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Effect of Lung Volume Reduction Surgery on Pulmonary Diffusion Capacity in a Rabbit Model of Emphysema[1]

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Background. **While there is renewed interest in lung volume reduction surgery (LVRS) for treatment of emphysema, many aspects of the operation such as patient selection and surgical end points of excision are uncertain. We studied the effects of LVRS on measured lung volumes and diffusion capacity in an animal model to investigate optimal resection volumes.** ***Methods.*** **Emphysema was induced in 32 New Zealand white (NZW) rabbits using aerosolized elastase. Helium dilution lung volumes and single breath DLCO were measured concurrently at baseline, following induction of emphysema (preop), and 1 week postoperatively (postop) following LVRS. Bilateral upper and middle lobe stapled lung resections were performed through midline sternotomies with excision of variable amounts of lung tissue from 1.8 to 5.8 g.** ***Results.*** **FRC increased following induction of emphysema and decreased postoperatively. DLCO improved with increasing lung tissue resection up to 3 g of tissue and then decreased as even greater amounts were removed (r = 0.54).** ***Conclusions.*** **Measured lung volumes increase with development of emphysema and appropriately decrease in response to LVRS in this rabbit model. DLCO improves with moderate resection but then decreases with excessive excision of lung quantities and may help define one physiologic operative end point. In this rabbit model, excision of approximately 30% of lung volume was optimal and prevented further decrease in diffusion capacity.**

Key Words: **lung volume reduction surgery; rabbit model.**

INTRODUCTION

Lung volume reduction surgery (LVRS) has recently been recognized as a potential palliative treatment for refractory obstructive emphysema. However, many aspects of LVRS procedures remain uncertain and require further investigation. There is currently little clinical data available regarding exchange of gases at the alveolar level following surgical lung volume reduction.

Gas exchange is a function of accessibility to the alveolar-capillary bed as well as the membrane characteristics of the alveolar lining. Obstructive emphysema decreases diffusion capacity by decreasing the ventilating compartment of the lung, thereby decreasing the available exchange surface area. Total diffusion capacity as measured by the diffusion of carbon monoxide (DLCO) is decreased, as is alveolar volume adjusted DLCO (DLCO/VA) [1].

LVRS has been suggested to improve airway support and thereby increase expiratory flows [2]. Volume reduction is designed to remove nonventilating or poorly ventilating lung segments. How this intervention affects the lung's overall ability to diffuse respiratory gasses is not well documented.

End points for optimal resection volumes during surgery are not known. Spirometric improvement with increasing lung volume resection may have to be weighed against potential reduction in gas exchange capabilities as larger volumes of lung tissue are resected.

For these reasons, we studied the effects of LVRS on measured lung volumes and single breath DLCO in a rabbit model of elastase-induced obstructive emphysema. Changes in lung volume and the resulting changes in diffusion capacity were followed through the development of emphysema and following LVRS to demonstrate physiologic interactions that must be assessed to optimize LVRS procedures.

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METHODS

This protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Irvine. All rabbits were cared for in accordance with the NIH Guidelines for the Care and Use of the Laboratory Animal.

Animal preparation. Thirty-two rabbits (3.0–4.5 kg) were anesthetized with 2:1 mixture of ketamine HCl (100 mg/ml):xylazine (20 mg/ml) at a dose of 0.75 ml/kg im. The rabbits were intubated with a 3-mm endotracheal tube and mechanically ventilated (Harvard Apparatus Dual Phase Control Respiratory Pump—Canine, Harvard Co., South Natic, MA) with a tidal volume of 50 ml and a frequency of 30–40/min. A 20-gauge iv catheter was placed in a marginal ear vein for iv access. Anesthesia was maintained with 0.3 ml of 1:1 mixture of ketamine HCl (100 mg/ml):xylazine (20 mg/ml) given as iv bolus as needed to maintain apnea throughout all procedures.

Induction of emphysema. Emphysema was induced in 32 rabbits under general anesthesia by aerosolizing 15,000 units (7.89 ml) of porcine elastase (Product E1250, Sigma Chemical Company, St. Louis, MO) through the endotracheal tube over approximately 1 h. The nebulizer (Respirgard, Marquest Medical Products, Inc., Englewood, CO) was placed in the inspiratory arm of the ventilator circuit with the tidal volume provided by the ventilator set at zero with a rate of 30 breaths/min. The O_2 flow through the nebulizer was adjusted to maintain the peak airway pressure at 20 cm H_2O, monitored by a pressure gauge placed at the side port of the endotracheal tube which provided the tidal volume during induction.

