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Measuring the Effect of Airway Pressure on Pulmonary Arterial Diameter in the Intact Rat Lung

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ABSTRACT

To study the relationship between transpulomnary pressure (Ptp), intravascular pressure (Pv), and the pulmonary arterial tree structure, morphometric measurements of pulmonary arterial trees were made in intact lungs from Sprague-Dawley rats. Using cone beam micro-CT and techniques we developed for imaging small animal lungs, volumetric CT data were acquired for Ptp from 0 - 12 mmHg and Pv from 5 - 30 mmHg. The diameter, D (measured range approximately 0.08-2.0 mm), vs. pressure, P, relation can be described by $D(P) = D(0)(1+ \alpha P)$, where α is a distensibility coefficient. Unlike studies performed in larger animals, where changes in either Ptp or Pv had nearly identical effect on vessel distensibility, we found that there is only a small dependence of arterial diameter on Ptp in the rat. For example, using the above relation where P=Ptp and Pv is held constant at 12mmHg, alpha = 0.55±0.42(SE) %/mmHg, compared with when P=Pv and Ptp is held at 12mmHg, alpha = 2.59±0.17(SE) %/mmHg.

Keywords: micro-CT, pulmonary arterial morphology, transpulmonary pressure

1. INTRODUCTION

The mechanical behavior of both intrapulmonary vessels is affected by the manner in which the vessels are embedded in the lung. This has been referred to as vascular interdepedence[3, 13, 19]. This interdependence has been diffucult to measure, especially for small vessels. Thus, the available information is on the larger intrapulmonary arteries or relatively large laboratory species, such as the dog and pig[1, 3, 7-9, 12, 14, 18, 20]. Also, most of the observations made using planar angiography are of vessel diameters but not lengths. Lumenal eccentricity can also confound measurements from planar roentgenograms[4]. Therefore, we approached the problem of pulmonary arterial-parenchymal interdependence using volumetric CT in the rat lung.

2. METHODOLOGY

2.1 Rat Preparations

Sprague-Dawley rats approximately 65 days of age, ranging in weight from 250-325 gm were used for the study. Each rat was anesthetized with sodium pentobarbital (50 mg/kg ip) and a midline sternotomy performed. The rat was heparinzed (200 IU/kg) by right ventricular injection. The trachea and pulmonary artery were cannulated (PE240 tubing) and the heart dissected away to allow free drainage out the severed pulmonary vein. The lungs were removed, supported by the two cannulae and the vasculature flushed with about 35 ml of a physiological salt solution (pss) containing 6 mg of papaverine hydrochloride at a flow rate of 10 ml/min, increased to 40 ml/min momentarily to clear blood. The lungs were ventilated with a 15% O_2, 6% CO_2 in N_2 gas mixture, 3 mmHg end expiratory pressure and 8 mmHg end inspiratory pressure and intermittent sighs with peak inspiratory pressures of 15-20 mmHg to eliminate any atelectasis that might have occurred during the excision and help clear blood from the vessels. Once the effluent was clear, the cannulae were clamped at end inspiration.

2.2 Imaging Methods

The lungs were placed in the imaging chamber and the vascular and tracheal cannulae attached to an adjustable pressure source. The tracheal pressure was initially set to 12 mmHg. The pleural pressure was atmospheric throughout the studies. Using a height adjustable reservoir, the pss in the arteries was replaced by perfluorooctyl bromide (Perflubron) to obtain high intravascular x-ray absorbance. It is important to note that the surface tension at the H_2O-Perflubron

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interface prevents the perflubron from passing through the capillaries at pressures used in these studies. Therefore, only the arteries were filled with this contrast medium. The arteries were "conditioned" [6] by varying the intravascular pressure between 5 and 30 mmHg several times ending at 30 mmHg. The lungs were then rotated in the x-ray beam at 1° increments to obtain 360 planar images. To maintain the integrity of the lungs, a typical experiment would entail holding Pv constant and varying Ptp or holding Ptp constant and varying Pv. For a given lung one of these protocols would be followed while performing CT scans at several incremental pressure values, after allowing time for the lungs to reach a steady state. Table 1 describes the various protocols used in this study. Whether Ptp or Pv was varied pressures were set to their highest value originally and a deflation was performed to obtain the image data set at each pressure.

