Effects of tidal volume and respiratory frequency on lung lymph flow

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LUNG LYMPH FLOW (QL) has long been known to be affected by ventilation (V), but the underlying mechanisms remain poorly understood (1, 12, 13, 17, 26). For example, increases in tidal volume (VT) and respiratory frequency (f) caused by the addition of dead space (1) resulted in a small but persistent increase in lymph flow from the efferent duct of the caudal mediastinal lymph node in intact sheep. Using the same experimental preparation, Koizumi et al. (13) found large increases in QL associated with the hyperpnea of exercise. They attributed this increased QL to the combination of hyperpnea and an increased pulmonary capillary pressure. Hyperpnea alone had no effect on QL, whereas an isolated elevation in pulmonary capillary pressure increased QL by only a small amount (13). The large V-induced increase in lymph flow in exercising animals was hypothesized to explain the ability of the lung to maintain normal extravascular lung water in the face of significant exercise-induced increases in pulmonary capillary pressures. These studies, however, were unable to determine the separate effects of changing VT and f. Moreover, lung lymph collected from the efferent duct of the caudal mediastinal lymph node may be contaminated with lymph from diaphragmatic lymphatics (5). The contribution of diaphragmatic lymph increases with diaphragmatic contraction (5) and thus could vary with the depth and rate of V.

Less is known about the effect of V on QL in injured, edematous lungs. Studies designed to quantify QL relative to total edema formation in animal models of acute pulmonary edema have concluded that lymph flow contributes little to overall edema clearance (9, 12, 14, 16, 23). QL correlated with lung fluid filtration rate rather than total lung edema, suggesting that lymph flow drains a small perimicrovascular compartment close to the site of fluid filtration that is connected in series to a larger interstitial compartment surrounding the extra-alveolar vessels (6, 8, 12, 16, 25). The contribution of QL to edema clearance in this situation may be underestimated, however, because these studies were performed with fixed levels of V in anesthetized animals or isolated lungs. If a large increase in QL results from the increased V triggered by early pulmonary edema (13), QL may play a more important role in overall edema clearance than previously realized. Moreover, the small perimicrovascular compartment drained by lymph flow may be important for gas exchange. Thus the relationship between V and QL in injured lungs may be relevant to the selection of ventilatory strategies designed to limit human ventilator-induced lung injury in the intensive care unit (7).

The purpose of the present study was to investigate the separate effects of VT and f on QL and fluid balance in isolated, in situ, perfused sheep lungs (17), which become injured due to ischemia and reperfusion (20). In the initial description of this preparation, we found that QL was directly proportional to f over a range of 0 to 30 min⁻¹, but the separate effects of VT and f on QL and fluid balance were not systematically examined (17). The major advantage of the isolated, perfused lung includes the ability to collect QL without contamination by systemic lymph while controlling key physiological variables. In this paper, we first examine the effects of independently changing VT and f over the same range of V but at different times during the course of edema formation. In a second group of experiments, changes in VT are followed for a longer time period to determine whether effects on QL are transient or sustained.

METHODS

Preparation

All animals received care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The study protocol was approved by the Johns Hopkins Animal Care and Use Committee. All animals were transported to the laboratory in a humidified environment and were allowed at least 30 min to adapt to room conditions before study initiation. Anesthesia was induced with propofol (1 mg/kg bolus) followed by isoflurane (1%) and was maintained with isoflurane (0.5%) and oxygen (2 L/min) throughout the experiment. The lungs were isolated with a 5-ml/kg bolus of heparin (5,000 U/kg) and were ventilated (VT 12.5 ml/kg, 10 min⁻¹; f 20 min⁻¹) under a constant VT (12.5 ml/kg) to obtain washout of the lungs with no change in QL. After 30 min of VT, VT was increased to 25 ml/kg (VT 100 min⁻¹). VT was then kept constant or changed to 20 or 3 ml/kg during a second 100-min period. Increases in VT or f increased QL and vice versa, without corresponding effects on the rate of edema formation. For the same change in VT, changing VT had a greater effect on QL than changing f. The change in QL caused by an increase in VT was significantly greater after the accumulation of interstitial edema. The change in QL caused by a sustained increase in VT was transient and did not correlate with the rate of edema formation, suggesting that V altered QL through direct mechanical effects on edema-filled compartments and lymphatic vessels rather than through V-induced changes in fluid filtration.

