Increased surface tension decreases pulmonary capillary volume and compliance

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Topulos, George P., Richard E. Brown, and James P. Butler. Increased surface tension decreases pulmonary capillary volume and compliance. J Appl Physiol 93: 1023–1029, 2002. First published April 15, 2002; 10.1152/japplphysiol.00779.2001.—Increased surface tension is an important component of several respiratory diseases, but its effects on pulmonary capillary mechanics are incompletely understood. We measured capillary volume and specific compliance before and after increasing surface tension with nebulized siloxane in excised dog lungs. The change in surface tension was sufficient to increase lung recoil 5 cmH2O at 50% total lung capacity. Increased surface tension decreased both capillary volume and specific compliance. The changes in capillary volume and compliance were greatest at the lung volumes at which the surface tension change was greatest. Near functional residual capacity, capillary volume postsiloxane was ~30% of control. Presiloxane capillary specific compliance was ~7%/cmH2O near functional residual capacity and ~2.5%/cmH2O near total lung capacity. Postsiloxane capillary-specific compliance was 3%/cmH2O, and was independent of lung volume. We conclude that in addition to their well-known effects on lung mechanics, changes in surface tension also have important effects on capillary mechanics. We speculate that these changes may in turn affect ventilation and perfusion, worsen gas exchange, and alter leukocyte sequestration.

Increased surface tension (γ) is an important component of the pathophysiology of several respiratory diseases, including acute respiratory distress syndrome (1, 12, 27), infant respiratory distress syndrome (2), and industrial exposure to toxic aerosols (11, 24). Changes in γ may alter pulmonary capillary mechanics (e.g., their volume, diameter, and compliance), which in turn affect the pattern of pulmonary perfusion, matching of ventilation and perfusion and hence gas exchange, and leukocyte sequestration. However, the direct effects of changes in γ on the pulmonary capillary bed are incompletely understood, largely because no technique has been available to directly study capillary mechanics in unfixed lungs with altered γ.

METHODS

Animal preparation. We studied lower lobes excised from four female mongrel dogs (18–20 kg). Each animal was anesthetized with preservative-free pentobarbital sodium, initial dose ~15 mg/kg iv, with additional doses as needed to maintain adequate anesthesia. The animal was placed supine, a tracheotomy was performed below the larynx, and the trachea was cannulated with auffed endotracheal tube. Heparin (1,000–2,000 units/kg iv) was given, and at least 2 min later the dog was exsanguinated via a 14-gauge carotid artery catheter. The blood was collected, and sodium bicarbonate was added periodically as needed to keep the pH near 7.40 (Ciba Corning model 248). After ~1,000 ml of blood had been collected, the animal was killed with an overdose of pentobarbital (iv). A median sternotomy was performed, and the heart and lungs were excised en bloc. Except for momentary episodes during lung excision, lung inflation was maintained by using positive pressure
at the airway opening (Pao). The lung was kept moist by spraying it with 0.9% saline.

A lower lobe was isolated, and its bronchus and pulmonary artery (PA) and vein (PV) were cannulated with large-bore catheters. To reduce intravascular air, the PV catheter was initially flushed with saline, and then both the PA and PV cannulas were connected to a perfusion circuit filled with autologous blood. The lobe was suspended by the cannulas. At a transpulmonary pressure (Pl) of ~10 cmH2O, three pleural markers (short pieces of black suture) were cemented to the pleural surface with cyanoacrylate glue, ~3.5 cm apart in a triangular pattern. Photographs of the pleural markers and a ruler held parallel to the lobe surface, were used to calculate changes in lung volume (described below).

Pulmonary venous pressure and pulmonary arterial pressure, relative to barometric pressure, were measured (Sorenson Abbott Transpac 2), with the transducer heights matched to the height on the lobe where the optical probe was placed (mid lobe and far from any margin). To keep the pleural surface moist and to prevent gas exchange through the pleural surface, a plastic bag, ventilated with the same gas used to ventilate the lobe, was placed around the lobe. Between measurements, described below, the lobe was ventilated with 5–6% CO2 in O2 (Siemens 900, pressure-control mode) and perfused. An adjustable roller pump (Stockert Shiley model 10) pumped blood (at ~100 ml/min) from a reservoir through a 37–38°C heat exchanger into the PA; blood drained passively from the PV back into the reservoir.