Pulmonary function testing. Lung function measurements were obtained at baseline prior to induction of emphysema, immediately preoperatively at 4 weeks following induction of emphysema, and 1 week postoperatively. Static lung compliance and gas dilution lung volumes were measured at each time interval.

Gas dilution lung volumes. The inhalation gasses consisted of 9.30% helium, 60.50% oxygen, 29.05% nitrogen, 0.87% C_2H_2, and 0.28% $C^{18}O$ (Liquid Carbonics Corp., Los Angeles, CA). All gas concentrations were measured continuously by an on-line mass spectrometer (MGA 1100, Perkins-Elmer Corp., Pomona, CA). Analog data were converted to digital information by an AD converter (Keithley System 570, Cleveland, OH) sampling at 20 Hz and stored on an IBM personal computer.

The anesthetized and intubated rabbits were taken off the ventilator and placed in a left decubital position. The sampling tube of the mass spectrometer was connected to the side port of the endotracheal tube through which the inspired and expired gas concentrations were continuously measured. A syringe was filled to 60 cc with inhalation gasses and connected to the endotracheal tube. A multibreath helium dilution maneuver was performed by manually insufflating and removing 50 cc of tidal volume by the syringe for 10 breaths at an approximate rate of 20–30 breaths/min. The initial and final helium concentrations were used to calculate the functional residual capacity (FRC). Two measurements of FRC were obtained at each trial and averaged. The rabbits were returned to mechanical ventilation following each procedure.

Single breath carbon monoxide diffusion capacity ($DLCO_{SB}$). Five-second breath-hold DLCO maneuvers were performed following the above FRC measurement on each rabbit. All gas concentrations were measured continuously through the mass spectrometer. Of the inhalation gas 60 cc was insufflated into the lung through the endotracheal tube and held for 5 s. Of the inspired volume 30 cc was then withdrawn and held to measure the gas concentrations at 50% expired volume. All data were sampled and digitized at 20 Hz.

For analysis, the breath hold time was measured from 0.5 s from the start of inspiration to 30 cc of exhalation. The duration of inhalation was rapid and peak concentrations were achieved within 1 s. The initial helium and $C_{18}O$ concentrations were measured at their respective concentration plateaus following gas insufflation. The final gas concentrations were measured at 30 cc of exhalation. DLCO was calculated from the standard formula and corrected to STPD. Adjustments were made for the rabbit body temperature and water vapor pressure.

Lung volume reduction surgery. LVRS was performed 4 weeks following elastase induction of emphysema. The anesthetized and intubated rabbits were shaved and placed in a supine position. Twenty-three rabbits underwent resection of varying quantities of lung tissue. Nine control rabbits underwent the operative procedure with no excision of lung tissue (sham surgery).

Hypothermia was prevented with a surgical warming pad, and lactated Ringers solution was infused through an iv catheter in a marginal ear vein at 5–15 cc/h. The rabbits were mechanically ventilated. Oxygen saturation (Ohmeda Biox 3700 Pulse Oximeter, BOC Health Care), tidal CO_2 (Ohmeda 5200 CO_2 Monitor, BOC Health Care), and EKG (Hewlett Packard 78353B Continuous EKG Monitor, BioMedical Services) were monitored continuously.

The chest was shaved, prepped with Betadine, and draped sterilely. The thorax was entered through a median sternotomy. Bilateral upper and middle lobes were excised using a linear thoracoscopic stapler (Endopath ELC, Ethicon Endo-Surgery) with 3.5-mm staples. Target quantity of lung tissue removed was 2–6 g. The quantity of excised lung weight was carefully escalated. The excised lung tissue weights were obtained intraoperatively to assess adequate target resection. In the sham operations, no lung tissue was excised. Hemostasis was obtained and a 12 Fr neonatal chest tube was placed under direct visualization into each pleural space. The two chest tubes were connected to a 10-cm water suction. The sternum was closed with 0 Silk and the chest wound closed in layers with absorbable monofilament sutures. The rabbits were awakened from anesthesia and extubated. There was usually a small airleak in the chest tubes but all leaks sealed spontaneously within 1 h. All chest tubes were removed within 1 h.