Effects of tidal volume and respiratory frequency on lung lymph flow. J Appl Physiol 99: 556–563, 2005. First published March 24, 2005; doi:10.1152/japplphysiol.00254.2005.—Ventilation (V) increases lung lymph flow (QL), but the separate effects of tidal volume (VT) and frequency (f) and the role of V-induced changes in edema formation are poorly understood. An isolated, in situ sheep lung preparation was used to examine these effects. In eight sheep with f = 10 min⁻¹, results obtained during 30-min periods with VT = 5 or 20 ml/kg were compared with values obtained during bracketed 30-min control periods (VT = 12.5 ml/kg). Eight other sheep with constant VT (12.5 ml/kg) were studied at f = 5 or 20 min⁻¹ and compared with f = 10 min⁻¹. Three additional groups of six sheep were perfused for 100 min with control V (10 ml/kg, 10 min⁻¹). VT was then kept constant or changed to 20 or 3 ml/kg during a second 100-min period. Increases in VT or f increased QL and vice versa, without corresponding effects on the rate of edema formation. For the same change in VT, changing VT had a greater effect on QL than changing f. The change in QL caused by an increase in VT was significantly greater after the accumulation of interstitial edema. The change in QL caused by a sustained increase in VT was transient and did not correlate with the rate of edema formation, suggesting that V altered QL through direct mechanical effects on edema-filled compartments and lymphatic vessels rather than through V-induced changes in fluid filtration.

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Hopkins Animal Care and Use Committee. Young sheep (20–30 kg) were anesthetized with intramuscular ketamine (30 mg/kg) and atropine (0.5 mg). Anesthesia was maintained by intravenous injections of ketamine (3–6 mg/kg) at 15- to 30-min intervals. A tracheostomy was performed, and mechanical ventilation was begun with oxygen-supplemented room air at a $V_t$ of 12.5 ml/kg and $f$ of 15 min$^{-1}$. A sternotomy was performed, and the superior thoracic duct was catheterized above the hilum of the left lung with silicone rubber tubing (inner diameter: 0.03 in.). Heparin (10,000 units) was then injected intravenously, and the animal was exsanguinated from a left atrial cannula into the perfusion reservoir. $V$ was maintained with warmed, humidified gas mixture containing 28% O$_2$ and 5% CO$_2$ at a tafo of 10 and end-expiratory tracheal pressure (Ptr) of 4 mmHg.

The pulmonary artery was cannulated and connected to an extracorporeal circuit described previously (17). After 30 min of ischemia, the lungs were perfused in situ at 100 ml kg$^{-1}$ min$^{-1}$ with a mixture of autologous blood (∼1,000 ml) and 3% dextran in Ringer lactate solution (∼300 ml). The perfusate was warmed (38–39°C) with a heat exchanger (Travenol Miniprime) and continuously filtered of clots and bubbles with a filter. To prevent the transient pulmonary hypertension that normally occurs with extracorporeal perfusion of isolated sheep lungs (18–20), indomethacin (100 mg), dissolved in a mixture of normal saline (10 ml) and 1 N H$_2$SO$_4$ (10 ml), was added to the perfusate in an amount sufficient to achieve a perfusate concentration of 20 μg/ml. Our laboratory previously showed that indomethacin has no effect on the vascular barrier dysfunction and edema resulting from ischemia and reperfusion in this preparation (4). Blood drained from the left atrium through a Starling resistor to a reservoir and pressure in the left atrial cannula were controlled by adjusting the pressure around the Starling resistor. The reservoir was suspended from a force transducer, and changes in lung weight ($\Delta W$) were measured as the opposite of reservoir weight change. Our laboratory previously showed that, in this preparation, circulating vascular volume remained constant and vascular anastomotic leaks were trivial, allowing $\Delta W$ to accurately track minute-to-minute changes in vascular fluid filtration (17). Moreover, the presence of significant edema clearance across the visceral pleura caused $\Delta W$ to more accurately quantify total filtered fluid compared with gravimetric measurements of extravascular lung water (21). When necessary, the slope of the relationship of $\Delta W$ and time was calculated at 10-min intervals to determine the rate of reservoir weight change ($W$), which was used as an estimate of lung fluid filtration (21).

