Experimental Lung Research, 31:497–512, 2005

ISSN: 0190-2148 print/1521-0499 online
DOI: 10.1080/019021490944241

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PULMONARY FUNCTION TESTS VERSUS COMPUTED TOMOGRAPHY IN SHEEP WITH EXPERIMENTAL EMPHYSEMA

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□ *The authors explored the relative utility of pulmonary function tests (PFTs) and computed tomography (CT) to characterize the progression of papain induced emphysema in sheep ($n = 12$). PFT included plethysmography (FRC_{pleth}), helium dilution (FRC_{He}), and expired reserve volume (ERV). Following papain, FRC_{pleth} and FRC_{He} were unchanged; ERV decreased hence residual volume increased significantly ($RV + 270$ mL, $+86\%$, $P = .02$). In contrast, FRC by CT increased in 10 of 12 sheep ($+264$ mL, $+21\%$, $P = .008$). We conclude that plethysmography was insensitive to emphysema, but the effect on ERV (i.e., trapped gas volume) and FRC by CT were very similar, and in line with the morphologic changes in this animal model.*

Keywords CT, emphysema, hyperinflation, papain, plethysmography, sheep, trapped gas

Hyperinflation is an essential feature of animal models of emphysema, but the sensitivity of standard pulmonary function tests (PFTs) for hyperinflation in animal models is unknown. There are several reasons to suspect

Received 6 August 2004; accepted 15 February 2005.
This work was supported by NIH grant HL 62266-03.
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that PFT lack sensitivity. For one, these measurements are made under general anesthesia, which substantially depresses functional residual capacity (FRC) [1–3] and residual volume (RV) [4]. There are several examples in the canine species where FRC changed little [5–7] or not at all [8] despite significant pathology. Where FRC and thus RV are depressed from anesthesia, the changes in expired reserve volume (ERV) may dominate the volumetric signal related to emphysema. Such reliance on ERV to model changes in RV is vulnerable to dynamic and gravity dependent airway closure [9] and ERV is defined arbitrarily by end points of tracheal or transpulmonary pressure. The extent to which these factors and others (e.g., body position, body weight) influence the assessment of hyperinflation in animals is unclear. What is clear is that changes in FRC and RV due to experimental emphysema have been significantly greater in small animals [10]. It has been assumed that this difference is related to greater lung pathology. However, it is entirely possible that the difference lies in the sensitivity of PFT for trapped gas in a small versus large animal species.

One opportunity to investigate this question is to compare PFT with analogous volumes derived from computed tomography (CT). The validity of CT as a measure of trapped gas volume in humans is well supported. For example, (1) CT can be used to effectively quantify lung morphometry and trapped gas in human emphysema [11–18], as well as asthma [19–21], lymphangioleiomyomatosis [22], and sarcoidosis [23]; (2) longitudinal measurements of lung volume and density by CT for α_1-anti-trypsin deficiency are more sensitive than pulmonary function tests [24]; (3) measurements of air trapping using CT was a better discriminator between cystic fibrosis and normals [25], and (4) CT is more sensitive to mild emphysema [26] and provides more specific information with respect to airspace enlargement [27]. Taken together, this information suggests that CT-derived measurements of lung volume offer a valid gold standard with which to compare functional tests (e.g., FRC, RV, total lung capacity [TLC]) during emphysema. Yet, a direct comparison between PFT and CT has not been described in large animal models of emphysema. Chino and colleagues [28] observed profound increases in lung volume by CT in dogs after treatment with elastase; lung volumes by PFT were not reported. Studies employing CT have also been performed in swine receiving elastase [29, 30], but these were not accompanied by PFT. Hence, there is a need to better understand the relationship between PFT and CT derived lung volumes in animal models.

We hypothesized that CT would be more sensitive to the progression of emphysema than standard PFT (i.e., FRC, RV, TLC) in sheep with papain-induced emphysema. In this study, we found that whole body

plethysmography failed to detect changes in lung volume, and that trapped gas assessment using PFT was less sensitive than CT.

