Department of Mechanical Engineering

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CONTRIBUTION OF ELASTIN TO THE CHANGE IN MECHANICAL PROPERTIES OF PULMONARY ARTERIAL TISSUES RESULTING FROM HYPERTENSION

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Background:

Pulmonary Hypertension

- Leads to vascular remodeling in the chronic situation. Resulting structural changes increase both the flow resistance of the distal arteries and the flow impedance of the proximal arteries.
- The increased afterload imposed on the heart leads to cardiac remodeling and eventual right ventricular failure.
- Stiffening of the proximal arteries increases the hydrodynamic load imposed on the heart, and exacerbates cardiac remodeling.

Artery Morphology

1. Typical structure of Elastic Arteries
   - Tunica Intima: Innermost layer consisting of a single layer of endothelial cells and a thin basement membrane.
   - Tunica Media: Elastic layer consisting of smooth muscle cells, elastic lamellae and collagen fibrils.
   - Tunica Adventitia: Helically oriented collagen bundles provide strength and rigidity at high strain levels.

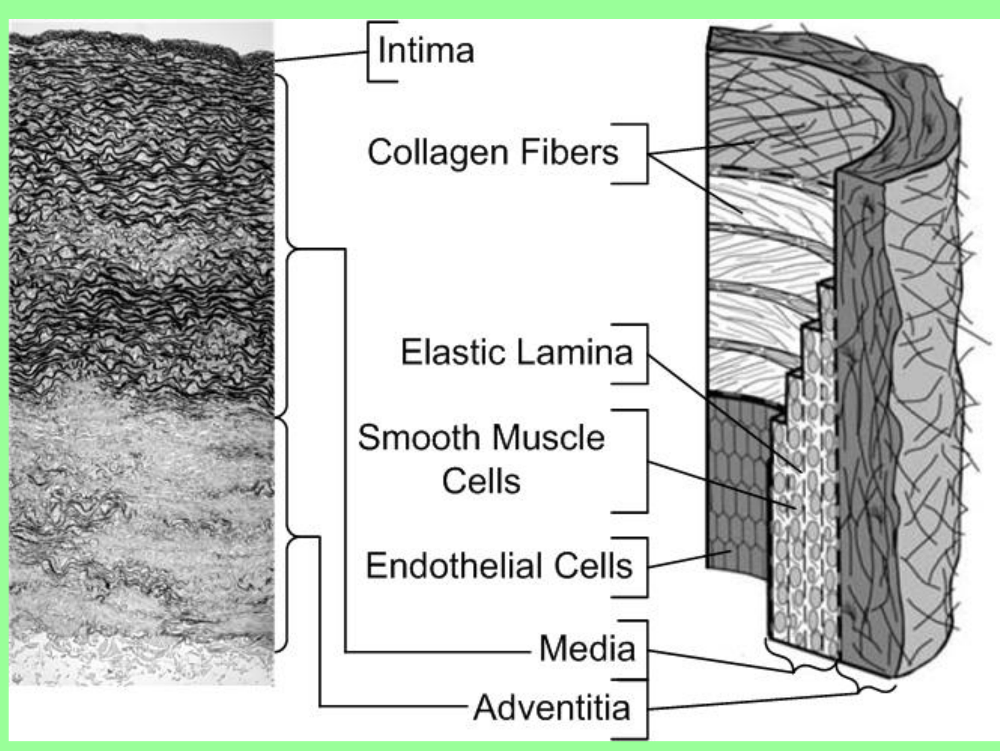


Fig. 1 Detail of artery structure and morphology. [Ref. 1]

Methods:

Animal Model

- 3-Control, 5-Hypertensive male Holstein calves (2-wks)
- Hypertension induced by hypoxia (2-wks, 30,000' equivalent air pressure)
- All studies were performed after approval by institutional animal care and use committees.

