Insights Into Regional Adaptations in the Growing Pulmonary Artery Using a Meso-Scale Structural Model: Effects of Ascending Aorta Impingement

Bahar Fata

Department of Bioengineering,
University of Pittsburgh,
Pittsburgh, PA 15104;
Center for Cardiovascular Simulation,
Institute for Computational
Engineering and Science,
Department of Biomedical Engineering,
University of Texas,
Austin, TX 78712

Will Zhang

Center for Cardiovascular Simulation,
Institute for Computational
Engineering and Science,
Department of Biomedical Engineering,
University of Texas,
Austin, TX 78712

Rouzbah Amini

Department of Biomedical Engineering,
Auburn Science and Engineering Center 275,
West Tower,
The University of Akron,
Akron, OH 44325

Michael S. Sacks^{1,2}

Professor
W. A. "Tex" Moncrief, Jr. Simulation-Based
Engineering Science Chair I,
Center for Cardiovascular Simulation,
Institute for Computational
Engineering and Science,
Department of Biomedical Engineering,
University of Texas,
Austin, TX 78712
e-mail: msacks@ices.utexas.edu

As the next step in our investigations into the structural adaptations of the main pulmonary artery (PA) during postnatal growth, we utilized the extensive experimental measurements of the growing ovine PA from our previous study (Fata et al., 2013, "Estimated in vivo Postnatal Surface Growth Patterns of the Ovine Main Pulmonary Artery and Ascending Aorta," J. Biomech. Eng., 135(7), pp. 71010–71012). to develop a structural constitutive model for the PA wall tissue. Novel to the present approach was the treatment of the elastin network as a distributed fiber network rather than a continuum phase. We then utilized this model to delineate structure-function differences in the PA wall at the juvenile and adult stages. Overall, the predicted elastin moduli exhibited minor differences remained largely unchanged with age and region (in the range of 150 to 200 kPa). Similarly, the predicted collagen moduli ranged from ~1,600 to 2700 kPa in the four regions studied in the juvenile state. Interestingly, we found for the medial region that the elastin and collagen fiber splay underwent opposite changes (collagen standard deviation juvenile = 17 deg to adult = 28 deg, elastin standard deviation juvenile = 35 deg to adult = 27 deg), along with a trend towards more rapid collagen fiber strain recruitment with age, along with a drop in collagen fiber moduli, which went from 2700 kPa for the juvenile stage to 746 kPa in the adult. These changes were likely due to the previously observed impingement of the relatively stiff ascending aorta on the growing PA medial region. Intuitively, the effects of the local impingement would be to lower the local wall stress, consistent with the observed parallel decrease in collagen modulus. These results suggest that during the postnatal somatic growth period local stresses can substantially modulate regional tissue microstructure and mechanical behaviors in the PA. We further underscore that our previous studies indicated an increase in effective PA wall stress with postnatal maturation. When taken together with the fact that the observed changes in mechanical behavior and structure in the growing PA wall were modest in the other three regions studied, our collective results suggest that the majority of the growing PA wall is subjected to increasing stress levels with age without undergoing major structural adaptations. This observation is contrary to the accepted theory of maintenance of homeostatic stress levels in the regulation of vascular function, and suggests alternative mechanisms might regulate postnatal somatic growth. Understanding the underlying mechanisms will help to improve our understanding of congenital defects of the PA and lay the basis for functional duplication in their repair and replacement.