MATERIALS AND METHODS

Animals

All protocols employed in this study were approved by the Institutional Animal Care and Use Committee at Tufts University. Twelve mixed breed female sheep aged 2 to 5 years (57.5 ± 1.3 kg, range 49 to 63 kg) were employed for study. Prior to the study, the sheep received prophylactic anthelminthic treatment with ivermectin (200 μg/kg intramuscular [IM]), were immunized for tetanus toxin and *Clostridium perfringens* types C and D enterotoxemia, and given 3 weeks acclimation. Grass hay was fed ad libidum throughout the experiment.

Study Design

Two groups of sheep were employed for this study. In one group ($n = 10$), measurements of FRC by helium dilution were performed while awake ('Awake Studies'). The remainder of studies described below were performed under anesthesia on a second group of sheep ($n = 12$), who served as their own controls to study the effects of papain.

The order of procedures for the group subject to papain was as follows: 1) baseline physiologic measurements (FRC, ERV, RV, etc.—see below) and imaging (CT), spaced 1 week apart; 2) induction of emphysema using papain over a 6-month period; and 3) repeat of the physiologic measurements and CT 6 weeks after the last exposure to papain. Body weights were recorded at each time period. Induction of anesthesia was performed using ketamine (7.5 mg/kg intravenous [IV]), midazolam (0.3 mg/kg), and propofol (30 mg IV) given as a bolus, and sheep maintained in prone position. Within each physiology measurement day, we placed an esophageal balloon, and first measured plethysmographic functional residual capacity (FRC_{pleth}) and maximum inspiratory pleural pressure measurements (MIP) with the sheep spontaneously breathing. We then delivered a constant infusion of propofol (50 to 100 μg/kg/min) to eliminate spontaneous respiration movement, and continued measurements as follows: ERV, lung and chest wall compliances, single breath diffusion capacity (DLco), alveolar volume (V_A), and FRC by helium dilution using a rebreathing method (FRC_{He}). All sheep were fasted for 24 hours prior to anesthesia for physiologic measurements and 16 hours prior to anesthesia for CT.

Induction of Emphysema

We induced emphysema in sheep using an escalating dosage regimen of papain (papain aqueous solution; Sigma Chemicals, St. Louis, MO) given by aerosol. For aerosol delivery, sheep were ventilated (Bear, Model 2, settings: FIO_2 0.6, f = 12/min, peak airway opening pressure 25 cm H_2O, inspiratory peak flow 30 L/min, inspiratory pause 1.0 seconds, and PEEP 5 cm H_2O) to promote uniform lung deposition [31, 32]. For each exposure, we loaded 2 parallel jet nebulizers (LC Plus; Pari, Midlothian, VA) with papain solution. These nebulizers were joined by a Y piece, the distal end of which was inserted into a side port of a spacer (200 mL), located in-line on the inspiratory side of the ventilator circuit. The starting dose was 50 IU/kg body weight (bw) (diluted 1:1 with sterile physiologic saline). In the absence of a clinical response (increased respiratory rate >60/min, increased respiratory effort, increased body temperature >42°C) to the previous exposure, the dose was increased by 25 IU/kg every other treatment until a maximum concentration of 100 IU/kg was reached. The total dosage of papain received by aerosol in 8 of the sheep was 750 IU/kg and in 4 sheep, 900 IU/kg. All sheep received these exposures over a total of 10 treatments, spanning 6 months.

Lung Volume Measurement of Awake FRC (Awake Group)

The rebreathing method of helium dilution was used, which incorporated a facemask, low dead space stopcock (120° angle stopcock, Hans Rudolph) and rebreathing bag (3-L nondiffusible bag; Rousch). The bag was partially filled (1.6 L—enough volume to avoid emptying of the bag) with test gas (10% helium, 21% oxygen, balance nitrogen) and once the initial concentrations were measured, was opened at end-expiratory lung volume to the sheep. The gas mixture was rebreathed from 45 to 60 seconds, at which time the exhalate was collected and analyzed (Morgan Scientific Heliometer, Kent, England). A rebreathing period of 45 seconds was demonstrated in our laboratory to constitute the point of steady state in normal and postemphysema sheep. The test was repeated at least once, and 2 runs within 5% were used for averaging.

Pulmonary Function Tests in Anesthetized Sheep (Emphysema Group)

Sheep were anesthetized as described herein, and placed on a cart in prone position with the abdomen positioned over a large (30 cm diameter) cut-out to minimize abdominal pressure. An esophageal balloon catheter was passed to the level of the mid-thorax, where maximal Pes and minimal

cardiac oscillations were visualized. The Pes (esophageal pressure) were subsequently used to measure expiratory reserve volumes, lung, and active and passive chest wall compliances.