The lymph catheter in the superior thoracic duct was allowed to drain into a small beaker suspended from a force transducer ~20 cm below the lungs to permit continuous determination of $Q_l$. This position ensured that the cannulated duct was always collapsed, thus maintaining a lymphatic outflow pressure of zero (17). Lymph collected in this fashion from our preparation was previously shown to be uncontaminated by lymph from unperfused organs above and below the diaphragm (17). Lymph and plasma colloid osmotic pressures were measured at 30-min intervals with an oncometer (Instrumentation Laboratories, model 186).

$P_t$, pulmonary arterial pressure ($P_{pa}$), and left atrial pressure ($P_{la}$) were referenced to the level of the left atrium. $P_{pa}$ and $P_{la}$ were determined at end expiration while the lung was mechanically ventilated. Pressures, flow, weights, and inspired O$_2$ and CO$_2$ concentrations were continuously recorded (Grass model 7D polygraph). Perfusate O$_2$ and CO$_2$ tensions and pH were measured at regular intervals using standard electrode techniques. Perfusate pH was adjusted to 7.35–7.45 at 30-min intervals with 1 N NaHCO$_3$. Perfusate glucose concentrations were monitored with Dextrostix and kept within 90–130 mg/dl by periodic addition of 50% glucose in water.

**Protocols**

**Protocol 1: changes in $V_t$ or $f$.** Figure 1 provides a diagram of the protocols. In the first part of the study, four groups of four lungs each were perfused for a total of 150 min (after a 30-min stabilization period), which was divided into five 30-min study periods. In two groups, $V_t$ was changed from the control setting (12.5 ml/kg) to either 20 or 5 ml/kg for 30 min before returning to control for another 30 min. $V_t$ was then changed in the opposite direction for 30 min followed by the final control period. The $f$ was kept constant at 10 min$^{-1}$ during the entire protocol. The other two groups underwent a similar protocol, except that $V_t$ was kept constant at 12.5 ml/kg and $f$ was changed to 30-min periods of either 20 or 5 min$^{-1}$, always bracketed by control frequency (10 min$^{-1}$). Changes in $V_t$ were further classified as early or late, depending on when they occurred during the course of the 150-min perfusion.

**Protocol 2: prolonged change in Vt.** As shown in the bottom of Fig. 1, three additional groups of six sheep were perfused for 200 min. After a control period of 100 min ($V_t = 10$ ml/kg, $f = 10$ min$^{-1}$), $V_t$ was changed to 20 or 3 ml/kg or kept constant during the subsequent 100-min experimental period.

**Statistics**

All time course data were compared with a two-factor (group, time), split-plot analysis of variance. Because of nonhomogeneity of variance, the Qt values were transformed to logarithms before statistical analysis to comply with the assumptions of the analysis of variance. When significant variance ratios were obtained, least significant differences were calculated to allow comparison of individual means. The relationship between Qt and W was determined by least squares linear regression. Values presented in the text are means ± SE. Differences were considered significant when $P \leq 0.05$.

**RESULTS**

Sheep body weight averaged 25.9 ± 0.6 kg and was not different among groups in either protocol. There were also no differences between or within groups in perfusate Hct, PO$_2$, PCO$_2$, or pH, which averaged 23.4 ± 0.6%, 148.0 ± 0.1 Torr, 28.0 ± 0.6 Torr, and 7.40 ± 0.003, respectively. The lympho-plasma colloid osmotic pressure ratio did not differ as a function of time within or between groups, averaging 0.57 ± 0.01.