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1 Introduction

Congenital abnormalities of pulmonary artery often necessitate surgical repair or the use of autologous tissue and synthetic biomaterials as vascular grafts [1–3]. The patency of synthetic conduit replacements remains limited, often requiring further surgical re-interventions due to lack of adaptation to the normal growth of the child and/or functional failure of the graft [4]. The autologous conduit replacements are limited in supply and may not adjust to

different flow environment of the graft site. Above all, an optimal vascular replacement should be able to accommodate somatic growth and closely mimic the structure, function, and physiologic function of the native vessel. In recent years, there has been a growing interest in the development of a living, autologous tissue graft that could address the critical need for growing substitutes for the repair of congenital cardiac defects [5–8], especially the pulmonary valve and artery (PA). The engineering foundation of such novel approaches must thus rest on an understanding of changes in the structure-function relationship that occur during postnatal maturation. Moreover, the distensibility of great arteries are important determinants of ventricular afterload and eventual dysfunction in the pulmonary hypertension, as well as many congenital defects [9]. Yet, relatively little known of the postnatal somatic growth characteristics of the PA.

In general, the altered mechanical properties of the great arteries are primarily associated with remodeling of the collagen

¹Present address: Institute for Computational Engineering and Sciences (ICES), The University of Texas at Austin, 201 East 24th Street, ACES 5.438, 1 University Station, C0200, Austin TX 78712-0027.

²Corresponding author.

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and elastin fiber networks. For example, biochemical studies in animals have shown a significant upsurge in the collagen and elastin synthesis and mass, as well as reorganization in hypertensive pulmonary arteries [10,11]. The perinatal period is associated with significant elastin and collagen accumulation in the pulmonary trunk and aorta in preparation for a marked postnatal increase in arterial pressure [12,13]. It is well known that newborn animals develop more severe pulmonary hypertension than adults with dramatic vascular changes [14], possibly due to the elastin and collagen synthesis being particularly sensitive to modulation by hypoxia during this time of rapid growth. Lammers et al. [15] have delineated the prominent role of elastin in the alteration of pulmonary artery mechanics in hypertensive calves. Moreover, structural and degradative alterations of medial elastin is found to be a major contributing factor in physiological phenomena such as aging, and the initiation and development of cardiovascular disease, such as aortic aneurysms [16,17].

We have recently demonstrated complex patterns of spatial growth in the growing ovine PA [18,19]. Our results indicated that the spatial and temporal surface growth deformation patterns of both arteries were heterogeneous, including an increase in taper in both arteries and increase in cross-sectional ellipticity of the PA. Interestingly, contact between the PA and AA resulted in increasing spatial heterogeneity in postnatal growth, with the PA demonstrating the greatest changes. Results of this study clearly underscored the fact that functional growth of the PA during postnatal maturation involves complex geometric adaptations. In a parallel study, we quantified the structural and biomechanical properties over the same age period [20]. Here, the PA wall demonstrated significant mechanical anisotropy, except in the posterior region where it was nearly isotropic, and overall modest changes in regional mechanical properties with growth. Perhaps our most interesting finding was that we found that the PA wall thickness was maintained over the entire growth period in spite of the substantial increase in vessel diameter. This suggests that the PA grows by in-plane tissue accumulation only, resulting in a 40% average increase in hoop stress over the growth period. Therefore, unlike the arterial wall remodeling due to hypertension [10,11,21], there is not strictly-held homeostatic maintenance of wall stress during the postnatal growth period. This rather surprising result opens the door for many questions, such as whether are there alterations in the effective moduli of the collagen and elastin networks during the growth period.

To begin to address these questions, one can utilize constitutive models which incorporate several important aspects of the underlying microstructure. Structurally based models can help elucidate the mechanisms governing the structure-function relationship of biological tissues and elucidate what happens during the remodeling period. Such approaches have been utilized for arterial tissue remodeling [22,23], as well as the PA [24–26]. However, the models developed so far have not addressed the mechanical behavior of normal growing vessels from the early juvenile to the adult states, either as a contiguous growth model or as a set of quasi-static steps. Moreover, the reliability of such models considerably depends on the accurate quantification of organization and load-bearing behavior of fibrous components of the tissue. In the case of the PA, these are collagen, elastin, and to a lesser extent, smooth muscle as the mechanically significant structural components. Thus, the elastin and collagen structure-function relationship of the normal PA and in connection to growth changes need to be utilized in such models.

