of the tissue slice that had been subjected to strain was expressed as a percentage of the airway lumen area of its partner that had not been strained, the percent increase in lumen area was greatest for the smallest-sized airways (61%), in between for the middle-sized airways (22%), and least for the largest-sized airways (5%). The percent increase in lumen area was statistically significant for the small- and the medium-sized airways (\( P < 0.01 \); Fig. 4B).

The tissue slices in 0.5 ml of solution were administered 10 μl of 10\(^{-3}\) M ACh, and the decrease in airway lumen area was recorded by videomicroscopy. Airway lumen area was ex-
in the midrange of lung volume and intact intraparenchymal bronchi. We used a Ptm of 7 cmH2O, impact of chronic strain on the physiological properties of airways that had not been strained (21% vs. 13% of preconstriction lumen area; *P<0.01). Effect of strain on airway narrowing was greatest in the group of small airways; postconstriction lumen size of the small airways that had been strained was greater than that of the small airways that had not been strained (21% vs. 13% of preconstriction lumen area; *P<0.01).

DISCUSSION

In the present study, we subjected airways to chronic strain at levels comparable to those that occur in vivo to evaluate the impact of chronic strain on the physiological properties of intact intraparenchymal bronchi. We used a Ptm of 7 cmH2O, which is achieved in the midrange of lung volume and produces a strain of ~50% for intrapulmonary airways (21). These airways are the most important determinants of airway responsiveness in vivo, and they are also the most likely to be the target of alterations in mechanical strain that might occur under pathophysiological conditions. We demonstrate that the imposition of physiological levels of chronic mechanical strain for 48 h on isolated rabbit intrapulmonary bronchial segments and lung parenchymal tissue slices results in significant alterations in the passive properties and active physiological responses of these tissues. A higher level of chronic pressure or strain resulted in a higher passive compliance of the bronchi and larger airway lumen size, suggesting that strain resulted in alterations in tissue structure. Chronic strain also resulted in changes in the contractile responsiveness of both preparations. Bronchi chronically subjected to a Ptm of 0 cmH2O were more responsive to ACh than bronchi subjected to a Ptm of 7 cmH2O. Similarly, lung slices that had not been subjected to chronic strain narrowed more than lung slices subjected to strain.

Our findings indicate that airways that are not strained and remain chronically without distension are stiffer than airways that remain chronically strained or distended. The specific compliance of the intraparenchymal airway segments maintained at a Ptm of 0 cmH2O was threefold lower than that of airway segments maintained at a Ptm of 7 cmH2O. Chronic exposure to zero Ptm may also result in a lower resting volume of the airways (Fig. 2). Technical limitations precluded us from obtaining pressure and volume measurements from smaller, more peripheral airway segments, so we used tissue explants to assess the effect of chronic strain on these airways. After subjecting tissue explants to chronic strain or to no strain, their lumen sizes were measured in the absence of strain. The airways that had not been subjected to strain had significantly smaller lumen sizes than those that had been subjected to chronic strain. This suggests that a chronic absence of strain had a similar effect on the airways in tissue explants as the effect observed for intact bronchial segments: the bronchial

Fig. 4. A: comparison of airway lumen area in lung parenchymal tissue slices after they were removed from the Flexcell where they had been maintained for 48 h under conditions of Strain (black) or No Strain (gray). Tissue slices were grouped by airway size (large, medium, and small). Airways that had been maintained in culture under Strain were significantly larger than the airways that had been maintained in culture with No Strain (*P<0.0001). B: to assess the effect of strain on airway lumen area, the airway lumen area of the tissue slice that had been subjected to strain was expressed as a percentage of the lumen area of its partner that had not been subjected to strain. The percentage increase in lumen area caused by strain was greatest for the smallest-sized airways (61%), less for the medium-sized airways (22%), and least for the largest-sized airways (5%). The percent increase in lumen area was statistically significant for the small- and the medium-sized airways, *P<0.01.
The effects of strain on different-sized airways differed in magnitude. In the tissue explants, chronic strain resulted in the greatest increase in airway lumen area in the smallest-sized airways, suggesting that the smallest, most compliant airways may be most susceptible to the effects of chronic strain. The effect of applying stress to the tissue explants is analogous to increasing Ptm in vivo or in isolated lungs, where the smaller, more compliant airways exhibit a greater percent increase in airway caliber than the larger, more central cartilaginous airways (17, 20).

The intact intraparenchymal airway segments that were maintained at a Ptm of 0 cmH2O generated greater pressures in response to ACh stimulation at constant volume than the airways that had been maintained at a Ptm of 7 cmH2O. Similarly, airways in the tissue explants that had been maintained in culture without strain narrowed more in response to ACh than airways that had been maintained under chronic strain. The effect of strain on airway narrowing was greatest in the smallest-sized airways. This difference in effect of chronic strain on airway narrowing in small and large airways paralleled the differences we observed in the effects of strain on the passive properties of these groups of airways.