No. lungs	Ptp	Pv
2	6	5, 12, 21, 30
2	2, 12	5, 30
1	2, 4, 8, 12	5
2	0, 2, 4, 8, 12	12
1	2, 4, 8, 12	21

Table 1. Experimental conditions.

2.3 Imaging System and Reconstruction

The micro-focal x-ray CT system is composed of a Feinfocus FXE/FXT 100.20 microfocal x-ray source (3um effective focal spot), a Thomson TH9438 HX H661 VR24 image intensifier optically coupled to a Silicon Mountain Design (SMD1M-15) CCD, and a New England Affiliated Technologies (NEAT) specimen micromanipulator stage, all mounted on a precision rail with positional information provided by Mitutoyo linear encoders accurate to 10μm. The geometry of the imaging system allows magnification of the specimen to be increased by decreasing the specimen's proximity to the x-ray source. Each planar projection image was a result of averaging 7 frames to minimize noise. The image data (512x512 pixels) was sent from the camera, via RS-422, to a frame grabber board mounted in a Dell 610 workstation running WindowsNT. Image acquisition and positional control were all preformed by window-based software, written in-house, running on the 610 workstation. The projection data was transferred via network to a Dell 340 workstation running Linux Red Hat 7.3 and after proper preprocessing, to compensate for field distortions and nonuniformities introduced by the imaging chain, isotropic reconstructions were obtained through an implementation of the Feldkamp cone-beam algorithm[5]. The typical reconstructed volume was 497^3 pixels with a typical pixel size of 70μm.

2.4 Measuring Arterial Lengths and Diameters

One measurement parameter available through CT imaging of contrast filled vessels is the lumenal diameter of the artery. Therefore, morphometric measurements were made, along the main pulmonary arterial trunk, on the isotropic reconstructed data sets using methods introduced in earlier work[10, 11]. Diameter measurements were made between successive bifurcations along the main trunk, while data on main trunk vessel length was obtained from bifurcation -to-bifurcation positional information. Lumenal measurements were made in arteries ranging in size from approximately 80 – 2000 um. To measure mechanical properties of the vessel wall, measurements of the each lung at several different intravascular or transpulmonary pressures were made at identical locations on the main trunk.

3. RESULTS

3.1 Constant Vascular Pressure

Under conditions when the intravascular pressure was maintained constant and the transpulmonary pressure was varied from 0 – 12 mmHg, vessel lengthening was seen as the most significant effect. Figure 1 shows differences in an isolated lung between Ptp = 0, 2 and 12 mmHg at a constant Pv of 12 mmHg. As Ptp is increased, there are large changes in longitudinal expansion of the arteries. There are very slight changes in the arterial diameters. Some impingement on the right lobar artery caused by expansion of the main bronchus is seen at Ptp = 12 mmHg. Figure 2 shows diameter vs. distance of subsequent bifurcations along the main trunk at Ptp = 0, 2, 4, 8, and 12 mmHg for the lung in figure 1. To quantify the change diameter as a function of Ptp we introduce a distensibily coefficient α, where the vessel diameter is describable by equation 1, in which diameter is assumed to change linearly with Ptp. Plots of vessel diameter as a function of Ptp for several vessel segments in a similar experiment are presented in