In the present study, we utilized the extensive experimental measurements of the growing PA to develop a structural model for the PA in the juvenile and adult states. Novel to this approach was the explicit treatment of the elastin phase as a population of fibers as opposed to single material phase as in other studies of the PA. The developed constitutive model also took into account the contributions of the ground matrix, consisting of smooth muscle cells and other noncellular materials. When applied to the available data, we demonstrate that we were able to delineate the structure-function relationship of PA wall in the postnatal growth period in the juvenile and adult states.

2 Methods

2.1 Mechanical and Structural Data. Extensive structural and biaxial mechanical properties and collagen and elastin fiber architecture measurements from four regions of the PA wall were taken from Ref. [20] (Fig. 1(a)). This data included elastin, collagen, and smooth muscle mass fractions obtained histologically (Fig. 1(b)). In addition to these data, collagen recruitment of the medial region (Fig. 1(b)) were available from Ref. [20]. Details of the exact available data and its pre- and post-processing are given in the following.

2.2 Considerations and Assumptions. While the PA is a multilayered structure (Fig. 1(b)) undergoing continuous growth, to make the present problem tractable we model the PA wall as a single homogenized layer at two quasi-static time points of postnatal growth: juvenile and adult. This is in part based on our previous observations that no changes in relative thickness of individual layers and total wall thickness were observed in the growing PA [20]. Next, the following comprehensive set of assumptions were observed:

- (1) The general form of the constitutive model was assumed to be maintained during the postnatal maturation period. This assumption is partially supported by the fact that the stress-strain curve shapes were conserved with growth [20].
- (2) The collagen and elastin fiber networks are considered to be the mechanically dominant components compared to the inactive smooth muscle cells [27].
- (3) Both elastin and collagen fibers bear load only along their fiber axes and have negligible resistance to compressive forces.
- (4) The elastin-collagen mechanical interactions were ignored and the net tissue response is considered to be the sum of the individual constituent responses only.
- (5) The contribution of both fiber systems is in the plane of the wall, with the radial mechanical behavior of the artery is governed by the incompressible muscle and ground matrix constituents.
- (6) The load required to straighten the collagen fibers is negligible compared to the load transmitted by elastin or stretched collagen. Thus, collagen bears load only when completely straight.

Fig. 1 (a) 3D reconstruction of the ovine ascending aorta (AA) and the pulmonary artery trunk showing the pulmonary trunk (PT) view, with the white dashed box indicating area of contact between the two great vessels. Also shown are the posterior, anterior, lateral, and medial regions of the PA. (b) Transmural micrograph of the ovine PA and volume fraction results of the PA at the juvenile and adult states. Note that all regions demonstrated an increase in collagen mass with age, with the medial region indicating the largest mass fraction in the adult state.

- (7) Based on the finding of Zulliger et al. [28], the contribution of inactive smooth muscle tone to load bearing structures was assumed to be very small compared to the fibers, and are thus modeled as a single material phase assuming an isotropic response

2.3 Tissue Level Strain Energy Function. The tissue level pseudohyperelastic strain energy density Ψ [29] of a representative volume element (RVE), which is assumed to be small enough so that the deformation gradient tensor $\mathbf{F}$ is homogenous (i.e., does not locally depend on position), yet large enough to allow local averaging of the constituent microstructure. We further assumed the mechanical contributions of elastin (e), collagen (c), and smooth muscle (m), are weighted by their respective volume fractions ϕ

$$\Psi(\mathbf{C}) = \phi_c \Psi_c(\mathbf{C}) + \phi_e \Psi_e(\mathbf{C}) + \phi_m \Psi_m(\mathbf{C}) \quad (1)$$

where $\mathbf{C} = \mathbf{F} \cdot \mathbf{F}^T$ is the left Cauchy-Green stretch tensor. The tissue level response in terms of the 2nd Piola-Kirchhoff stress tensor $\mathbf{S}$ is derived using