**Protocol 1**

Figure 2 shows the effects of the changes in $V_t$ (Fig. 2A) and $f$ (Fig. 2B) on peak Ptr. As expected, the changes in $V_t$ caused corresponding changes in peak Ptr. For example, increasing the $V_t$ from 12.5 to 20 ml/kg caused a 53% increase in peak Ptr, whereas decreasing to 5 ml/kg decreased peak Ptr by 50%. The two time courses of peak Ptr differed from each other during both control periods, in addition to the experimental period where $V_t$ was changed ($P < 0.001$, ANOVA interaction). The differences in control Ptr likely resulted from persistent effects of the immediately preceding experimental intervention (Fig. 1). Increasing $f$ from 10 to 20 min$^{-1}$ increased peak Ptr by 5%, whereas a decrease in $f$ to 5 min$^{-1}$ had no effect on peak Ptr. The two protocols resulted in Ptr values that differed from each other only at the onset of the experimental period and during the second control period ($P < 0.05$, ANOVA interaction).

As shown in Fig. 3, changes in $V_t$ had significant effects on Qt. The increase in $V_t$ from 12.5 to 20 ml/kg caused a rapid 74% increase in Qt by the first 5-minute measurement at 20 ml/kg. When $V_t$ was returned to the control level, Qt decreased by 30%. All Qt values measured during the increased
VT were greater ($P < 0.01$, ANOVA interaction) than corresponding $Q_t$ measured during the decrease in VT. The increase in $f$ from 10 to 20 min$^{-1}$ caused a smaller, more gradual increase in $Q_t$ than that caused by increased VT (30 vs. 74%; Fig. 3B). This time course differed significantly ($P < 0.05$, ANOVA interaction) from the decrease in $Q_t$ caused by decreased in $f$ but only at the 10-min time point.

Figure 4 shows the effects of changing $V$ on end-expiratory $P_{pa}$ and the average $W$ during each $V$ condition. End-expiratory $P_{pa}$, which averaged 19.4 ± 1.5 mmHg at the beginning of the first control period, was not affected by changes in VT. The lungs subjected to changing $f$ differed in the time course of $P_{pa}$ as a function of protocol order ($P < 0.05$, ANOVA interaction) because of a small but significant increase in $P_{pa}$ beginning during the control period preceding the $f$ of 5 min$^{-1}$. $W$ averaged 2.3 ± 0.4 g·h$^{-1}$·kg$^{-1}$ over the two control and experimental time periods. The only significant difference in the time course of $W$ that occurred as a function of $V$ was between the two control periods before the change in VT.

The independent effects of VT and $f$ on $Q_t$ and $W$ per unit change ($\Delta$) in $V$ are shown in Fig. 5. Increasing VT or $f$ caused similar significant increases in $\Delta Q_t/\Delta V$ ($P < 0.05$), but decreasing VT produced a larger decrease in $\Delta Q_t/\Delta V$ compared with decreasing $f$ over the same range of $V$ (ANOVA interaction term; $P < 0.005$). Increasing VT but not $f$ increased $\Delta W/\Delta V$ compared with baseline, but decreases in $V$ or $f$ did not affect $\Delta W/\Delta V$, and the effects of VT and $f$ on $\Delta W/\Delta V$ did not differ from each other as a function of the change in $V$ (ANOVA interaction term; $P > 0.05$), indicating a disconnect between the effects of $V$ on $Q_t$ and $W$.

The design of protocol 1 (Fig. 1) allowed a comparison of the effects of the same ventilatory changes as a function of when they occurred during the total perfusion time. For example, increases in VT from 12.5 to 20 ml/kg occurred at both 30 (early) and 90 min (late), measured from the beginning of the first control period. This allowed comparisons at different phases of edema formation, because all lungs steadily gained weight throughout the protocol (data not shown). As shown in Fig. 6, the time course of $Q_t$ resulting from the increase in VT from 12.5 to 20 ml/kg differed significantly, depending on whether it occurred early or late in the experimental protocol ($P < 0.002$; ANOVA interaction). Specifically, the late increase in VT caused an increase in $Q_t$ that peaked 5 min after the increase in VT followed by a decrease in $Q_t$, despite the continued increase in VT. In contrast, $Q_t$ continued to gradually increase after the initial VT-induced increase when the same protocol occurred earlier in the protocol. To allow a specific comparison between the early and late immediate changes in $Q_t$ following the increased VT, we performed an ANOVA of the $Q_t$ values expressed as the relative change from the initial control value. This analysis revealed that the immediate changes in $Q_t$ caused by increasing VT were significantly greater when performed later compared with earlier in perfusion ($P < 0.02$; ANOVA interaction). These differences in $\Delta Q_t$ were not secondary to differences in $\Delta P_{tr}$, which were similar at the two time points (data not shown).
Protocol 2