Histologic preparation. The animals were sacrificed at 1 week following LVRS. The lungs were removed en bloc and inflated with formalin (20 cm pressure) for histologic preparation. The lung sections were prepared at 0.2 to 0.4 cm thickness and embedded in paraffin. Slides were stained with hematoxylin–eosin and studied by light microscopy.

Statistical analysis. All helium dilution lung volume data and DLCO data for each rabbit were tabulated corresponding to baseline, preoperative, and postoperative measurements. Comparisons of baseline to preoperative values and preoperative to postoperative values were made using paired Student's *t* test. The percentage change in DLCO from preoperative to postoperative data was analyzed and graphed in relation to the excised lung weights. A best-fit curve was generated from a polynomial equation and correlation value calculated.

RESULTS

Mortality from elastase induction of emphysema was 10%. Rabbits died from acute pulmonary hemorrhage or pneumothorax following induction. Of the 23 rabbits surviving induction, all survived LVRS. Excised lung mass ranged from 1.85 to 5.81 g. Nine rabbits underwent sternotomy with no lung resection.

Microscopic evaluation confirmed histologic evidence of moderate to severe emphysema in all rabbits (Fig. 1).

Helium dilution FRC volumes showed an increase in FRC in response to induction of emphysema. LVRS returned these volumes toward preemphysema values (Table 1).

DLCO was adjusted for the VA (alveolar volume). Both the DLCO and DLCO/VA decreased in response to induction of emphysema. The effect of LVRS in DLCO was evaluated as percentage change in DLCO from preoperative to postoperative values. Sham LVRS caused a further decrease in the average DLCO and DLCO/VA (Table 1). As larger volumes of lung were excised, the DLCO increased until an average of