The sheep on the cart was first moved to a whole body plethysmograph for measurement of $FRC_{plethys}$ as previously described [31]. Box pressure, calibrated with a known volume (30 mL) of air, was measured using a low-pressure range transducer (± 10 cm H_2O; SCXL004; Invensys Sensor Systems, Milpitas, CA) and preamplifier (Max2270; Buxco Electronics, Sharon, CT) with the transducer referenced to an adjacent chamber equilibrated with atmosphere ($\tau \approx 10$ seconds). A second pressure transducer (Validyne DP45-28; Northridge, CA; ± 56 cm H_2O) was used for airway pressure (Pao) measurements, and the signal conditioned with a carrier demodulator amplifier (Max2215; Buxco Electronics), and commercial data acquisition system (Biosystem *XA*; Buxco Electronics), with sampling rate set at 100 Hz. At end-expiration, visible on the trace, we activated an occlusion shutter (Model 4285 Pneumatic Shutter; Hans Rudolph, Kansan City, MO) positioned at the oral end of the endotracheal tube. Sheep made inspiratory efforts (10 to 30 cm H_2O negative Pao) against the shutter, permitting measurement of FRC (FRC_{pleth}) from Pao and the respective change in plethysmographic volume using the method of Dubois and colleagues [33]: FRC_{pleth} (L): $[(\delta Vbox/\delta Pao \times (P_B - P_{H2O})]$. The dead space in the endotracheal tube and trachea (average 124 mL) was subtracted from FRC_{pleth} for comparison with FRC derived from CT.

ERVs were measured by slowly extracting gas from the lung using a precision syringe (3-L calibration syringe; Hans Rudolph) from FRC to 2 different end-points of transpulmonary pressure. The transpulmonary pressure dropped linearly with volume up to a point (RV′), usually -4 cm H_2O, where it dropped sharply in a nonlinear fashion until degassing was stopped at -25 cm H_2O transpulmonary pressure (RV_{-25}). The extracted volumes were termed ERV′ and ERV_{-25}, respectively. The average of two measurements were reported here, and used to compute RV′ and RV_{-25}.

Deflation pressure-volume curves were generated using a precision-volume syringe (3-L calibration syringe; Hans Rudolph), and recordings of respective transpulmonary ($Ptp = P_{esophageal} - P_{tracheal}$) pressure. Following at least 2 large inflations (25 cm H_2O) to establish volume history, the lung was inflated from FRC to a minimum of 30 cm H_2O, and pressure recorded at each deflation step (0.25 L) of 4 seconds. The average Ptp-volume data points from 2 runs for each sheep were fit to the exponential Salazar-Knowles equation by visual inspection [34, 35]:

$$V(P) = V_{max} - Ae^{-kP}$$

where k is the exponential curve 'shape factor,' V_{max} the volume corresponding to the apparent asymptote of distending pressure, V_{min}

the intercept which simulates residual volume, and A is the volume difference($V_{max} - V_{min}$) analogous to vital capacity. The pressure-volume curves were plotted using $FRC_{plethys}$ and the corresponding Ptp as the lowest (i.e., anchor) point.

Measurement of TLC incorporated the lung Ptp-volume and active chest wall compliance curves. Sheep breathed 60% oxygen and received 3 deep inflations to $\geq$30 cm H_2O airway pressure, prior to occlusion. Maximum negative deflections of Pes, defined as >2 equivalent or declining pressures, were recorded after occlusion at (1) FRC (relaxed end-expired lung lung volume) and (2) FRC plus 1.5 L (i.e., the static inflation volume that produced 8 to 10 cm H_2O Pes). The two MIP (Maximum Inspiratory Pressure) (i.e., MIP_{FRC} and $MIP_{FRC} + 1.5$ L) were plotted against their respective volumes (FRC_{pleth}, $FRC_{pleth} + 1.5$ L), representing the active chest wall compliance curve. The intersection of the active chest wall and Ptp-volume curves dictated TLC using the Campbell diagram [9, 36] (TLC_{pleth}). For comparison, we computed TLC a different (second way) commonly reported in the literature as the sum of FRC (pleth) plus V_{max}.