$$\mathbf{S} = \frac{\partial \Psi}{\partial \mathbf{E}} - p \cdot \mathbf{C}^{-1} = \phi_c \frac{\partial \Psi_c}{\partial \mathbf{E}} + \phi_e \frac{\partial \Psi_e}{\partial \mathbf{E}} + \phi_m \frac{\partial \Psi_m}{\partial \mathbf{E}} - p \mathbf{C}^{-1} \quad (2)$$

where $\mathbf{E} = (\mathbf{C} - \mathbf{I})/2$ is the Green-Lagrange strain tensor and $\mathbf{I}$ the identity tensor, along with the Lagrange multiplier p to enforce incompressibility.

We developed the strain energy function for each tissue phase as follows. First, based on assumption 7 above, we model the muscle component as a Neo-Hookean material with small shear modulus. This is consistent with the inactivated state of the tissue investigated. Using a shear modulus of $\kappa_m = 1$ kPa (divided by two to be consistent with linear elasticity), the 2nd-Piola Kirchhoff stress for the nonviable smooth muscle and nonfibrous ground matrix phases and water is given by

$$\mathbf{S}_m = \phi_m \frac{\kappa_m}{2} (\mathbf{I}_1 - 3) - p \mathbf{C}^{-1} \quad (3)$$

The contributions of the nonfibrous components and fluid phases are assumed to be responsible for the incompressibility of the tissue. Details for the fibrous components are given in the following.

2.4 Constitutive Model for Elastin. Novel to this study is the approach to model the elastin network as a discrete fibers oriented in the plane of the tissue, rather than a material phase (e.g., Ref. [26]). This has been made possible by the quantification of the elastin structure in Ref. [20]. The results from Fata et al. [20] and related studies [24–26] suggest that a linear $\mathbf{S}$ - $\mathbf{E}$ relation is observed in the low stress region. We assume that elastin dominates the low stress. This assumption was further supported in our previous study that collagen recruitment does not occur until strain range of the low stress region was exceeded [20]. Thus, we utilize the following elastin fiber strain energy

$$\Psi_e^f(\mathbf{E}_f) = \frac{\kappa_e}{2} \mathbf{E}_f^2 \quad (4)$$

where κ_e is the effective elastin fiber modulus. Assuming affine transformation for the RVE, the elastin fiber strain $\mathbf{E}_f$ was derived from the bulk tissue strain using $\mathbf{E}_f = \mathbf{N}^T \mathbf{E} \mathbf{N}$, where $\mathbf{N}(\theta) = \cos(\theta) \hat{\mathbf{X}}_1 + \sin(\theta) \hat{\mathbf{X}}_2$ with θ measured from the circumferential axis (Fig. 1(a)). Next, measured elastin orientation distributions from the multiphoton imaging data from Ref. [20] were normalized to unit area to obtain $\Gamma_e'(\theta)$ (Fig. 2). Next, since elastin fibers do not appear to undergo any type of recruitment the initial expression for the total elastin stress is given by

$$\begin{aligned} \mathbf{S}_e(\mathbf{E}) &= \phi_e \frac{\partial \Psi_e}{\partial \mathbf{E}_f} \frac{\partial \mathbf{E}_f}{\partial \mathbf{E}} = \phi_e \int_{\theta} \Gamma_e'(\theta) \mathbf{S}_e^f(\mathbf{E}_f) \frac{\partial \mathbf{E}_f}{\partial \mathbf{E}} d\theta \\ &= \phi_e \kappa_e \int_{\theta} \Gamma_e'(\theta) \mathbf{E}_f \mathbf{N} \otimes \mathbf{N} d\theta, \quad \theta \in \left[-\frac{\pi}{2}, \frac{\pi}{2}\right] \end{aligned} \quad (5)$$