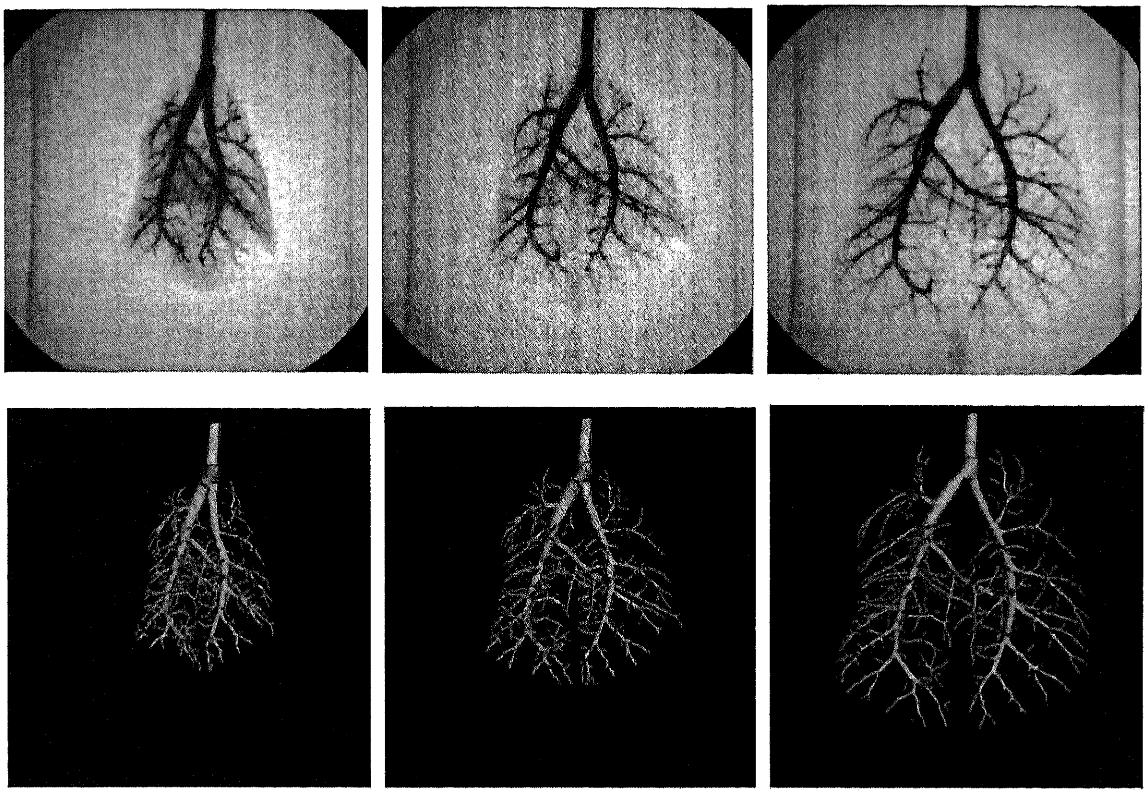


Figure 1. (Top row) Planar arteriograms of a rat lung with intravasuclar pressure (Pv) 12 mmHg and transpulmonary pressures (Ptp) of 0, 2, and 12 mmHg from left to right. (Bottom row) Surface shaded renderings made from 3-D micro-CT reconstructions of the same lung under the same conditions.

$$D(P_{tp}) = D_o(1 + \alpha P_{tp}) \qquad (1)$$

figure 3, which shows for the Ptp range used in this study, equation 1 appears to be an acceptable model for these data. Although vessel diameters tend to increase with increasing Ptp, the increase is much less than seen in studies where intravascular pressure was varied as shown by Karau et. al.[11] and data presented below in this paper. Using data from two lungs (66 vessels segments) in which Pv =12 mmHg and Ptp = 0 – 12 mmHg α was estimated at 0.55±0.42(SE) %/mmHg.

3.2 Constant Transpulmonary Pressure

Under conditions when the transpulmonary pressure was held constant and the intravascular pressure was varied from 5 – 30 mmHg, there was very little vessel lengthening. Figure 4 shows changes in an isolated lung between Pv = 5, 12 and 30 mmHg at a constant airway pressure of 6 mmHg. As Pv is increased, there are large increases in vessel diameter. The changes in the arterial lengths, under these conditions, are minimal. Pulmonary arterial diameter is largely dependent on Pv as seen in figure 5. Calculating α from two lungs (70 vessel segments) where Ptp = 6 mmHg and Pv