**Optical methods.** Point illumination of the pleural surface results in a distinctive pattern of backscattered light from an ~1.5-cm³ volume of lung. The fractional change in blood volume can be derived from this pattern; the methods have been previously described in detail (25, 26) and are summarized here. Optical fibers delivered laser light of two wavelengths (683 and 808 nm) to a point on the lobe surface and carried backscattered light from three other points on the lobe back to sensing photodiodes (Fig. 2). The animal end of the two source and three receiving fibers were held securely in a plastic block (1.9 × 5 cm area), such that the fibers were in a fixed position relative to each other. The fibers and block were held gently against the lung. The lasers were cycled alternately on and off with a period of 8 ms. The signal from each photodiode alternately provided wavelength-specific light intensity at each known radial distance from the source fibers.

**VL calculation.** VL at each Pl was calculated from digital photographs of the pleural markers using the technique of Lehr et al. (16, see also Ref. 6). From each photograph the distance between each pair of pleural markers was measured (NIH Image version 1.61). To reduce variability, all picture analyses were done by the same individual. VL was calculated from the 3/2 power of the triangle area and expressed as a percent of presiloxane (control) TLC, defined as lung volume at Pl = 30 cmH2O. (In one animal, one of the three pleural markers was dislodged during the course of the experiment, and VL was calculated from the distance between the remaining two markers.) Note that this VL is the sum of lung tissue and gas volume, and ~44% TLC = functional residual capacity (FRC; end-expiratory VL at rest) (10). Postsiloxane it was not possible to determine VL by using pleural markers below Pl = 7 cmH2O because regions of the lobe began to collapse. The absence of atelectasis at higher Pl was indicated by the gross appearance of the lung, the uniformity of the pressure-volume (P-V) changes between lungs and within lobes (6), and the smoothness of the light intensity data with changes in position of the optical probe.

The exponential expression of Salazar and Knowles (20),

\[ V = V_0 + VC [1 - \exp(-Pao/P_0)] \]

was fit to the pressure and volume data by least squares. V0 is lung volume at zero transpulmonary pressure, VC is the change in volume from V0 to its asymptotic value at high transpulmonary pressures (essentially equivalent to 30 cmH2O), and P0 is the pressure at which lung volume reaches 69% of its asymptotic volume. This was done separately pre- and postsiloxane for purposes of interpolating recoil pressures and optical data at isovolume points, which are re-
required for calculation of Vc postsiloxane as a percent of its control value.

**Experimental protocol.** Data were collected with the perfusion pump off and the PV cannula occluded, during slow (10–60 s) oscillations in vascular pressure generated by raising and lowering the blood reservoir, while P<sub>L</sub> was held constant at one of several levels (see Fig. 2). Microcirculatory intravascular pressure was defined as PV pressure, because the PV cannula was clamped during the maneuver and there was therefore minimal flow-resistive pressure loss between the capillaries and the PV. Vascular Ptm was defined as the difference between microcirculatory intravascular pressure and airway opening pressure. Vascular Ptm ranged from approximately −5 to +20 cmH<sub>2</sub>O. The design of our experiments kept PV pressure ∼PA pressure, so the vasculature was in West’s zone 1 when vascular Ptm < 0 and in zone 3 when vascular Ptm > 0 (30). The microvasculature may have transiently gone through zone 2 conditions as vascular Ptm went through zero. Before each data collection period, the lung and vasculature were exposed to standard volume histories; Pt was cycled from 2 to 30 cmH<sub>2</sub>O three times and lung and vasculature were exposed to standard volume histories, i.e., V<sub>L</sub> at Pt = 30 cmH<sub>2</sub>O presiloxane. After siloxane, lobes have increased recoil below ∼90% TLC due to increased γ. The change in recoil at 50% TLC was −5 cmH<sub>2</sub>O. At ∼90% TLC, the P-V curves meet, and the γ postsiloxane is probably little changed from control.