However, in the multiphoton images of the PA wall, elastin appeared both as oriented fibrous and sheetlike isotropic structures. These contiguous sheetlike structures, although visualized, were not reliably quantifiable in terms of mass fraction. Supportive evidence of their mechanical contribution came from the low stress regions where we observed significant stress development in the longitudinal direction, even though there were no measurable elastin fibers aligned in the longitudinal direction in the juvenile and adult states (Fig. 2). Therefore, we modified Eq. (5) to model the elastin fiber network as the sum of an oriented phase of a measured orientation function $\Gamma_e'(\theta)$ and an isotropic, randomly distributed fiber phase. Thus, the total fiber orientation distribution function is

Fig. 2 Final average elastin and collagen measured orientation distributions and the Modified Beta distribution probability distribution fits in the juvenile and adult states

$$\Gamma_e(d_e, \theta) = d_e \cdot \Gamma'_e(\theta) + (1 - d_e)/\pi \quad (6)$$

The resulting final expression for the elastin phase stress is given by

$$\mathbf{S}_e = \phi_e \kappa_e d_e \int_{\theta} \Gamma_e(\mu_e, \sigma_e, d_e, \theta) \mathbf{E}_f(\mathbf{E}, \theta) \mathbf{N} \otimes \mathbf{N} d\theta \quad (7)$$

with ϕ_e and Γ'_e taken from experimental measurements (Figs. 1 and 2, respectively) and κ_e and d_e estimated from the biaxial mechanical data, as detailed in the following sections.

2.5 Constitutive Model for Collagen. Following Ref. [28], we treat collagen fibers with a common orientation as a fiber ensemble that undergoes a uniaxial strain $\mathbf{E}_{\text{ens}} = \mathbf{N}^T \mathbf{E} \mathbf{N}$. As in related work on collagenous tissues [28,30], we assume a linear fiber stress strain relationship for individual collagen fibers

$$\Psi_c^f(\mathbf{E}_f) = \frac{\kappa_c}{2} \mathbf{E}_f^2 \quad (8)$$

where κ_c is the elastic modulus of individual straight collagen fibers. Due to their crimped structure, we express individual collagen fiber's true fiber strain using

$$\mathbf{E}_t = \frac{\mathbf{E}_{\text{ens}} - \mathbf{E}_s}{1 + 2\mathbf{E}_s}$$

where $\mathbf{E}_s$ is the collagen fiber slack strain. The resulting *individual* collagen fiber strain energy is then

$$\Psi_c^f(\mathbf{E}_t) = \frac{\kappa_c}{2} \mathbf{E}_t^2 \quad (9)$$

While it is evident that $\mathbf{E}_{\text{ens}} = \mathbf{E}_f$, we make the distinction between fiber and ensemble strains here since individual collagen fibers will have a different strain levels due to their undulations.

Next, we account for the gradual recruitment of the collagen fiber in each fiber ensemble with strain stochastically using the function $D = D(\mathbf{E}_s)$, defined over the ensemble strain range $\mathbf{E}_{\text{ens}} \in [\mathbf{E}_{\text{lb}}, \mathbf{E}_{\text{ub}}]$, where $\mathbf{E}_{\text{lb}}$ and $\mathbf{E}_{\text{ub}}$ represent the lower and upper bounds of collagen fiber ensemble recruitment strain levels, with $\mathbf{E}_{\text{ub}} > \mathbf{E}_{\text{lb}} > 0$. The ensuing fiber ensemble strain energy and stress-strain relation are then described as the sum of individual fiber strain energies of the ensemble weighted by the distribution of slack strains D , as

$$\begin{aligned} \Psi_c^{\text{ens}} &= \frac{\kappa_c}{2} \int_0^{\mathbf{E}_{\text{ens}}} D(x) \left(\frac{\mathbf{E}_{\text{ens}} - x}{1 + 2x} \right)^2 dx \\ \mathbf{S}_c^{\text{ens}} &= \kappa_c \int_0^{\mathbf{E}_{\text{ens}}} D(x) \frac{\mathbf{E}_{\text{ens}} - x}{(2x + 1)^2} dx \end{aligned} \quad (10)$$