We measured DLco using the single breath determination method [37], and corrected the measurements for effective alveolar volume (V_A). The circuit was previously calibrated with test gas (10% He, 0.3% CO, 21% O_2, and 68.7% N_2) to $\pm$0.1%, and corrected for FIO_2 of 60% and BTPS (Body Temperature and Pressure Saturated). We used the measured inspired and expired plateau values in a calculation of DLco as follows: $DLco = [V_A/(Pb - PH_2O) \times 60\,s/10\,s \times \ln (Fi_Aco/Ff_Aco)$, where V_A was the alveolar volume computed from helium dilution. We also performed measurements of FRC_{He} by forcing a known volume of test gas (0.5 L below V_{max}) in and out (20 times) from end-expiratory volume after several deep inflations to establish volume history. As for FRC_{pleth}, the average dead space volume from the endotracheal tube and trachea (124 mL) was subtracted from FRC_{He} for better comparison with FRC_{CT}, which excludes these structures in volume determination.

Computed Tomography for Measurement of FRC

Sheep were ventilated (FIO_2 0.6, f 12, V_T 10 mL/kg, peak inspiratory flow 30 L/min, PEEP = 0, square wave) in prone position while receiving propofol anesthesia (50 μg/kg/min or to affect) until the time of imaging. A series of $\geq$3 deep inflations (>30 cm H_2O) preceded each imaging. Three separate CT were taken, including one each at PEEP = 0, for determination of FRC (FRC_{CT}), and lung volumes at Pao equal to 10 and 25 cm H_2O. The CT scans were acquired with a PQ 5000 helical single slice

scanner (Picker International, Cleveland, OH). The settings were 120 kVp, 300 mA, 8 mm thickness, with 4-mm table movement between slices, helical pitch = 1.5, 0° tilt for scanning. The algorithm employed for imaging lung was ahi.sres.s (Pickard), i.e., sharp. Each scan required 30 seconds to perform. Each image of the CT image set was analyzed to obtain a total lung volume. An automatic object detection algorithm based on thresholding (−300 HU) was used to detect the lung within the image. The percentage of air in each pixel, within the detected region of lung, was determined from Equation 1. Multiplying the percent air by the volume of a single voxel, we obtained the air volume per pixel (Equation 2). Summing over all pixels within the lung slice results in the lung volume that is air, per slice (Equation 3); summing over all slices results in the total air volume of the lung (Equation 4).

$$\%\mathrm{Air}_{\mathrm{pixel}} = \left(\frac{\mathrm{HU}_{\mathrm{pixel}}}{-1000}\right)100 \quad \text{for pixels with } -1000 > \mathrm{HU} > 0 \tag{1}$$

$$\mathrm{Air}_{\mathrm{pixel}} = \%\mathrm{Air}_{\mathrm{pixel}} \times \mathrm{V}_{\mathrm{voxel}} \tag{2}$$

$$\mathrm{Air}_{\mathrm{slice}} = \sum_{\mathrm{pixel}} \mathrm{Air}_{\mathrm{pixel}} \tag{3}$$

$$\mathrm{Air}_{\mathrm{lung}} = \sum_{\mathrm{slice}} \mathrm{Air}_{\mathrm{slice}} \tag{4}$$

Linear Mean Intercepts (Lm)

Lung morphometry was undertaken as the gold standard for emphysema severity in this animal model. Although the quantitative relationship between changes in Lm and function can not be directly compared, we assumed that the percentage change in each variable (function-image-tissue morphometry) should be similar.

The lung was removed en bloc at postmortem, and after degassing was filled with formalin (10%) to a level of 20 cm H_2O pressure to standardize morphometry. At least 6 samples of lung (1 cm^3) representing dorsal and ventral regions equally were sectioned (4 μm) and stained with hemotoxylin and eosin. Evaluations of morphometry were performed by light microscopy (200× magnification) by a single observer (EI). The linear mean intercept (Lm) was measured from 4 randomly selected high power fields (with >40 alveoli per field), from each section per sheep. One sheep without emphysema was used as control.

Statistical Analysis

For comparison between awake and anesthetized sheep we employed the Student's *t* test. To evaluate the effects of papain on static lung function and CT variables in the anesthetized group, we employed the paired *t* test (1-tailed). Pearson's product moment correlation coefficient was used to test the association between independent measurements of images, function, or morphometry. A $P \leq .05$ was considered statistically significant. Values are expressed throughout as mean $\pm$ SD.