Measurement Locations and Dissection Geometry

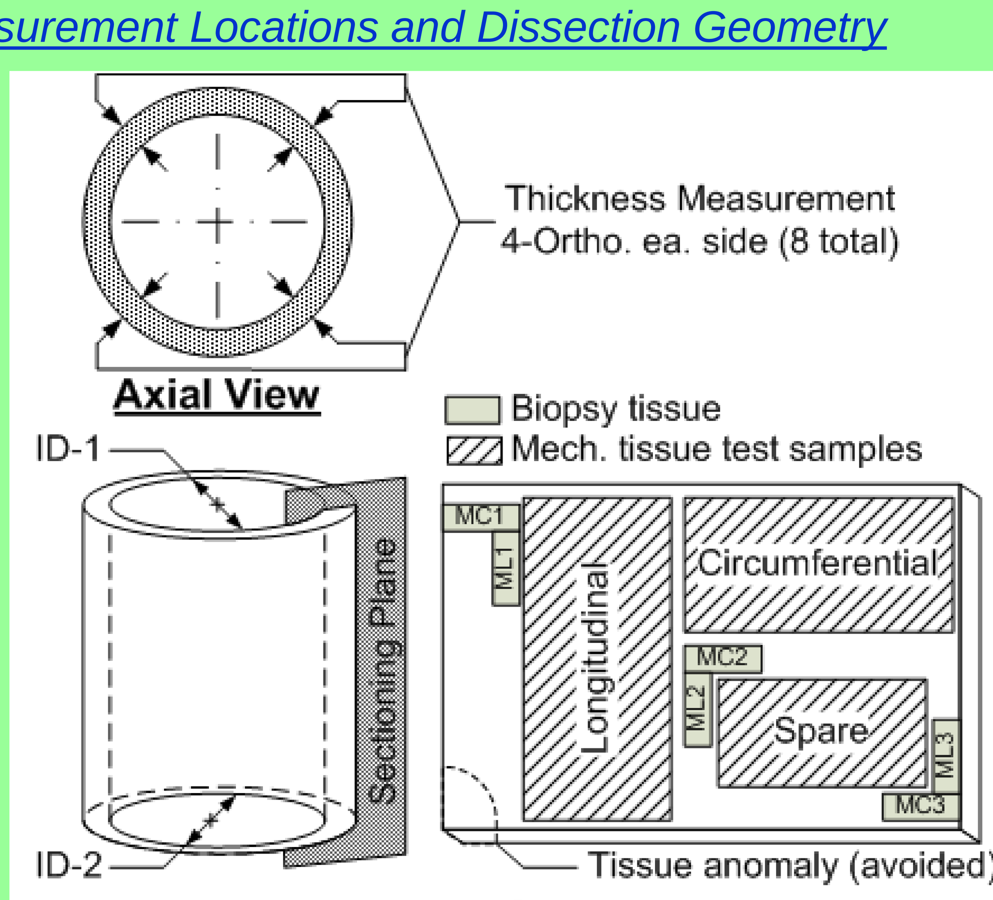


Fig. 2 Thickness measurements, tissue dissection and biopsy locations (typical)

Histology and Elastin Area Fraction Analysis

- Biopsy samples were fixed in 4% formaldehyde, embedded in paraffin, sectioned and stained with Verhoeff's Van Gieson stain for elastic fibers.
- Photomicrographs: 100X magnification, sequential images were merged with Photoshop (Photomerge).
- Area fraction determined using Matlab image processing toolbox.
  - Intensity Thresholding
  - Morphological operations

Methods (contd.):

Uni-axial Stress-Strain Tests

- An MTS Insight-2 material testing was system used to test arterial tissues in circumferential and longitudinal directions.
- All tissues tested in Ca+-free PBS buffer, 0.01M, pH 7.4