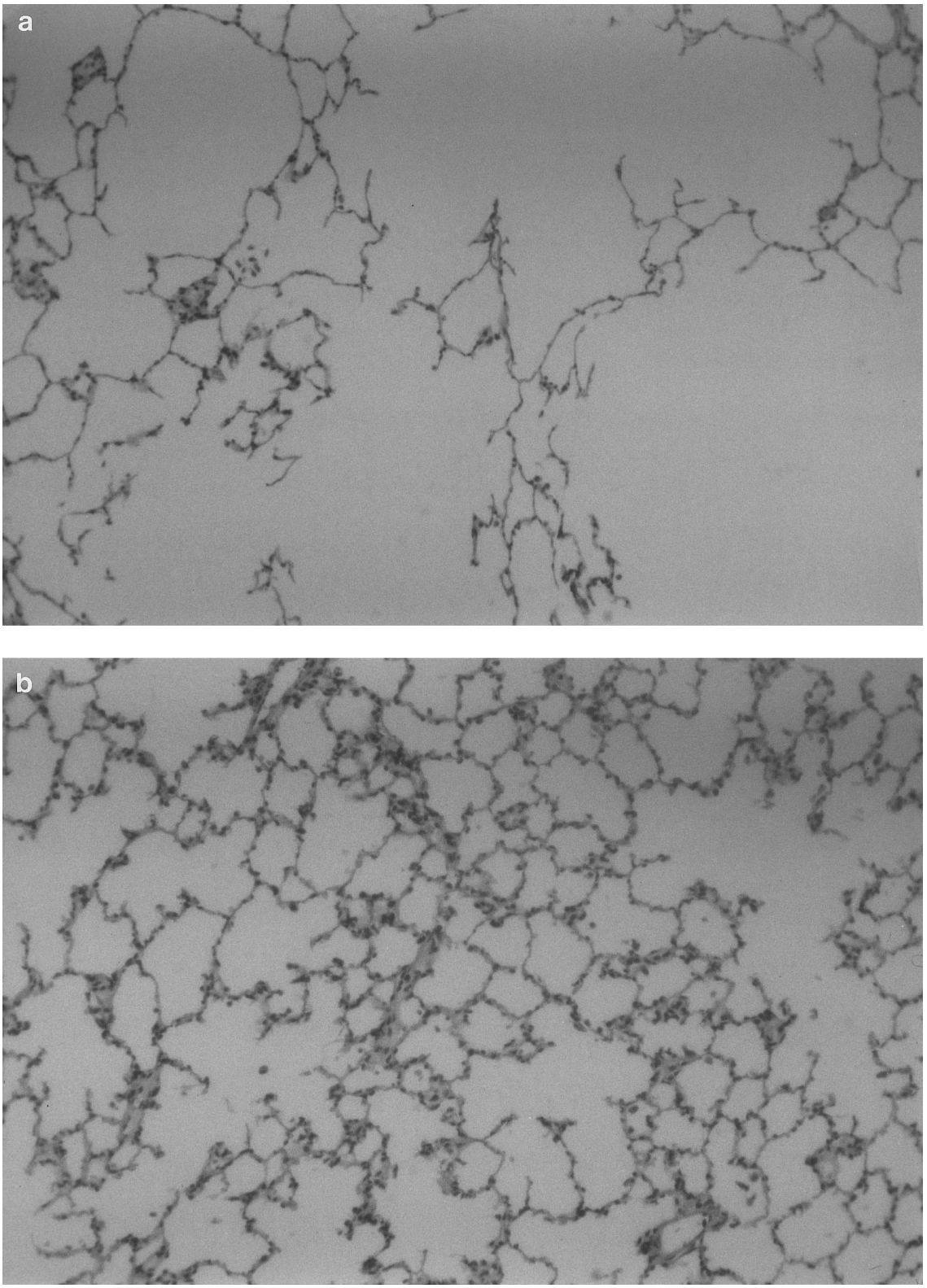

FIG. 1. (a) Histology of elastase-induced emphysema: histologic changes in rabbit lung 5 weeks following elastase induction. Magnification 10×, hemotoxylin–eosin stain. (b) Histology of normal rabbit lung tissue. Magnification 10×, hemotoxylin–eosin stain.

2.8 g was resected. At progressively higher resections above 2.8 g, DLCO decreased again to below preoperative values (Fig. 2).

The rabbits with emphysema that did not receive actual volume reduction showed declining diffusion capacity. In contrast, the diffusion capacity of the rabbits that underwent excision of 2–3 g of lung tissue showed no deterioration, and therefore a benefi-

TABLE 1

Changes in Diffusion Capacity

				P values[a]	
	Baseline	Preoperative	Postoperative	Baseline–preop	Preop–postop
FRC (cc)					
All excised	30.45	35.41	32.46	<0.001	0.02
DLCO (cc/min/mm Hg)					
All excised	0.62	0.57	0.51	0.09	0.02
DLCO/VA (cc/min/mm Hg/L)					
Sham[b]	6.46	6.12	5.49	0.49	0.10
DLCO/VA (cc/min/mm Hg/L)					
2–3 g[b]	7.07	5.79	5.76	0.03	0.93
DLCO/VA (cc/min/mm Hg/L)					
>3[b]	6.62	6.05	5.31	0.13	0.01

[a] Statistical analysis by paired *t* test.
[b] Subgroups: Sham ($n = 9$), 2–3 g excision ($n = 12$), >3 g excision ($n = 10$).

cial effect, in DLCO from LVRS. However, the diffusion capacity of the rabbits that underwent greater than 3 g showed a significant decrease in DLCO from preoperative to postoperative values (Fig. 3).

DISCUSSION

This elastase-induced model of pulmonary emphysema has been shown to have increased static compliance and decreased expiratory flows with development of emphysema [3, 4]. We have previously shown that LVRS reverses these changes by decreasing compliance and improving expiratory flows in these animals [4]. How LVRS affects diffusion capacity of the lungs has not been previously studied in animal models. It is essential to evaluate the relationship between lung mechanics and gas transfer functions following LVRS in order to optimize surgical approaches.

It is predictable that, within limits, removal of increasing amounts of lung tissue would result in greater improvement in spirometry following LVRS in animals with severe emphysema. However, beyond some point, lung function would be expected to deteriorate as greater resected tissue volumes would leave too little residual lung parenchyma to function adequately. Analogous predictions would also be made for gas exchange properties and diffusing capabilities of the lung. A major issue requiring investigation is whether optimal volumes of tissue removal for spirometric improvement are similar to the optimal volume of tissue removal for the best gas exchange measurement outcomes. Therefore, we examined the relationship between improvement in spirometry as a function of amount of lung tissue removed and compared this to change in DLCO in this model.