RESULTS

Effects of Papain on Pulmonary Function Tests

Mean FRC (mL/kg) was significantly lower in the anesthetized sheep (FRC_{He}, –48%; FRC_{pleth}, –29%) than in the awake group (1.92 L, 41.8 mL/kg) ($P < .001$). The changes in pulmonary function associated with emphysema development in anesthetized sheep are summarized in Table 1. The mean FRC_{He} (1.24 L $\pm$ 0.43, 21.8 mL/kg) and FRC_{pleth} (1.70 $\pm$ 0.41 L, 29.6 mL/kg)

TABLE 1 Pulmonary Function Testing and Body Weight Values before and after Exposures to Papain to Induce Panlobular Emphysema in Sheep (n = 12)

Variable	Preemphysema	Postemphysema	Difference (%)	*P* value
Body weight	57.5 ± 4.36	63.7 ± 4.62	+6.2 (+10.8%)	<.0001
FRC_{pleth}	1704 ± 413	1625 ± 490	−79 (−4.9%)	.17
FRC_{He}	1240 ± 430	1069 ± 271	−171 (−12.5%)	.068
ERV′	1382 ± 361	1028 ± 247	−354 (−25.6%)	.0008
ERV_{-25}	1424 ± 116	1103 ± 286	−321 (–23.5%)	.0024
RV′	322 ± 412	597 ± 484	+270 (+85.4%)	.018
RV_{-25}	280 ± 421	522 ± 479	+242 (+86.4%)	.032
Vmin	825 ± 374	817 ± 617	−8 (−1.0%)	.49
TLC_{pleth}	3293 ± 577	3588 ± 883	+295 (+8.9%)	.037
TLC_{Vmax}	3788 ± 466	4063 ± 661	+275 (+7.3%)	.036
V_A	3277 ± 415	3099 ± 373	−177 (−5.4%)	.07
DL_{co}	18.17 ± 3.7	11.56 ± 2.24	−6.61 (−34.8%)	.00003
$DLco/V_A$	5.59 ± 1.48	3.78 ± 0.83	−1.81 (−30.4%)	.00016
k	0.137 ± 0.039	0.202 ± 0.042	+0.065 (+60%)	.00011

Units: volumes (mL), DLco (mL/mm Hg/min), DL/V_A (mL/mm Hg/min/L), k (unitless).

FRC_{pleth} = FRC by body plethysmography; FRC_{He} = FRC by helium dilution; ERV′ = expiratory reserve volume at lowest linear negative transpulmonary pressure; ERV_{-25} = expiratory reserve volume at −25 cm H_2O transpulmonary pressure; RV′ = residual volume derived from $FRC_{pleth} - ERV'$; RV_{-25} = residual volume derived from $FRC_{pleth} - ERV_{-25}$; Vmin = RV derived by Salazar-Knowles exponential model of pressure-volume curve; TLCpleth = volume from Campbell diagram: intersection between lung and active chest wall compliance curves; $TLC_{Vmax} = FRC_{pleth} + V_{max}$; V_A = effective alveolar volume; DLco = transfer factor (mL/min/mm Hg); k = lung pressure-volume curve exponential shape factor derived using Salazar-Knowles model.

as a function of body weight were significantly correlated ($r = .581$, $P = .001$).

There was no significant change in FRC_{pleth} or FRC_{He} with emphysema. Only 3 out of 12 sheep demonstrated an increase in FRC_{pleth}. Expiratory reserve volumes ERV′ and ERV_{-25} were significantly reduced, by 26% and 24% respectively. Residual volumes, RV′ and RV_{-25}, were significantly increased, by 6% and 60% respectively. Salazar-Knowles RV (i.e., V_{min}) was unchanged from before to after emphysema. Total lung $capacity_{pleth}$ (via Campbell diagram) and V_{max} (via Salazar-Knowles equation) were increased significantly, but these changes were on the order of 7% to 9%. Effective alveolar volume (V_A) was slightly reduced in this model but this change was not statistically significant.