where

$$\int_{\mathbf{E}_{\text{lb}}}^{\mathbf{E}_{\text{ub}}} D(x) dx = 1$$

A scaled Beta distribution $B(\alpha, \beta)$ was used for D as follows

$$D(x) = \begin{cases} \frac{x^{\alpha-1}(1-x)^{\beta-1}}{B(\alpha, \beta)(\mathbf{E}_{\text{ub}} - \mathbf{E}_{\text{lb}})}, & \text{for } x \in]0, 1[\\ 0, & \text{otherwise} \end{cases} \quad (11)$$

$$x = (\mathbf{E}_{\text{ens}} - \mathbf{E}_{\text{lb}})/(\mathbf{E}_{\text{ub}} - \mathbf{E}_{\text{lb}})$$

where α and β are the shape constants with mean μ_r and standard deviation σ_r determined using

$$\begin{aligned} \mu'_r &= (\mu_r - \mathbf{E}_{\text{lb}})/(\mathbf{E}_{\text{ub}} - \mathbf{E}_{\text{lb}}) & \sigma'_r &= \sigma_r/(\mathbf{E}_{\text{ub}} - \mathbf{E}_{\text{lb}}) \\ \alpha &= \frac{(\mu'_r)^2 - (\mu'_r)^3 - (\sigma'_r)^2 \mu'_r}{(\sigma'_r)^2} & \beta &= \alpha \frac{1 - \mu'_r}{\mu'_r} \end{aligned} \quad (12)$$

The resulting total collagen fiber phase 2nd Piola-Kirchhoff stress is the sum of individual fiber 2nd Piola-Kirchhoff stress weighted by the angular probability function Γ_c

$$\mathbf{S}_c = \phi_c \kappa_c \int_{\theta} \Gamma_c(\theta) \left[\int_0^{\mathbf{E}_{\text{ens}}} D(\mu_r, \sigma_r, x) \frac{\mathbf{E}_{\text{ens}} - x}{(2x + 1)^2} dx \right] \mathbf{N} \otimes \mathbf{N} d\theta \quad (13)$$

2.6 Complete Constitutive Model Form. Since complete structural information was only available from the medial region, some final modifications were necessary to provide maximum fitting fidelity and to facilitate parameter optimization. As for the treatment for elastin we simulated the measured collagen fiber orientation distribution using

$$\Gamma_c(\mu_c, \sigma_c, d_c) = d_c \cdot \Gamma'_c(\mu_c, \sigma_c) + \frac{(1 - d_c)}{\pi} \quad (14)$$

where the oriented component Γ'_c is modeled using a Beta probability distribution function with mean (μ_c) and standard deviation (σ_c), along with a weighting term (d_c). Note that when applied to the measured medial region data the collagen baseline response given by d_c serves only to improve the model by quantifying randomly oriented collagen fibers observed in the actual measured data (Ref. [20], Fig. 2). Thus, by combining Eqs. (2), (3), (6), and (12), we have the final expression for the total 2nd Piola-Kirchhoff stress $\mathbf{S}$ for the PA wall

$$\begin{aligned} \mathbf{S} &= \phi_c \kappa_c \int_{\theta} \Gamma_c(\mu_c, \sigma_c, d_c, \theta) \left[\int_0^{\mathbf{E}_{\text{ens}}} D(\mu_r, \sigma_r, x) \frac{\mathbf{E}_{\text{ens}} - x}{(2x + 1)^2} dx \right] \\ &\quad \times \mathbf{N} \otimes \mathbf{N} d\theta + \phi_e \kappa_e d_e \int_{\theta} \Gamma_e(\mu_e, \sigma_e, d_e, \theta) \mathbf{E}_f(\mathbf{E}, \theta) \mathbf{N} \otimes \mathbf{N} d\theta \\ &\quad + \phi_m \frac{\kappa_m}{2} (\mathbf{I}_1 - 3) - p \cdot \mathbf{C}^{-1} \end{aligned} \quad (15)$$

The final model has a total of twelve parameters (Table 1).