Before the lungs were treated with siloxane (see below), control measurements were made at Pt of 4, 7, 10, 12.5, 15, 20, and 30 cmH<sub>2</sub>O, chosen to cover the range from below FRC to TLC. After treatment, measurements were repeated at Pt of 7, 10, 12.5, 15, 17.5, 20, 25, and 30 cmH<sub>2</sub>O, chosen to cover the same range of lung volumes.

After control data were obtained, γ was altered by ventilating the lobe with 5 ml of nebulized, constant-γ, siloxane (polydimethylsiloxane, γ = 20.6 dyn/cm; Nye Lubricants, New Bedford, MA) as described in detail elsewhere (6). This treatment has been found to change the lung’s P-V relationship in a characteristic and repeatable fashion. The change in lung recoil pressure at any given V<sub>L</sub> is an index of the effective dose of the nebulized siloxane. The change in Pt was −5 cmH<sub>2</sub>O at 50% TLC and fell to zero at ∼90% TLC (Fig. 3). The volume of siloxane deposited has been determined to be 0.0045 ± 0.0026 ml/g lung (mean ± SD) (6).

The siloxane was nebulized into the inspiratory limb of the ventilator circuit over a period of 5–10 min during pressure ventilation at a respiratory rate of 20 breaths/min. During nebulization, the end-inspiratory pressure limit was ∼20 cmH<sub>2</sub>O. Over the course of nebulization, γ and lung recoil increased, the positive end-expiratory pressure (initially ∼5 cmH<sub>2</sub>O) was increased to ∼10 cmH<sub>2</sub>O to maintain end-expiratory V<sub>L</sub> at approximately its presiloxane level. During nebulization, the perfusion pump was off and the vascular reservoir was below the level of the lung, so vascular pressures were low.

**Data acquisition.** Raw signals from each pressure transducer (PA, PV, and airway) and the wavelength-specific light intensities at the three known distances from the source fibers were sampled at 100 Hz per channel and stored on a computer using a 12-bit analog-to-digital board and data acquisition software (Dataq Instruments, Akron, OH). All raw data were averaged over 1-s intervals. These averages were used in all subsequent analyses.

**Data analysis and statistics.** Diffuse light scattering allows calculation of fractional changes in Vc (relative to a reference Vc) at any given V<sub>L</sub>, but does not provide a measure of absolute Vc (25, 26). Light-scattering data were reduced to two lung volume-specific outcome variables, both measured at vascular Ptm of 5 cmH<sub>2</sub>O: 1) microvascular Cc and 2) the microvascular volume after changes in γ (Vc postsiloxane), expressed as a percent of its control values. At each V<sub>L</sub>, a vascular P-V loop was made separately before and then after increasing γ with siloxane (examples are shown in Fig. 4). Cc is essentially the slope of each microvascular P-V loop at the reference vascular Ptm (Fig. 4, point on the upper loop and square on the lower loop). Specifically, the fractional change in Vc with vascular Ptm was regressed quadratically against vascular Ptm and constrained to equal zero at the reference Ptm of 5 cmH<sub>2</sub>O. Cc is the slope of each regression at vascular Ptm = 5 cmH<sub>2</sub>O.

Vc postsiloxane was also calculated at the reference vascular Ptm of 5 cmH<sub>2</sub>O but required data collected both pre- and postsiloxane exposure, as shown in Fig. 4. Changes in

![Fig. 3. Deflation pressure-volume (P-V) data (means ± SD), control (●) and postsiloxane exposure (○) for all 4 dog lobes. Lung volumes (V<sub>L</sub>) are normalized to control total lung capacity (TLC), i.e., V<sub>L</sub> at Pt = 30 cmH<sub>2</sub>O presiloxane. After siloxane, lobes have increased recoil below ∼90% TLC due to increased γ. The change in recoil at 50% TLC was −5 cmH<sub>2</sub>O. At ∼90% TLC, the P-V curves meet, and the γ postsiloxane is probably little changed from control.](http://jap.physiology.org/)