Figure 7 shows the time course of $Q_L$ over 200 min of perfusion in three groups of lungs with different VT during the second half of the protocol. The data are normalized to the $Q_L$ measured at 100 min because the time course of $Q_L$ over the first 100-min period did not differ between groups, increasing to $0.11 \pm 0.02$, $0.18 \pm 0.06$, and $0.15 \pm 0.05$ ml·h$^{-1}$·kg$^{-1}$ by 100 min in the control, low-VT, and high-VT groups, respectively. $Q_L$ continuously increased in control lungs over the entire time course. This increase was linearly related to a continual increase in W ($Q_L = 0.12 W + 0.002; r = 0.98; P < 0.0001$), such that the change in W accounted for 96% of the variance in $Q_L$ (data not shown). Increasing the VT from 10 to 20 ml/kg at 100 min of perfusion resulted in nearly a threefold increase in $Q_L$ compared with control lungs at the same time point. This increase in $Q_L$ was transient, however; and $Q_L$ in the high-VT group was not different from control levels over the last 60 min of the experimental period. The decrease in VT from 10 to 3 ml/kg in the low-VT group caused a 60% decrease in $Q_L$ that remained significantly different from both control and high-VT values throughout the experimental period ($P < 0.005$ by ANOVA interaction). The relationship between W and $Q_L$ in the high-VT group ($r = 0.77$) had a significantly lower $r$ value compared with that in the control group ($r = 0.98$). The $r$ value for this regression was also lower in the low-VT group ($r = 0.90$), but this was not statistically different from the control group.

The changes in VT did not have a significant effect on the average W determined over the last 40 min of each experimental period. All three groups demonstrated a significant increase in W from just before the VT change to the last 40 min of the experimental period, but the magnitude of this increase did not differ among groups (Fig. 7, inset).

**DISCUSSION**

Increasing lung V is known to increase $Q_L$, but the relative contributions of VT and f and the role of V-induced changes in fluid filtration have been difficult to separate utilizing intact animal preparations (1, 13, 26). Moreover, there are few data...
describing these effects in lungs with ongoing endothelial barrier dysfunction. We, therefore, chose to examine the separate effects of VT and f on Q₁ in the isolated, perfused sheep lung (17). The advantages of this preparation include the ability to 1) collect pure lung lymph without contamination from systemic lymphatics, 2) control many variables that can affect lung fluid balance, 3) measure minute-to-minute fluid filtration, and 4) separately change VT and f without altering blood-gas tensions or pH (17, 19–21). In addition, this preparation develops progressive interstitial edema and increasing Q₁ (18–20) with preserved lymphatic contractions (17). The pulmonary vascular barrier dysfunction and edema are reproducible, resulting from an oxidant injury triggered by ischemia and reperfusion (4, 20). In the initial description of this preparation, we found that Q₁ was directly proportional to f over a range of 0 to 30 min⁻¹, but the separate effects of f and VT on Q₁ and fluid balance were not systematically examined (17).

To reproduce the same range in V generated by doubling and halving a control f, we varied VT from a low of 3 to a high of 20 ml/kg. Although this VT may fall within a VT range capable of causing ventilator-induced lung injury in rodent models (7), a VT as large as 30 ml/kg was well tolerated in adult sheep (24) and lambs (3) for several hours without evidence of significant lung dysfunction.

Previous studies suggest at least three mechanisms to explain the increased Q₁ observed with increases in V. First, inspiration is thought to compress a low-compliance perimicrovascular space near alveolar vessels and enlarge the interstitial space surrounding extra-alveolar vessels, airways, and conduit lymphatics (1, 13, 17, 22). These effects may phasically increase the upstream pressure for lymphatic drainage while simultaneously decreasing downstream pressure and...
lymphatic resistance by dilating conduit lymphatic vessels (6). Because peribronchovascular interstitial pressure increases with increasing accumulation of edema fluid (2, 15), at some point in time increasing lung volume may serve to phasically compress conduit lymphatics simultaneously with the perimicrovascular interstitial space. The presence of lymphatic valves ensures that lymph flow remains unidirectional, regardless of the direction of the pressure gradient between the two interstitial compartments. Second, the phasic circumferential and longitudinal stretch of interstitial lymphatics occurring with each breath could trigger an active lymphatic contraction (11, 12). If this mechanism predominated and the efficiency of each lymphatic contraction was independent of VT, one might expect that the major effect of hyperpnea on lymph flow would be through increases in f rather than VT. Lastly, the increased QT associated with increased V was hypothesized to result, in large part, from a V-induced increase in the rate of fluid filtration across extra-alveolar vessels (1).