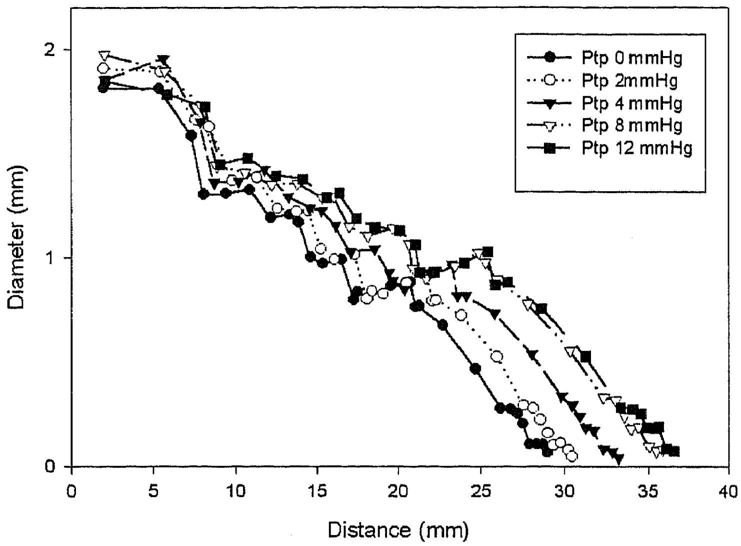


Figure 2. Vessel diameter vs. main pulmonary trunk length (cumulative distance along the main trunk to subsequence bifurcation sites) measured from CT data for intrapulmonary pressures (Ptp) equal to 0, 2, 4, 8, and 12 mmHg.

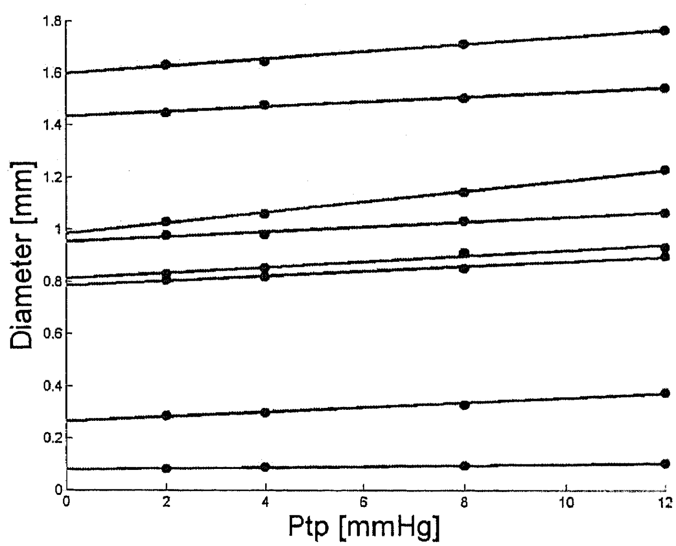


Figure 3. Transpulmonary pressure (Ptp) vs. vessel segment diameter measured in several vessels along the main arterial