![Fig. 4. Sample P-V loops for the pulmonary microcirculation. Loops were generated separately pre- (upper) and postsiloxane exposure (lower). Each loop shows the change in microvascular volume (Vc) during slow oscillations of vascular pressure, while V<sub>L</sub> was held constant. The slope of each P-V loop, at the reference vascular transmural pressure (Ptm) of 5 cmH<sub>2</sub>O (vertical dashed line), is the specific compliance (Cc). Postsiloxane the loops are flatter; i.e., increased γ decreased Cc (see also Fig. 6). In this example, Vc postsiloxane falls to −30% of control (vertical dashed line; scale not shown). Note that postsiloxane the reference volume for Cc (●) and Vc (○) are different, although both are measured at vascular Ptm = 5 cmH<sub>2</sub>O. Data shown are to demonstrate the method and are not at the same V<sub>L</sub>.](http://jap.physiology.org/)
optical data with changes in \( V_L \). Presiloxane were interpolated to match each of the postsiloxane \( V_L \). Because of the need for interpolation and the fact that the pre- and postsiloxane measurements were separated by an hour or more, our estimates of the change in \( V_c \) after increasing \( \gamma \) are not as robust as our estimates of \( C_c \). Note that the reference \( V_c \) used at each \( V_L \) to calculate \( V_c \) postsiloxane is the \( V_c \) at PtM 5 cmH\(_2\)O before siloxane exposure (e.g., Fig. 4 point on upper loop) and that this is different from the reference \( V_c \) used to calculate \( C_c \) postsiloxane, described above.

Data were pooled by nominal PtM for analysis of \( V_c \) postsiloxane and \( C_c \), which are presented as means \pm SE, and statistical significance was defined as \( P < 0.05 \).

**RESULTS**

Increasing \( \gamma \) increased lung elastic recoil at volumes up to \(-90\%\) TLC, as demonstrated by the rightward shift of the P-V curves postsiloxane (Fig. 3). The change in \( V_L \) at \( V_L = 50\% \) TLC was similar in all animals, \(-5\) cmH\(_2\)O.

The change in \( V_c \) postsiloxane varied significantly with \( V_L \) (Fig. 5). At \( V_L \) where postsiloxane \( \gamma \) was increased, \( V_c \) postsiloxane was less than control. At FRC (\(-45\%\) TLC), \( V_c \) postsiloxane was \(-30\%\) of control. At high \( V_L \) (where postsiloxane \( \gamma \) was decreased or little changed), \( V_c \) postsiloxane was greater than control.

In the control state, \( C_c \) fell significantly as \( V_L \) increased, from \(-7%/cmH_2O\) near FRC to \(-2.5%/cmH_2O\) at TLC (Fig. 6), similar to our previous results (25). After siloxane, \( C_c \) was \(-5%/cmH_2O\) and did not change significantly with \( V_L \). Near TLC (where \( \gamma \) was little changed by siloxane), \( C_c \) was unchanged after siloxane.

**DISCUSSION**

Increasing \( \gamma \) enough to shift the deflation P-V curve \( 5\) cmH\(_2\)O at \( 50\%\) TLC caused decreased \( C_c \) and capillary volume (and hence absolute capillary compliance) over most of the vital capacity. Capillary volume and compliance decreased substantially near FRC, where the changes in \( \gamma \) were large. In the following paragraphs, we discuss technical limitations of this study, compare our findings with those of others, and discuss the physiological implications of our findings.

**Technical limitations.** The two primary technical issues that affect our estimation of changes in \( V_c \) and \( C_c \) using diffuse light scattering are uncertainties about changes in lung geometry after siloxane and the presence of noncapillary blood in the field of view. These effects are addressed below. They are opposite in sign (i.e., they tend to cancel each other) and probably result in a small net underestimation of the magnitude of the changes in \( C_c \) and \( V_c \) with changes in \( \gamma \). Other minor technical limitations of light-scattering measurements with changes in \( \gamma \) are discussed in detail in the companion paper (6).