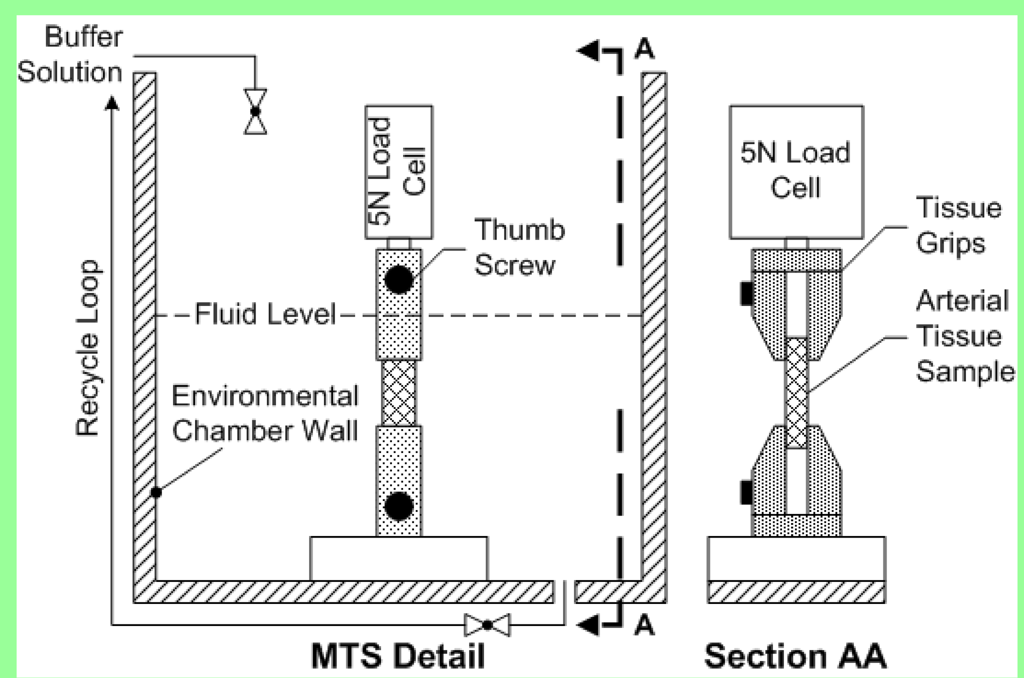


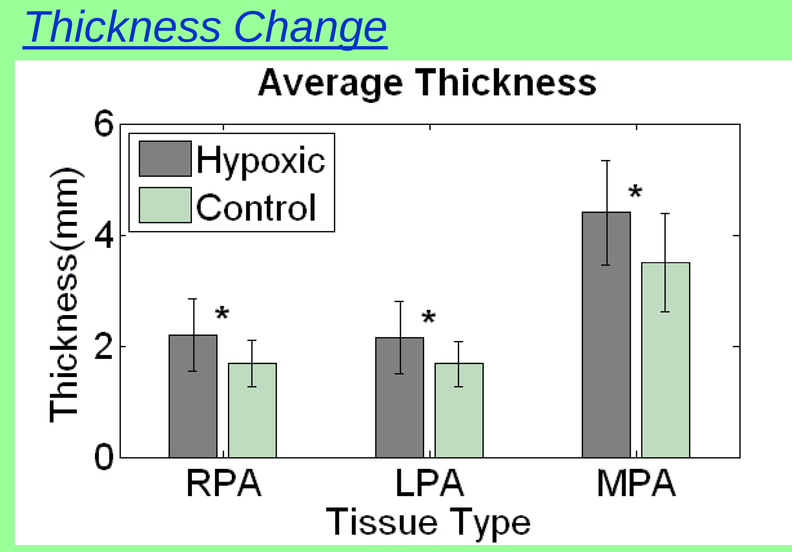
Fig. 3 Detail of material testing procedure and apparatus.