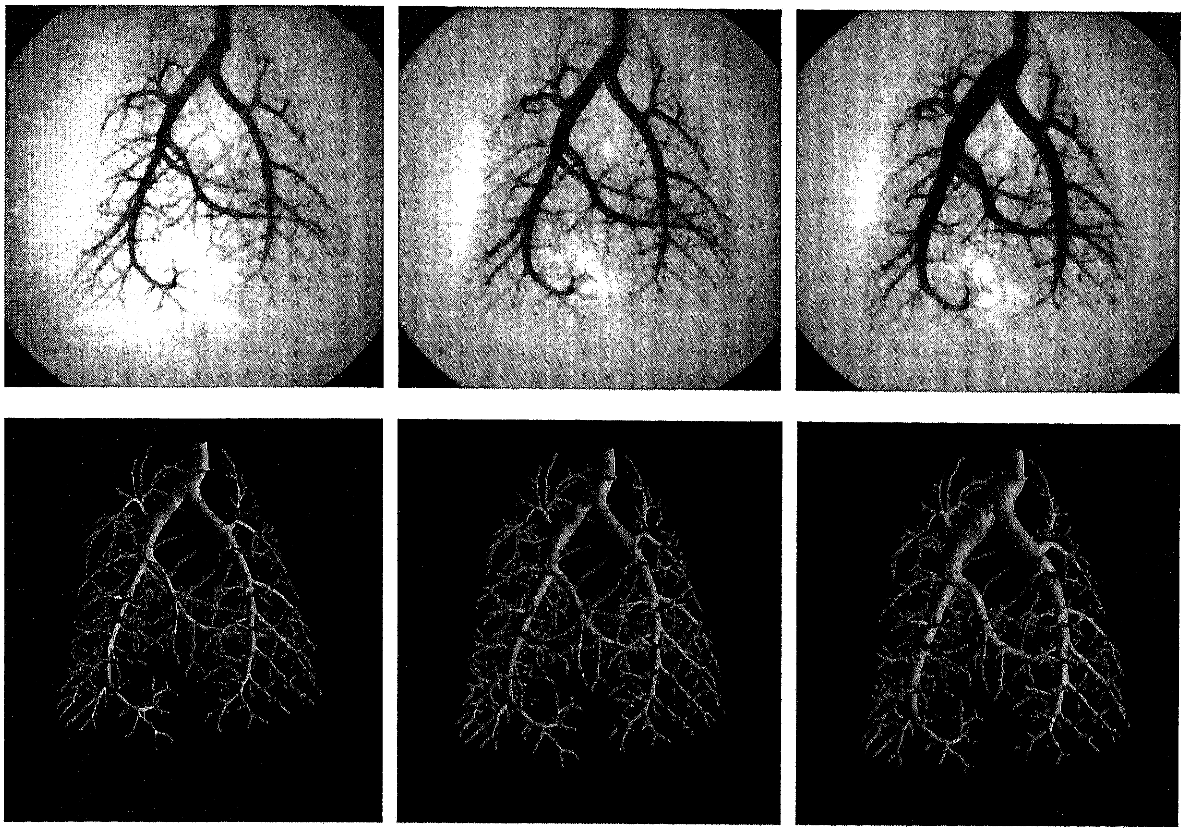


Figure 4. (Top row) Planar arteriograms of a rat lung with transpulmonary pressure (Ptp) 6 mmHg and intravascular pressures (Pv) of 5, 12, and 30 mmHg from left to right. (Bottom row) Surface shaded renderings made from 3-D micro-CT reconstructions of the same lung under the same conditions.

varied from 0 - 30 mmHg resulted in alpha = 2.59±0.17(SE) %/mmHg. To investigate if there was a size depedent effect on α, vessel segments were separated according to the value of D(0). Figure 6 is a plot of the average± S.E. pressure-diameter relationship for 3 groups of vessel segments, large (D(0) > 1.4) mm, Medium (1.4 > D(0) > 0.5 mm), and small (D(0) < 0.5 mm) for an experiment in which Pv was varied and one where Ptp was varied.

4. DISCUSSION

4.1 Comparison to Previous Findings

A fairly large body of work has investigated bronchial diameter and length with lung inflation[8-9, 14-15] fewer references exist on vascular morphology and its interdependence on lung inflation (lung volume). Some of the early work in this area was accomplished by Howell's group[7, 18] in which excised dog lungs were studied to determine the effect of lung inflation on the pulmonary vasculature. In Howell's studies, experiments were designed so that there would be no difference in the results whether positive or negative inflation was used. The resting state of the vasculature was set such that the height of dextran in connected burettes was equal to the height of the top of the lung. A completely opposite response in Pv was seen if an additional 11 ml of dextran was added to the vascular system. Changes in vascular volume were also a function of biases in Pv from the resting state. This led to an experimentally supported conclusion that the vascular space was, in essence, behaving like two separate compartments. The large vessels (the expanded portion) that displayed volume expansion with increased lung inflation and the small perialveolar vessels (the compressed portion) that lost volume as lung volume was increased. It was also impossible in their studies to determine the diameter-length characteristics.