Light-scattering measurements assume a constant acinar geometry at a fixed \( V_L \). Siloxane could change acinar geometry directly, because of its volume, or indirectly as a result of changes in \( \gamma \). The volume of siloxane deposited, if spread uniformly, would result in a siloxane film layer \(-0.1\mu m\) thick at TLC and about twice that at FRC. This thickness is a negligible fraction of alveolar diameter and on the order of magnitude of the 0.1-\mu m thickness of the normal surfactant layer (15); thus it is thin enough to avoid changes in acinar architecture simply because of its volume. There is, however, significant uncertainty regarding the nature and degree of spreading (which is discussed in detail in the companion paper; see Ref. 6).

As discussed extensively by Wilson (32), an increase in \( \gamma \) is expected to cause a decrease in septal area at a given \( V_L \), but the magnitude of the change is uncertain. The change in area has been quantified morphometrically by Bachofen et al. (3) in rabbit lungs fixed after rinsing with a detergent and by us in unfixed rabbit lungs (6) after siloxane nebulization. Decreasing septal area at a given \( V_L \) would change the optical properties of the lung, and diffuse light scattering would then overestimate the actual change in \( V_c \) (25). If septal area at FRC fell by \(-17\%\) after siloxane treatment (6), then the corrected \( V_c \) postsiloxane would be \(-50\%\), rather than \(-30\%\), of control. This technical issue does
not affect our estimates of Cc, because these are based on measurements made at a single Vl and γ (either pre- or postsiloxane).

We are primarily interested in septal capillaries, but some of the blood in the volume sampled by diffuse light scattering is in larger extra-alveolar vessels. Our estimates of Cc and Vc postsiloxane with changes in γ are an average of all the blood in the sampled volume. More than two-thirds of the blood in lung parenchyma is in pulmonary capillaries (8, 13), and the contribution of capillary blood to our measurements is likely to be larger because we are sampling far from the hilum, in a region where large and moderately sized vessels are scarce. Further, the effective pressure outside extra-alveolar vessels is pleural pressure. In our experimental model, pleural pressure equals ambient pressure, and the effect of increasing γ was an increased Pt at all Vl up to ~90% TLC. Because we controlled vascular Ptm, the intravenous pressure also increased as γ increased. Therefore, the extra-alveolar vessels would be expected to increase, not decrease, their volume with increased γ. Our finding of a net decrease in Vc (an average for all the vessels in the sampled volume) with increasing γ is therefore an underestimate of the true fall in capillary volume.

Change in lung P-V curve and γ. The magnitude of the change in γ postsiloxane varies with Vl, and is manifested by the difference in Pt. This effect is large at low Vl, and falls to zero at high Vl (Fig. 3) for reasons explained below. The change in Pt at 50% TLC was similar in all animals, ~5 cm H2O, similar to the lavage preparation of Smith and Stamenovic (22). The lavage preparation is thought to result in a lung with an approximately constant γ independent of lung volume (14, 33), and to the degree that this is so our preparation is likely to be nearly the same. At the Vl at which the deflation pre- and postsiloxane P-V curves meet, there is no change in lung configuration (6, 22); thus γ in the siloxane-exposed lungs is equal to its value in normal lungs at this Vl. The deflation P-V curves met at ~90% TLC, a result similar to those of (22), and near the volume at which Schürch et al. (21) found γ in normal rat lungs during deflation of 20 dyn/cm. These findings suggest that the γ in our preparation after siloxane is probably near 21 dyn/cm (6, 22).

Change in Vc and Cc. Increased γ resulted in decreased Vc and Cc at Vl from ~40 to 90% TLC. That is, at Vl at which the siloxane exposed lungs had increased γ (an isovolume increase in Pt), both Cc and Vc postsiloxane were decreased. The changes in capillary volume and compliance were greatest at the lung volumes at which the γ change was greatest. At Vl at which the normal and postsiloxane γ were probably unchanged so were pre- and postsiloxane Cc and Vc. This last finding suggests that our treatment with nebulized siloxane resulted in a change in γ without injury to the lung parenchyma or pulmonary vasculature. We are unable to measure the absolute capillary compliance; however, at most Vl, the reference Vc is smaller after treatment (Fig. 5), therefore the fall in absolute capillary compliance is greater than that for specific compliance, Cc (Fig. 6).