There are several possible reasons why the imposition of chronic strain might have had a greater impact on small airways than large airways in these experiments. We subjected tissue explants containing both small and large airways to a constant level of stress; this would result in greater strain on the smaller, more compliant airways. Thus the magnitude of the mechanical stimulus to the small airways may have been larger. Mechanical stimulation may trigger signaling pathways that regulate cellular responses that modulate bronchial elasticity and/or contractility (2, 12, 19, 25–27, 29, 30). Alternatively, the same mechanical stimulus may elicit different responses from large and small airways, due to differences in the sensitivities of their respective tissue components to mechanical stimulation.

Mechanical strain has previously been shown to modulate the active and passive mechanical properties of trachealis muscle strips (5–7, 14, 16, 22, 31). The contractile responsiveness of isolated trachealis smooth muscle strips can be increased by maintaining them at a short length for prolonged periods ranging from hours to days. Furthermore, trachealis tissues maintained at a short length for at least 24 h exhibit higher levels of passive tension and greater stiffness (14, 18, 31). The fact that we observed analogous effects in isolated intraparenchymal airway segments and lung tissue explants suggests that many of the effects of chronic strain may result from the direct effects of mechanical forces on the airway smooth muscle. In airway smooth muscle, integrin-linked signaling pathways sensitive to mechanical strain can transduce external mechanical stimuli to downstream signaling pathways that regulate cytoskeletal and contractile proteins (26, 27). These signaling pathways may also regulate gene expression and thus regulate phenotypic changes in the muscle. In addition, cytokines released from other tissue components as a result of mechanical strain may stimulate phenotypic changes in the muscle, extracellular matrix synthesis, or the proliferation of smooth muscle cells or fibroblasts (12, 18, 19, 29, 30). However, the molecular mechanisms responsible for the effects of chronic strain on the mechanical properties of the airways remain to be fully elucidated.

Our observation that the absence of significant chronic strain causes stiffening of the airways may have important physiological implications. In vivo, conditions that shift the balance of forces within the lung tissue so as to chronically alter the amount of strain imposed on the airways may stimulate airways to undergo mechanical adaptations that lead to stiffer and more contractile airways. Conditions such as obesity, atelectasis, sleep, or airway inflammation resulting in edema, collagen deposition, or an increase in smooth muscle tone that stiffens the airway wall could all result in poor distension of the lungs and lower lung volumes for extended periods of time. Stiffening of the airways induced by a decrease in the level of chronic strain could ultimately lead to a heightened airway responsiveness that does not improve with a deep inspiration, as is a characteristic of subjects with asthma (1, 8, 13, 15, 23).

We used a computational model of rabbit airway narrowing to estimate how the mechanical properties of airway segments that result from different conditions of chronic strain might affect airway responsiveness across the entire airway tree (9). We assumed differences in airway compliance and airway muscle stress generation in our model that were comparable to the relative differences that we observed in bronchial segments subjected to chronic Ptm values of 0 cmH2O (No Strain) or 7 cmH2O (Strain). On the basis of our present results, we also assumed an intercept and slope for the pressure-area curve at zero pressure that were 30% lower for airways subjected to no strain than for airways subjected to strain (Fig. 2), and a maximal smooth muscle stress generation that was 50% greater for the unstrained airways (Fig. 3). On the basis of these parameters, the model predicted a higher baseline airway resistance and a greater increase in resistance during maximal

![Graph](image)

Fig. 6. Prediction of changes in airway resistance with increasing doses of bronchoconstrictor (−Log Agonist Dose) using a computational model of the rabbit airway tree. A Ptm of 2 cmH2O is assumed. In the model the intercept and slope of the pressure-area curve at zero pressure were assumed to be 30% lower than for airways subject to chronic strain (No Strain) and lower for airways subject to chronic strain (Strain). With the use of these parameters, a higher baseline airway resistance and a greater increase in resistance with maximal narrowing for airways was predicted for airways that had been not been subjected to strain (No Strain) compared with airways that had been subjected to strain (Strain).
narrowing for the airways that were not subjected to strain, compared with airways subjected to strain (Fig. 6).

The model predicts airway properties that are analogous to those of asthmatic individuals, who characteristically exhibit a greater baseline resistance and increased airway responsiveness (3, 24, 28). In addition, local differences in Ptm, airway tone, airway narrowing, or airway closure could result in regional differences in chronic strain among airways within the lung. These differences in chronic strain might then lead to local remodeling of the tissues and contribute to the heterogeneity of airway responsiveness within the lung that is also characteristic of asthmatic individuals (4, 10, 11).

In summary, we demonstrated that the absence of chronic strain on intraparenchymal airways results in smaller, stiffer airways that generate greater pressures and narrow more in response to contractile stimulation than airways that have been subjected to chronic strain. The effects of strain are most prominent in the smaller, more compliant airways. Pathologic conditions in vivo may cause alterations in the strain on the airways that could lead to changes in the structure and/or function of the airways and contribute to the progression of airway disease.

GRANTS

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REFERENCES