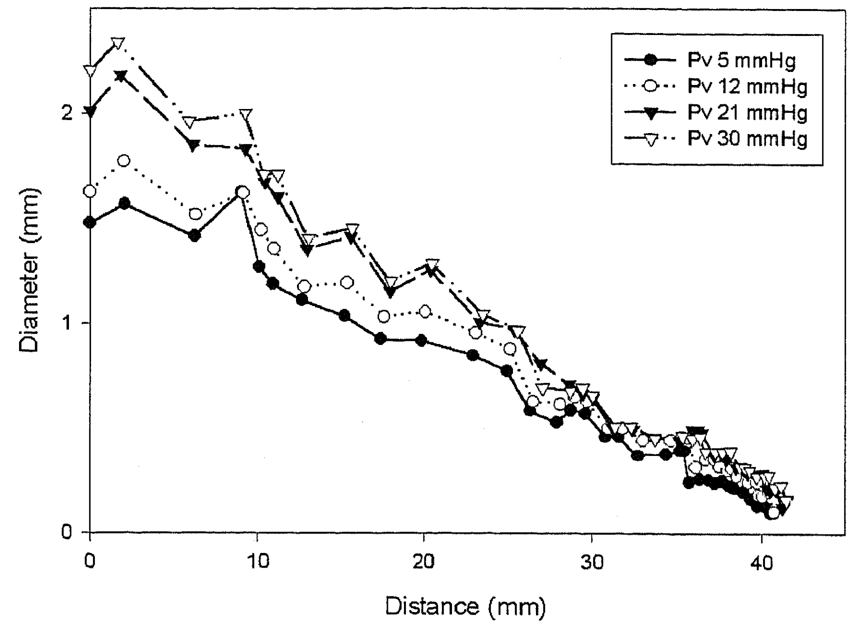


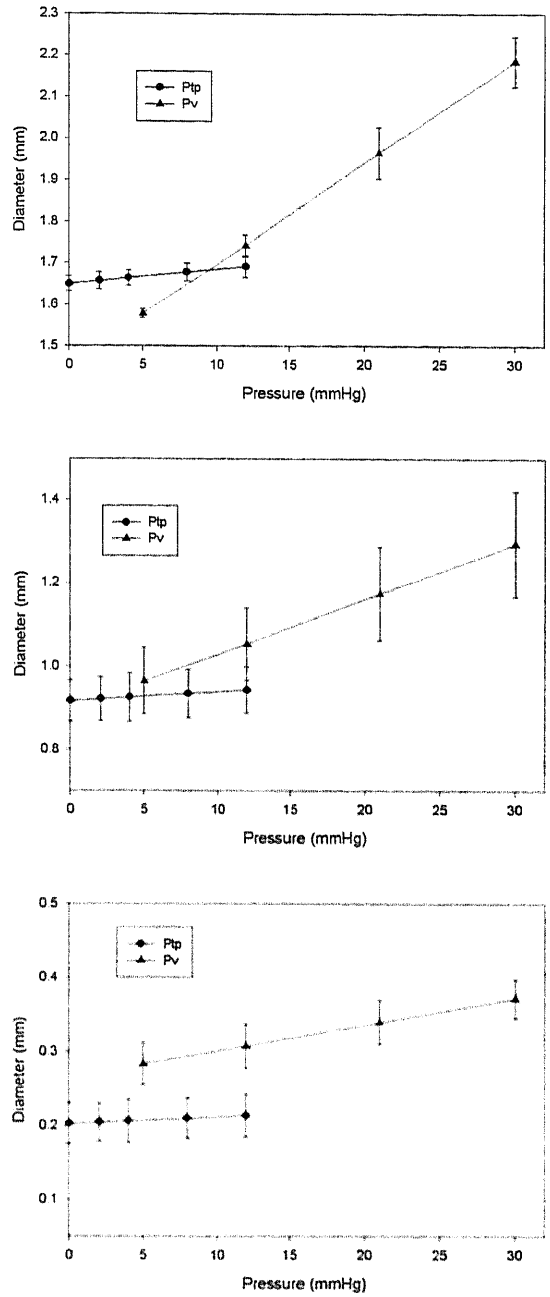
Figure 5. Vessel diameter vs. main pulmonary trunk length (cumulative distance along main trunk measured at subsequent bifurcation sites) measured from CT data for various intravascular pressures.