Measurement of diffusing capacity requires concurrent measurement of alveolar lung volumes. In this study, multibreath helium dilution lung volume mea-

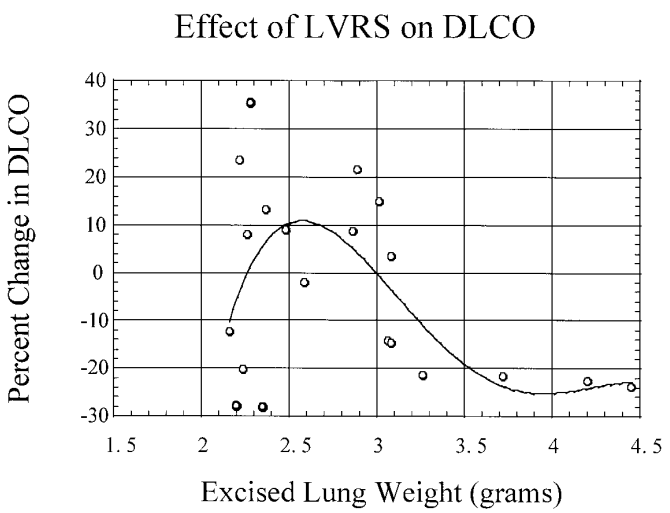

FIG. 2. Effect of LVRS on DLCO. Increasing mass of lung excised results in increasingly declining DLCO at higher lung masses ($y = -2616.8 + 3125.4\,x + -1352.7\,x^2 + 252.17\,x^3 + -17.2\,x^4$, $r = 0.54$).

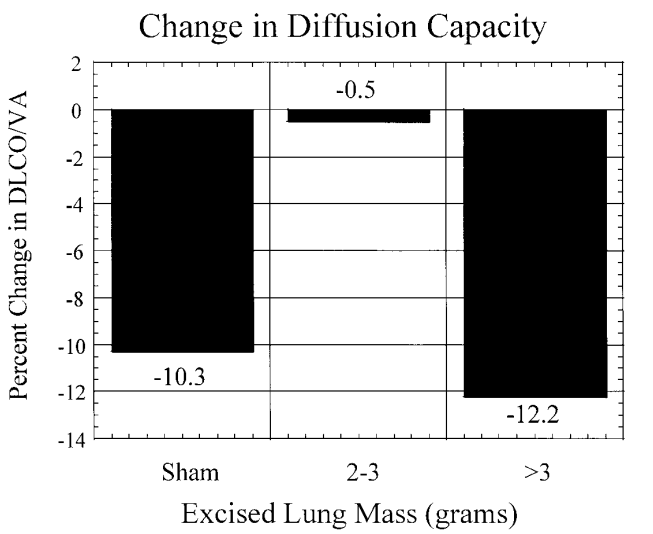

FIG. 3. Change in diffusion capacity. Percentage change in alveolar volume adjusted DLCO from preoperative to postoperative values. The data are divided into three groups based on resected lung mass: sham operations, no lung resection ($n = 9$); 2–3 g ($n = 12$); and greater than 3 g ($n = 10$).

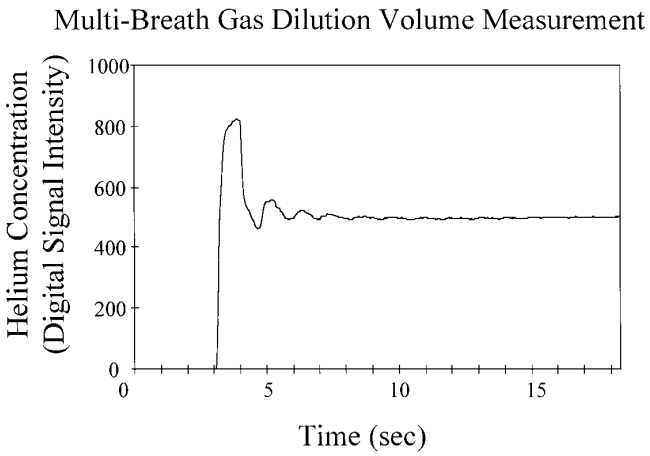

FIG. 4. Multibreath helium lung volume measurement. Helium reaches a stable final concentration within four to five breaths with no delayed mixing.

surements were used. Helium dilution lung volume may be inaccurate in severe obstructive lung disease due to localized air trapping. However, helium dilution appears to be accurate in this animal model as evidenced by flat helium dilution curves within 5 to 10 s of rebreathing. This rapid equilibration likely reflects the small size and rapid respiratory rate capabilities of rabbit lung (Fig. 4).