Elastin Digestion

- Elastin digestion procedure described by Lu *et al.* [Ref.2]
- Arteries were submerged in a 70% formic acid, 50 mg/mL Cyanogen Bromide (CNBr) solution and gently stirred for 19 hours at room temperature followed by 1 hour at 60ºC, boiled for 10 min.
- Stored at pH 7.4 with PBS, 0.01M

Results

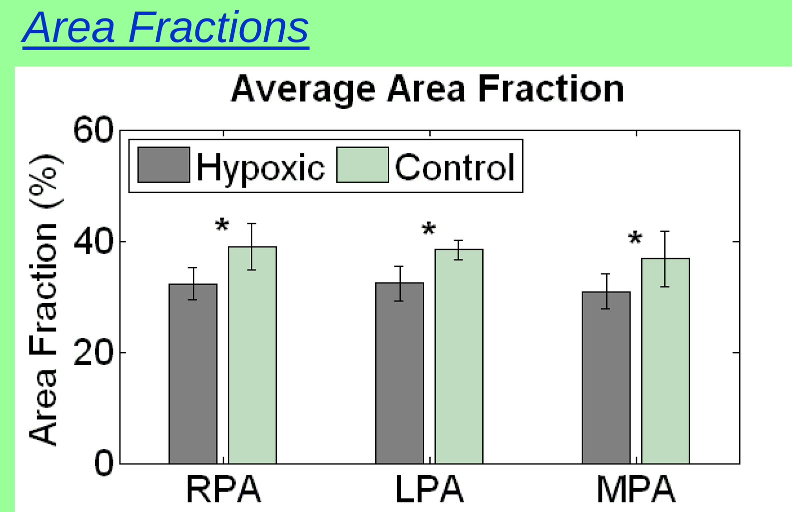
Thickness Change



% Change in thickness due to hypertension
- RPA: 30% (P << 0.05)
- LPA: 28% (P << 0.05)
- MPA: 26% (P = 0.007)

Fig. 4 Comparison of average thickness values for control and hypertensive arteries.

Area Fractions



% Change in area fraction due to hypertension
- RPA: -17% (P = 0.03)
- LPA: -16% (P = 0.01)
- MPA: -16% (P = 0.03)

Fig. 5 Comparison of average elastin area fraction values for control and hypertensive arteries.

Change in Elastin Content as a Result of Hypertension

- Elastin Content Factor (ECF): The ECF is equal to the elastin area fraction multiplied by the average tissue thickness.
- The ECF is analogous to the volume of elastin present in each tissue.
- % Change in ECF due to Hypertension:
  - RPA: 7.6%
  - LPA: 7.7%
  - MPA: 5.5%

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Results (contd.):

Physiologic strains

- Systolic and diastolic pressures were used with the hoop stress equation to determine the range of physiologic strains.
- Curvature of the stress-strain curve was used to determine the elastin-dominant, transitional and collagen-dominant regions of the stress-strain curve.
  - Average strain at systole: 39% (σ = 8%) Ctl., 44% (σ = 7%) Hyp.
  - Average strain at diastole: 26% (σ = 5%)
  - Average transitional strain: 51% (σ = 8%)