Our results confirm previous studies in intact animals (1, 3, 13), which showed that increases in V caused increases in QT and, in addition, demonstrate that changing VT had a greater effect on QT than changing f over the same range of V (Figs. 3 and 5) in a preparation without diaphragmatic lymph contamination. Specifically, we found that increasing and decreasing VT from the control level of V had similar, significant corresponding effects on ΔQT/ΔV (Fig. 5). The increase in f from control V had an equivalent effect on ΔQT/ΔV to increasing VT, but the decrease in f had a smaller, nonsignificant effect on ΔQT/ΔV, suggesting a flatter dose-response curve for f compared with VT at the lower end of V.

The time to maximal ΔQT associated with the ΔV changes was rapid, occurring within 5 min of a change in VT and within 10 min of a change in f (Fig. 3). The ΔQT caused by an increase in VT was significantly greater if VT was altered later in the protocol after the accumulation of interstitial edema (Fig. 6). The V-induced changes in QT were not correlated with changes in W (Figs. 4 and 5), suggesting that changes in V altered QT through direct mechanical effects on edema-filled compartments and lymphatic vessels rather than through V-induced changes in extra-alveolar fluid filtration. Despite the overall disconnect between QT and W, we did observe that the ΔW/ΔV increased in association with the increase in ΔQT/ΔV in the condition where VT was increased from control (Fig. 5B), raising the possibility that increased fluid filtration could have contributed, at least in part, to the increase in QT in this situation.

To further address the possibility that the changes in QT caused by changing VT were occurring because of changes in fluid filtration, we performed the experiments described in protocol 2. In designing this protocol, we considered a model of lung lymphatic drainage (16) similar to those previously proposed by other investigators (6, 8, 12, 25) to explain the effects of V on ΔQT. In this model (Fig. 8), the perimicrovascular fluid space is represented by a compliance (Cpmv), which simultaneously drains 1 through a resistance into a second compliance within the peribronchovascular space (Cpbv) and 2 into the lymphatics, which course through, but have no communication with, the peribronchovascular space (9). Thus QT is dependent on the rate of microvascular fluid filtration rather than the absolute amount of interstitial edema surrounding extra-alveolar vessels (6, 8, 12, 16, 25).

As described above, inspiration would compress the Cpmv and increase the perimicrovascular pressure (Ppmv) through increases in the surrounding alveolar pressure. Before the accumulation of significant interstitial edema, inspiration could simultaneously enlarge the Cpbv and decrease the peribronchovascular pressure (Ppbv), creating a phasic pressure gradient for fluid movement into the lymphatics and the Cpbv (10, 16). After the accumulation of a critical quantity of interstitial edema, inspiration could simultaneously compress both the Cpmv and Cpbv (2). This would tend to decrease the extra-lymphatic gradient for fluid movement from the Cpmv to the

![Figure 7](http://jap.physiology.org/)

**Fig. 7.** Time course of QT in control (VT = 10 ml/kg), high (VT = 20 ml/kg), and low (VT = 3 ml/kg) lungs in protocol 2. QT is normalized to the value obtained at 100 min just before changing VT. The inset graph shows the average W determined between 160 and 200 min normalized to the W measured just before the change in VT (60–100 min). *P < 0.005 vs. control (ANOVA interaction)."
Cpbv (2) but could theoretically further enhance the phasic increase in Ql because of the additional volume of lymph being compressed by each inspiration and the presence of one-way valves in the lymphatic vessels. Changes in f would function in a similar manner through changes in the mean alveolar pressure, Ppmv, and Ppbv at any given VT. In addition, changes in f could trigger changes in the frequency of lymphatic contractions, which, in the presence of valves, could influence Ql (11, 12). Our observation that ΔQl was larger when the increase in VT was performed later in the experiment (Fig. 6) is consistent with the notion that an edematous Cpbv may increasingly compress peribronchovascular lymph vessels during inspiration. This would imply that the direct compression of these valved lymphatics, which may have occurred when VT was increased later in the time course, was more effective at driving forward flow than the decrease in lymphatic resistance from the early increase in VT before the development of significant interstitial edema.