There was a marked decrease in DLco (−35%) and DLco/VA (−30%), and these changes were highly significant ($P < .001$). The measurement of Salazar-Knowles exponential shape factor for the pressure-volume curve of the lung (k) was also significantly increased ($P < .001$).

CT Measurements of Lung Volume, and Combined Use of CT and Pulmonary Function Tests

There were significant increases in all CT derived lung volumes (Table 2) ($P < .05$), ranging from 264 mL at PEEP = 0, to 566 mL increase at Pao = 25 cm H_2O. Increased FRC_{CT} was observed in 10 of 12 sheep following emphysema.

The difference between plethysmographic and helium derived FRC ($FRC_{pleth} - FRC_{He}$), representing physiologic trapped gas, was 341 ± 285 mL before and 433 ± 412 mL after emphysema (+92 mL, NS). An analogous variable substituting FRC_{CT} for FRC_{pleth} (i.e., $FRC_{CT} - FRC_{He}$) revealed a significant change in trapped gas (+435 ± 130 mL, $P = .0032$), from 11.8 ± 431 mL to 446 ± 256 mL, a change observed in 9 out of 12 sheep.

TABLE 2 Measurements of Air Volumes Obtained from CT Images of Lung in Sheep (n = 12) before and after the Induction of Experimental Emphysema

Variable	Preemphysema	Postemphysema	Difference (%)	*P* value
FRC_{CT} (PEEP = 0)	1251 ± 330	1515 ± 283	264 (+21%)	.008
Lung volume by CT (PEEP = 10)	1865 ± 516	2199 ± 378	334 (+17.9%)	.028
Lung volume by CT (PEEP = 25)	2524 ± 617	3089 ± 523	566 (+22.4%)	.01

Measurements (mean ± SD) were made at positive end-expiratory pressures of 0, 10, and 25 cm H_2O.

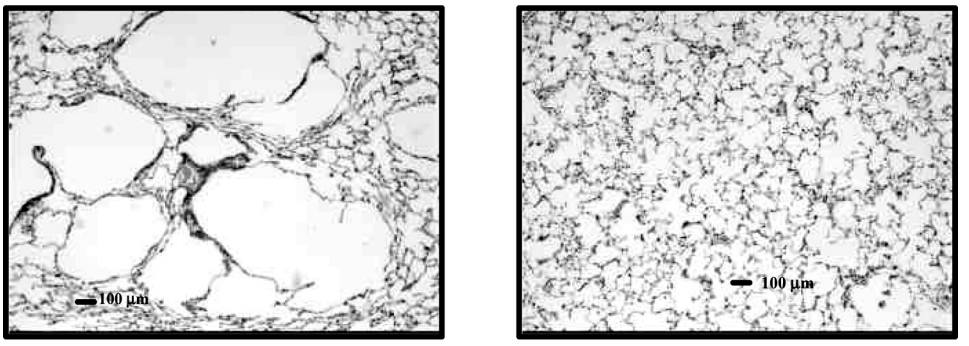

FIGURE 1 Photomicrographs of representative lung tissue specimens in sheep: (*A*) exposed to papain or (*B*) unexposed control; hemotoxylin and eosin stain, grey scaled.

Linear Mean Intercepts

Papain caused pan-acinar emphysema with no evidence of airway pathology on light microscopy in all sheep. Alveolar sizes were microscopically heterogeneous. Large abnormal airspaces were sporadically lined discontinuously with fibroblasts or cuboidal epithelial-like cells. Examples of light microscopic findings in a normal sheep and one with typical emphysema are provided in Figure 1*A*, *B*. Papain caused the Lm to increase to $89.1 \pm 12.7\,\mu m$ from a control value of $59.9\,\mu m$ ($+48.7\% \pm 21\%$), with Lm differences (from control) ranging from 13 to $54\,\mu m$, or 21% to 90% above control.

DISCUSSION

This is the first study to demonstrate the complete failure of plethysmography to detect hyperinflation in a large animal model of emphysema. As the result of this problem, data derived from plethysmography (e.g., RV, $FRC_{pleth} - FRC_{He}$) may also be inaccurate. In contrast, measurements of lung volume employing CT appear coincided with morphologic changes. These findings are important insofar as PFT and CT are commonly employed as end points for animal models of emphysema, and precise end points assure efficient and safe implementation of the model.