Diffusing capacity reflects the ability of alveolar membranes to exchange pulmonary gases. In obstructive lung disease, air trapping, airway disease, and ventilation perfusion inequality in lung units can effectively remove the involved regions from participating in gas exchange. This may lead to decreases in measured gas diffusing capacity. We used single breath helium DLCO measurement techniques in this study analogous to those used in patients with emphysema undergoing LVRS. DLCO measurements were reliable and reproducible in these studies.

There are several key points illustrated by the data from this study. First, the elastase-mediated destruction of lung parenchyma is associated with a decrease in DLCO. The coalescence of the air spaces in the lung decreases the surface area for gas exchange. Second, DLCO remains stable following reduction of appropriate lung tissue volumes. In marked contrast, the control rabbits undergoing sternotomy with no lung resection (sham surgery) continue to experience deteriorating diffusion capacity, probably due to progression of emphysema. This suggests that LVRS has a beneficial effect in lung mechanics, and that this improvement in lung mechanics can compensate for the continuing decrease in diffusing capacity.

This model also demonstrates possible critical end points for lung volume reduction. Resection beyond 3 g of lung tissue resulted in further deterioration of diffusion capacity. As predicted, animals with sham surgery or small volume tissue resection (<2 g) had decreases in DLCO following surgery. In contrast, animals with moderate-sized resected lung volumes (2–3 g) had improvement in DLCO compared to controls ($P < 0.05$). Animals with very large volume resections (>3 g) had reduction in DLCO.

Given the small number of animals in our trials, there is a fair amount of variability in our data. Furthermore, predicting the optimal range of lung mass resection is probably a complex function of lung spirometry, oxygenation, pulmonary pressures, and other outcome variables. Although we controlled for the amount of elastase aerosolized, there was a variable amount of induced emphysema in individual rabbits which may have added further variability to our data.

Similarly, clinical data have shown variable improvements in DLCO following LVRS. Improvement in DLCO has ranged from minimal without statistical significance to near 200% of preoperative values [2, 5–7]. The improvements in diffusion capacity following clinical LVRS may be attributed to excision of a larger proportion of nonventilating lung volume in humans. Removal of lung tissue that does not participate in gas exchange does not decrease diffusion capacity, but can actually increase diffusion capacity by increasing ventilatory flows to previously partially obstructed areas.

The optimal resected lung volume of 2–3 g in this model for improvement in DLCO is in sharp contrast to the optimal spirometry response to tissue removal. In this same animal model, larger lung volume resections were associated with progressively greater improvements in elastic recoil, expiratory flows, and residual volumes. These findings suggest that gas exchange measures, rather than deterioration in spirometry, may define the upper limits of optimal tissue resection for LVRS emphysema surgery. Such findings will need to be confirmed in humans.

There are a number of limitations to the current study techniques. Single breath DLCO measures may still be limited in accuracy in this animal model of obstructive lung disease due to regional inhomogeneity. The lung tissue removed was measured by weight rather than true volume. There is currently no method for measuring the exact volume of lung tissue removed. Resected lung weight and volume may not correlate well, particularly in severely diseased, hyperinflated lung regions. The disease distribution patterns in rabbits may not be exactly analogous to those in humans. There is regional heterogeneity although the degree of localization of airtrapping as well as the overall extent of emphysema is not as extensive as seen in human disease. The emphysema is centri-lobular and diffusely distributed. Rabbit lung function was measured in the lateral decubitus position in this study. Finally, chest wall compliance is considerably greater in rabbits than in humans and may affect the relationship between excised tissue and resultant changes in lung volume. Nonetheless, despite these limitations, this study elucidates important issues regarding measurement end points to be followed when assessing optimal LVRS techniques.

Lung volume reduction surgery has resulted in improvement in physiologic and functional status in some

patients following LVRS surgery. Yet, difficult questions regarding this procedure remain. This model helps to demonstrate physiologic principals of concern in LVRS and should be useful in ongoing investigations of a number of unanswered questions. These studies suggest that careful investigation of various pulmonary function and gas exchange parameters will be needed in order to determine the limiting physiologic factors for assessing the optimal extent of LVRS procedures.

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