Decreased Cc and Vc with increased γ suggests that the direct effect of γ on capillaries dominates any indirect effect of decreased septal tissue stress associated with modest septal retraction (which would have increased Cc and Vc). This further suggests that most capillaries have a positive radius of curvature (i.e., they bulge out into the alveolus). [The photomicrographs Figs. 1, 11, 12, and 19 in Ref. 28 show this feature. By contrast, cryo-scanning electron microscopy studies (4) report relatively smooth alveolar surfaces with little capillary bulging, but these were done at 15 cm H2O transpulmonary pressure with the lung in zone 1 or 2 and so are not directly comparable.] Alternatively, decreased Cc and Vc in the presence of increased γ could be caused by extreme septal retraction (see, e.g., Fig. 16 in Ref. 28) such that the capillaries become physically compressed. On the basis of our estimates of the change in surface area after siloxane (6), we feel that this is unlikely. On the other hand, because our primary data are a measure of blood volume per unit volume, they do not allow us to distinguish between these possible mechanisms for the change in Cc and Vc with γ, but they also are not dependent on any specific morphometric model for their fundamental interpretation.

Our findings compared with others. We have demonstrated a decrease in capillary size and compliance with increased γ, which is in agreement with work suggesting that γ exerts a compressive force on capillaries (28, 29, 31). Although we did not measure resistance directly, smaller and stiffer capillaries should have increased resistance. This conclusion is opposite to that previously drawn by others on the basis of vascular pressure-flow data during inflation compared with deflation (5, 18, 23) or on video microscopy of subpleural alveoli (17).

Sun et al. (23) compared middle-segment resistance, estimated from vascular occlusion data, at iso-lung volume points during deflation compared with inflation. [They also compared isovanealveolar pressure points, but we have no isovanealveolar pressure data and so restrict our comparison to their isovolume data. Their vascular Ptm (defined as intravascular-alveolar pressure) were lower during inflation than during deflation, which would affect resistance independent of changes in γ. They concluded that, at fixed Vl, increased γ decreases capillary resistance, but an alternative interpretation of their data suggests otherwise. Specifically, at ~50% TLC (the only lung volume at which they present data during both inflation and deflation), they found a smaller pressure drop across the middle segment, 5.0 mm Hg, during deflation (when γ is lower) than during inflation, 10.2 mm Hg (when γ is higher). The lower pressure drop during deflation is consistent with a lower, not higher, resistance when γ is lower and is in agreement with our data. Pain and West (18) and Bruderman et al. (5) found pulmonary vascular pressure-flow relationships consistent with lower resistance during inflation (when γ
is higher) than during deflation. Their findings were dependent on the entire pulmonary circulation, not just the microvasculature; nonetheless their data suggest that increased $\gamma$ decreased microvascular resistance. We cannot explain the apparent inconsistency between our volume findings and their flow findings.

Nieman et al. (17) report that capillary (middle segment) resistance decreased after surfactant depletion with detergent, which they report increased the minimum $\gamma$ to 24 dyn/cm. This change in $\gamma$ should have increased the $P_t$ near 50% TLC (where normal $\gamma$ is much lower than 24 dyn/cm), while causing only small changes in the lung P–V curve at high $V_L$ (where normal $\gamma$ is somewhat higher than 24 dyn/cm) (21). However, they report (Fig. 1 in Ref. 17) that control changes in the lung P–V curve at high $V_L$ (where $\gamma$ is somewhat higher than 24 dyn/cm) (21).

In summary, we have found significant changes in capillary mechanics associated with modest changes in $\gamma$. Importantly, these are changes that are directly attributable to $\gamma$ and are independent of the many other factors associated with diseases which may also influence capillary mechanics. Furthermore, the findings in this study are underestimates of the magnitude of changes directly associated with altered $\gamma$ seen in diseases such as acute respiratory distress syndrome, if in those cases $\gamma$ is significantly higher than in our experiments. We conclude that alterations in $\gamma$ in a variety of pulmonary diseases have direct effects on capillary mechanics and may play a significant role in the pathophysiology of gas-exchange deterioration and increased leukocyte sequestration in the lung.

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