In studies that investigated lung volume affect on vessel diameter, different groups found that, in the intact lung, vessel diameter became larger at higher transpulmonary pressures[3, 12, 13, 20], although these studies examined relatively large (2.0 mm and larger) vessels, Albert et. al. (Albert) found a similar relationship in vessels of even smaller dimension (0.2 - 1.3 mm). Typical studies of this type found only slight differences between the behavior of vein and arteries[3, 12, 20]. It is generally held that during lung inflation, as the lung expands, the parenchyma exerts radial traction on the intraparenchymal vessels walls, lowering the perivascular pressure and in turn causing an increase in vascular diameter. Conclusive evidence of what is occurring at the site of periaveolar vessels is still elusive. According to the data in figure 6, small vessels down to the range of 0.08 mm do not show a loss in diameter with increases in Ptp. With the resolution currently provided by the micro-CT data, diameter measurement ranges still do not approach that of the percapilary and capillary vessels and therefore it is still unsure if the compression region of vessels does in fact exist.

In the present study we utilize microfocal angiography / computed tomography to investigate the morphmetric (diameter-length) behavior of the pulmonary arterial tree as a function of lung inflation, in intact, excised rat lungs. Accordingly, the present study was carried out to extend knowledge of these relationships to smaller animals, which enabled investigation of smaller vessels (down to the 0.08 mm range), closer to the perialveolar level, and do it in such a way as to obtain novel three-dimensional data on the intact lung structure. To aid in resolving various issues raised in this paper, our group is also refining the experimental techniques to obtain direct measurements of total pulmonary arterial volume and changes in arterial volume with either changes in Pv or Ptp.

4.2 Vessel Eccentricity

Caro[4] had shown at low Pv values the vessels may become eccentric. A mesh plot of an artery approximately 1.2 mm in diameter from a CT reconstruction in an experiment where Ptp = 12 mmHg and Pv = 5 mmHg is shown in figure 7. Even at relatively low Pv values the vessel cross section is only slightly eccentric, therefore it is a reasonable to use the assumption that the vessel cross-section is circular. This assumption is used in the model for estimating vessel diameter in this study.



A

B

C

Figure 6. Average±S.E. pressure-diameter relationship for 3 groups of vessel segments, A. large (D(0) > 1.4) mm, B. Medium (1.4 > D(0) > 0.5 mm), and C. small (D(0) < 0.5 mm) for an experiment in which (Pv) was varied and one where (Ptp) was varied.

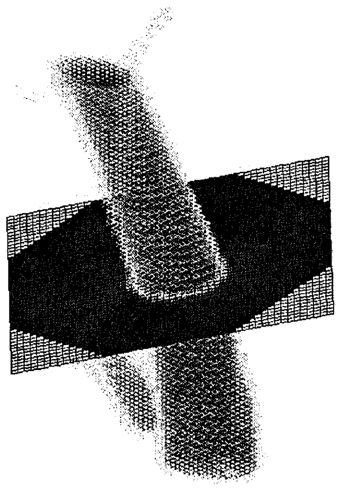


Figure 7. A mesh plot displays a local region of interest from a lung studied at Pv = 5 mmHg and Ptp = 12, data in the plane is used to estimate the diameter of the vessel.

4.3 Development Related Changes in Vessel Mechanics

Although previous studies have shown a significant change in the mechanical properties of the rat lung during postnatal development[17], under 40 day of age, all rats used in this study were beyond their 60^{th} day of age before experimentation and should not be effect by this.

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