Based on this model, we hypothesized that, if the added compressive effect caused by an increased VT did not affect transmircovascular fluid movement or alter the way in which fluid was distributed between the two parallel drainage pathways (lymphatic vs. peribronchovascular), the outcome would be a transient increase in Ql that would return to the control level of Ql associated with the original VT, despite the continued larger VT. Under these circumstances, increasing VT from control to 20 ml/kg would cause a transient increase in Ppmv and decrease in Ppbv. As a result, Ql would increase until the additional volume in the Cpmv discharged, returning Ppmv and Ql to control levels. The opposite effect would be anticipated with a decrease in VT. This would be analogous to the similar transient changes in exhaled gas CO₂ content that occur with changes in VT, despite a constant rate of CO₂ production in the tissues. If the effect of a VT change on Ql were solely due to enhanced extra-alveolar fluid filtration, the change in Ql should persist for as long as the change in VT was present. To test this hypothesis, we altered VT in two separate groups of lungs over a longer observation period and compared the effects on Ql with a third constant V group that allowed an estimation of control Ql over time. As shown in Fig. 7, the increase in VT did, in fact, cause a transient increase in Ql, returning to control levels by the end of the protocol. Interestingly, after a decrease in VT, the resulting decrease in Ql did not recover to control levels. Despite the significant effects on Ql, the changes in VT did not cause concomitant changes in W (Fig. 7, inset), adding independent support for a mechanical explanation behind the changes in Ql.

The prolonged decrease in Ql from the reduction in VT differs from the study by Sakuma et al. (23), in which completely eliminating left lung V by clamping the left bronchus in intact sheep did not affect the increase in caudal mediastinal lymph flow following instillation of serum in the left lower lobe. These data are not easily compared with the present study, however, because right lung f was increased in the bronchial occlusion group to maintain blood gases and the distribution of blood flow was likely altered between lungs because of hypoxic vasoconstriction in the clamped lung. The exact explanation for the lack of Ql recovery after decreasing VT shown in Fig. 7 is unknown, but this finding suggests that the effective resistance to lymphatic drainage increased with lower VT. For example, if a reduction in VT from 10 to 3 ml/kg resulted in a significant increase in lymphatic resistance to flow because the associated less negative Ppbv failed to adequately stent open the peribronchovascular lymphatics, then one might expect the recovery of the transient decrease in Ql to be prolonged. The same phenomenon would occur if the mean lymphatic driving pressure fell below a downstream lymphatic critical pressure (27) in some areas of the lung. Although Ql represents a small fraction of overall edema clearance from the lung (21), this steady-state reduction in Ql could theoretically result in a greater accumulation of perimicrovascular fluid over time if fluid filtration remains constant. Whether this happens and has a detrimental effect on lung function would depend on the ability of alternative edema clearance pathways to remove this excess interstitial fluid (21). Additional experiments will be required to settle this issue.

In summary, both increases and decreases in V caused significant rapid changes in Ql in the same direction that did not correlate with changes in W. Changing VT had a greater effect on Ql compared with changing f over the same range of V. The ΔQl caused by an increase in VT was significantly greater if VT was altered after the accumulation of interstitial edema. The ΔQl caused by a sustained increase in VT was transient and did not correlate with W, suggesting that VT altered Ql through direct mechanical effects on edema-filled compartments and lymphatic vessels rather than through V-induced changes in extra-alveolar fluid filtration. A sustained decrease in VT resulted in a sustained decrease in Ql, suggesting that the decrease in VT in some way increased the downstream lymphatic resistance. The functional significance of the prolonged decrease in Ql caused by decreasing VT and the potential interactions of simultaneous changes in VT and f will require further study.

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