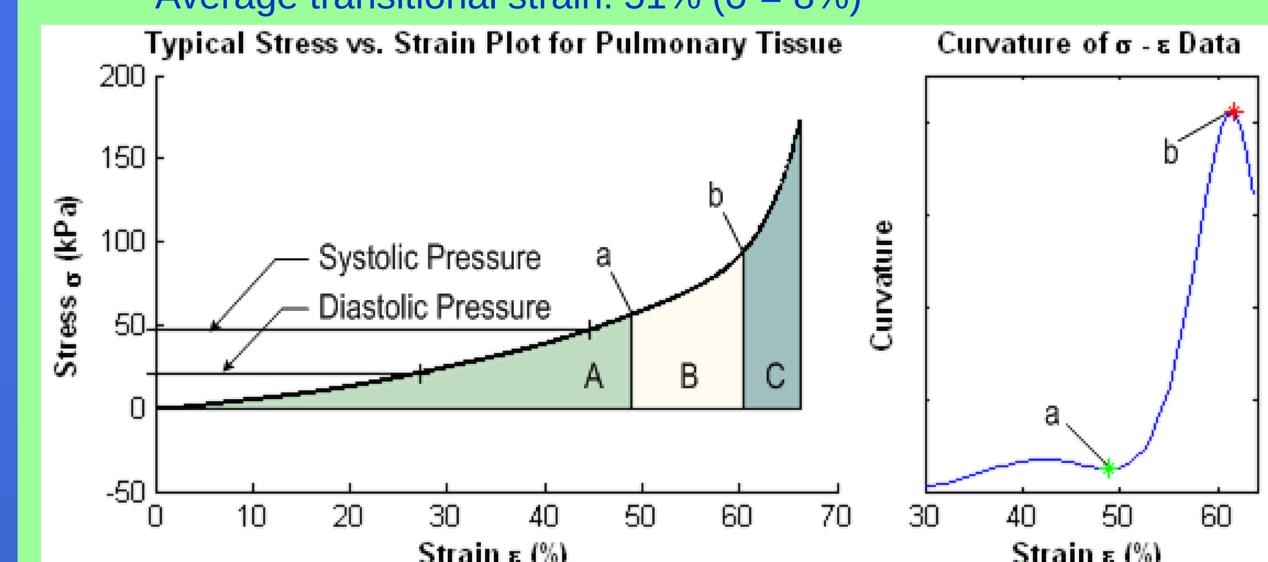

Fig. 6 Typical stress-strain curve, and associated curvature, for proximal (MPA,RPA and LPA) arterial tissues. Elastin-dominant (A), transitional (B) and collagen-dominant (C) regions identified by shaded areas.

Material Properties of Arteries

- Average stiffness: Hypertensive 235 mN/mm (σ = 45 mN/mm)
  Control 130 mN/mm (σ = 41 mN/mm)
- Stiffness change due to hypertension: 81% (σ = 23%, P<<0.05).
- Average modulus: Hypertensive 114 kPa (σ = 37 kPa)
  Control 80 kPa (σ = 30 kPa)
- Modulus change due to hypertension: 46% (σ = 13%, P=0.02).

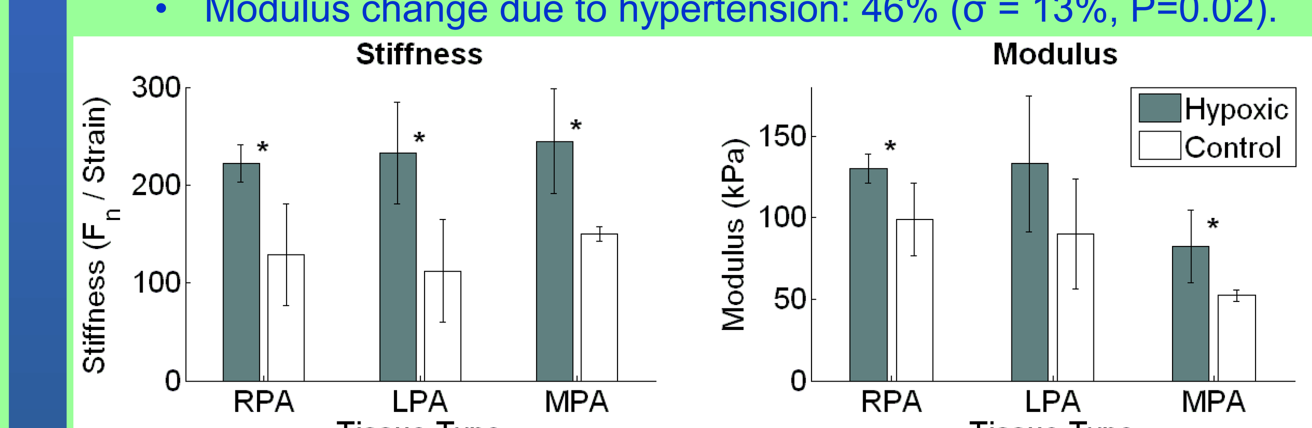

Fig. 7 Comparison of stiffness and modulus values for control and hypoxic arteries.