A major assumption of this paper was that the physiological determinants of RV, FRC, and TLC are the same as in humans where those determinants have been well established. For example, RV is a function of FRC, expiratory muscle force, and airway closure. Whether this is true for normal or emphysema groups of sheep is not established, especially where anesthesia is involved. The determinants of FRC in the sheep are also thought to be

the same as in humans, i.e., the force balance between chest and lung recoil pressures. In the awake fasted state, there are no doubt other factors that determine end-expiratory lung volume such as breathing pattern, rumination, and body condition (discussed below). During anesthesia, the anesthetic depth, body position, atelectasis, and rumen distension are considerable factors. The factors that determine TLC in this study other than chest muscle strength and lung elastic recoil (as for awake humans), presumably include the depth of anesthesia, contributions to negative pressure generation from the diaphragm versus chest wall, and external restriction from the abdominal contents. Active chest wall compliance did not change as a function of emphysema in this study or in previous studies, so the shape of the PV curve would largely dictate changes in TLC_{pleth} or TLC_{Vmax}. However, the many factors involved, both physiologic and experimental, make TLC measurement during anesthesia somewhat complicated, yet TLC or related variables (V_{max}) are commonly reported, and therefore included in this study for comparison.

In previous studies, we were able to detect low levels of hyperinflation at FRC, for example an 18% increase in FRC_{pleth} [31]. It was therefore perplexing why we failed to do so in the current study despite a greater severity of disease, e.g., greater loss of diffusion capacity (Dlco, −38% versus −35% previously) and shift in the pressure-volume curve (k, +60% versus 0% previously), and unmistakable alveolar destruction (Lm, +48%) in this study. One important explanation for the failure of plethysmography in this study might be the anesthetic effects to lower lung volumes (FRC_{He}, −34%) in the anesthetized versus awake sheep. Similar findings were reported in humans [1, 2, 37]. Clearly any signal associated with hyperinflation would be subject to the 'noise' created by anesthesia and recumbency. Another contributing factor in this study might be the observed gain in body weight (+10.8%), in contrast to the net loss of body weight in our previous studies (−2.7%). This shift in body condition may have exacerbated the anesthetic effects to reduce lung volume. Collie and coworkers [38] found that increased body weight was associated with *decreased* V_A in healthy anesthetized sheep. Furthermore, in a study of rabbits [39] where weight gain was observed (from 3.55 to 4.37 kg, or +23%), the effects of papain to increase FRC were highly variable and not statistically significant despite marked histologic changes. The mechanism by which anesthesia exerts this confounding effect is through a change in rib cage and diaphragmatic dimensions, and an increase in trapped gas [40], observations that worsen with morbid obesity [41]. Whether weight gain has similar effects in sheep is uncertain. The net result was that changes with FRC_{pleth} that ordinarily characterize hyperinflation, or differences in FRC_{pleth} and FRC_{He} that characterize trapped gas, were not features of this animal model.

Traditional explanations put forth for the failure of plethysmography to accurately assess lung volumes include the presence of abdominal gas [42], nonhomogenous pleural (alveolar) pressures during inspiratory efforts against an occlusion [43], and airway obstruction [44], which may introduce a pressure drop from the alveolar to the mouth pressure compartments. Abdominal gas is negligible on CT scans performed in fasted sheep immediately after anesthetic induction, when plethysmography is performed (personal observations). The effect of anesthesia to reduce the active movement of, and to deform the chest wall, may contribute to nonhomogeneities in alveolar pressure. This effect could dampen the signal obtained specific zones of hyperinflation that are unexpanded during inspiratory efforts. There was no evidence that airway obstruction was a contributing factor in this study, based on measurements of airway resistance (data not shown).