Material Properties of Arterial Elastin

- Average stiffness: Hypertensive 116 mN/mm (σ = 45 mN/mm)
  Control 68 mN/mm (σ = 36 mN/mm)
- Stiffness change due to hypertension: 100% (σ = 62%, P=0.01).
- Average modulus: Hypertensive 126 kPa (σ = 34 kPa)
  Control 70 kPa (σ = 20 kPa)
- Modulus change due to hypertension: 84% (σ = 45%, P<<0.05).

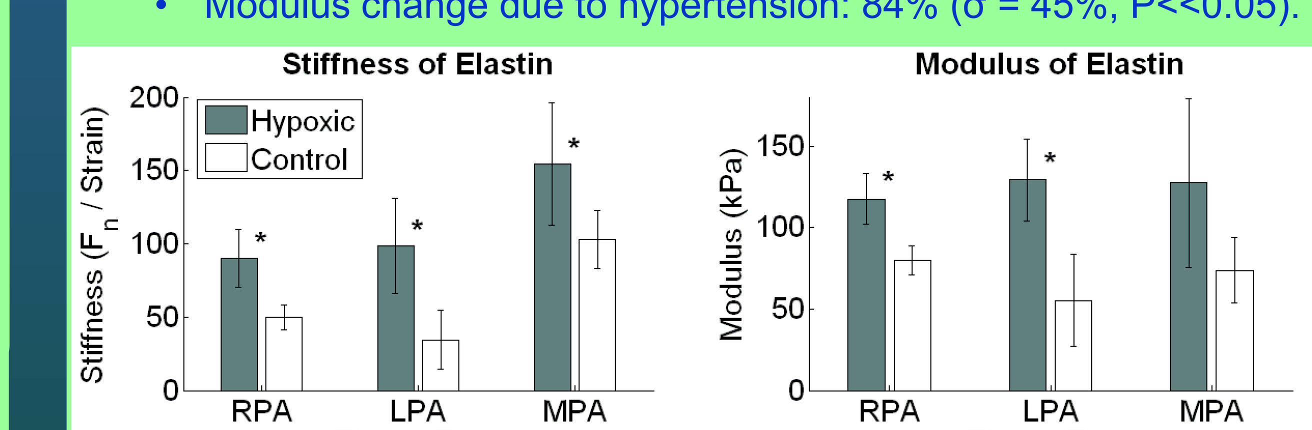

Fig. 8 Comparison of stiffness and modulus values for control and hypoxic arterial elastin samples

Conclusions

- Physiologic pressures correspond to strains which reside entirely within the elastin dominant region of the stress-strain curve.
- Hypertension results in a thickening of the arteries and in a reduction in the area fraction of elastin. However, the ECF indicates that hypertension results in an overall increase in the amount of elastin.
- There is a significant increase in the stiffness and modulus of both fresh arteries and arterial elastin tissue as a result of the disease.
- Our results show that the stiffness of elastin accounts for 49% of the stiffness of the arteries. However, it is likely that the modulus of the digested elastin samples is lower than the modulus of the tissue in its native state due to the removal of interstitial tissue during digestion.

1.) Holzapfel, G. A., T. C. Gasser, et al. (2000). "A new constitutive framework for arterial wall mechanics and a comparative study of material models." Journal of Elasticity 61(1-3): 1-48.

2.) Lu, Q., K. Ganesan, et al. (2004). "Novel porous aortic elastin and collagen scaffolds for tissue engineering." Biomaterials **25**(22): 5227-37.