The only traditional PFT that consistently characterized emphysema was ERV, i.e., ERV′ and ERV_{-25}. The change in volumes observed for ERV′ and ERV_{-25} were, respectively, −353 mL (−26%) and −321 mL (−23.5%), which was similar to the increase in FRC_{CT} (+264 mL or +21%). This suggests that these volumes both represent hyperinflation due to loss of elastic recoil or gas trapping. The finding of decreased ERV is almost universal in animal models of emphysema [4–6, 45, 46], and makes up a large portion of the RV signal. Our data support the use of ERV under carefully controlled conditions. That ERV represents a degree of gas trapping was further supported by the combined measurements of FRC_{CT} and FRC_{He} (i.e., $FRC_{CT} - FRC_{He}$), which demonstrated a significant increase (+435 mL) of trapped gas. A similar quantity of trapped gas was characterized by Krayer and coworkers, who also combined CT and PFT (i.e., the difference between CT and N_2 washout derived lung volume) [40]. Caution should be added that ERV should be reported independent of FRC_{pleth} because RV measured at baseline using $FRC_{pleth} - ERV$ is depressed as an artifact of the depressed FRC_{pleth} due to anesthesia. As a result, the percentage changes in RV due to emphysema may be exaggerated. Changes in absolute RV would better represent the degree of hyperinflation observed in animal models that exhibit this artifact.

That ERV was less than FRC_{He} was curious. The measurements of FRC_{He} were approximately 1 hour after ERV, so it is likely that some progressive atelectasis or bloat contributed to this difference. In a recent study with much shorter anesthetic periods, we found the difference between FRCHe and ERV (i.e., RV) to be positive 186 ± 156 mL (unpublished data). Hence the FRC_{He} measurements in this study appear to be further depressed by the effects of anesthesia.

The difficulties of expressing RV precisely during the progression of emphysema caused us to focus our attention on the utility of CT. We found that baseline FRC_{CT} (1250 ± 330 mL) was very close to FRC_{He}

(1240 ± 430 mL) following correction of the latter for extrapulmonary dead space, but lower than FRC_{pleth} (1704 ± 413 mL or +36%). We speculate that FRC_{CT} compares better with FRC_{He} as the depth of anesthesia is similar for these measurements (i.e., the anesthesia was maintained for measurement of FRC_{pleth} at a lighter plane to promote spontaneous breathing efforts). This once again highlights the difficulties in comparing various measurements under different anesthetic regimens or time periods. In other studies, values for FRC_{CT} were significantly lower than FRC_{He} in humans [47, 48], and in anesthetized, supine dogs [38] FRC_{CT} was lower by 15% to 35%. Wandtke and coworkers [37] surmised that a majority of this difference is due to technical limitations of measuring lung volume by CT, including partial volume effects, beam hardening, irregular borders of the lung, and sharp density gradients. It seems likely that these technical issues impacted our data as well, and therefore just as likely that our FRC_{CT} measurements are an underestimation, especially considering the low values for FRC_{He} obtained in this study (discussed above).

Despite the absence of plethysmographic evidence of emphysema, 10 out of 12 sheep exhibited an increase in FRC_{CT} with emphysema. This finding strongly supports the notion that hyperinflation was profoundly underestimated by plethysmography. Previous studies have shown a good correlation between CT derived lung volumes (RV, TLC) [17] and that CT morphometry has greater sensitivity for pathologic changes in lung volume than pulmonary function tests [25, 49]. In the present study, CT volumetric data corresponded better with the degree of tissue destruction. The changes in lung volume were observed in 10 of 12 sheep at all levels of PEEP. Therefore, 'stenting' open lung with PEEP did not improve the chance of detecting hyperinflation. The two sheep without increases in FRC_{CT} demonstrated Lm changes (+41.8%, +38.2%) slightly below the mean (+48.7% versus control), but all other measurements (DLco, k, ERV, etc.) were deranged to a greater extent than their cohorts. Their weight gain was similar to the rest of the group. Hence, the reason why two sheep lost lung volume on CT cannot be surmised other than the generally unpredictable effects of anesthesia, body positioning, and rumen distension in this species.

We were unable to correlate the change in the index of hyperinflation (FRC_{CT} or $FRC_{CT} - FRC_{He}$) with DLco, k, and Lm. The reason that these values did not correlate may relate to the small number of animals and narrow range of values in each category. Greaves and Colebatch [50] correlated Lm with the lung pressure-volume exponent k in human emphysema, but the range of disease was much broader in that study. Several studies suggest that CT morphometry does not correlate well with DLco [13, 51, 52], but a recent study show good correlation with CT evaluation of emphysema and Dlco [53].

In conclusion, we have found that CT was a more sensitive method to detect and quantify changes in lung volume and the presence of trapped gas in a sheep model. The use of CT alone or in conjunction with traditional PFT holds promise to more precisely characterize the progression of emphysema in animal models and reduce the likelihood of following imprecise end points.

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