2.7 Model Parameter Estimation. Direct parameter estimation for such a highly nonlinear model would be ill-advised with respect to uniqueness and confidence of a global minimum. Rather, we developed the following systematic means to obtain optimal parameter values by dividing the total model parameter estimation effort into the determination of the effective fiber properties, followed by the complete in-plane behavior. For the medial region, due to the availability of the requisite data (Table 2), we only had to fit the collagen and elastin moduli and d_e . Moreover, while important regional differences were observed in the measured biomechanical responses [20], the overall qualitative shape of the stress-strain curves were similar. We, thus, utilized both the

Table 1 Constitutive model parameter definitions

Parameter	Description
κ_e	Elastin modulus
μ_e	Mean of the elastin fiber orientation distribution
σ_e	Standard deviation of the elastin fiber orientation distribution.
d_e	Fraction of anisotropic elastin
κ_c	Collagen modulus
μ_c	Mean of the collagen fiber orientation distribution
σ_c	Standard deviation of the collagen fiber orientation distribution.
d_c	Fraction of anisotropic collagen. Preliminary data shows nearly isotropic collagen distribution for the adult posterior region.
μ_r	Mean of the collagen fiber recruitment distribution
σ_r	Standard deviation of the collagen fiber recruitment distribution
$\mathbf{E}_{\text{lb}}$	The lower bound for the collagen fiber recruitment distribution
$\mathbf{E}_{\text{ub}}$	The upper bound for the collagen fiber recruitment distribution

Table 2 Table of the directly measured and estimated parameters. Here, J: juvenile; A: adult; M: measured experimentally or from interpolated EB strain data; and P: Predicted via parameter optimization

Region	Age	Parameters											
		Elastin						Collagen					
		Orientation			Orientation			Recruitment					
		K_e	μ_e	σ_e	d_e	K_c	μ_c	σ_c	d_c	μ_r	σ_r	E_{lb}	E_{ub}
Posterior	J	P	P	P	P	P	P	P	P	P	P	M	P
	A	P	P	P	P	P	P	P	P	P	P	M	P
Anterior	J	P	P	P	P	P	P	P	P	P	P	M	P
	A	P	P	P	P	P	P	P	P	P	P	M	P
Lateral	J	P	P	P	P	P	P	P	P	P	P	M	P
	A	P	P	P	P	P	P	P	P	P	P	M	P
Medial	J	P	M	M	P	P	M	M	M	M	M	M	M
	A	P	M	M	P	P	M	M	M	M	M	M	M

predicted and measured responses of the medial region as initial estimates for the remaining three regions. Details of the fitting approach are provided in the following.

As presented in our work on pericardium [30], when utilizing a structural model, intrinsic *fiber ensemble* responses (i.e., the S_{ens} - E_{ens} relation) can be successfully determined directly from an equi-biaxial strain loading path (that is, $S_{ens} = S_{11} + S_{22}$, $E_{ens} = E_{11} = E_{22}$, $E_{12} = 0$) since there are no fiber rotations in this kinematic mode. This simple method allows one to directly fit fiber moduli and recruitment model parameters independent of fiber splay (and its associated parameters). However, the available mechanical testing data [20] was performed under stress control with no equibiaxial strain data available (Fig. 3(a)). To obtain this data, we interpolated the equibiaxial response from the experimentally acquired multiprotocol data using bicubic Hermite elements to fit the 2nd Piola Kirchhoff stress surfaces. Smoothness of the surface was enforced in the Sobolev-norm [31]. An interpolated protocol where $E_{11} = E_{22}$ from the resulting surface was used to simulate the equi-biaxial response (Fig. 3(a)).

Initial inspections of the S_{ens} - E_{ens} responses revealed a noticeable and consistent increase in stiffness for $S_{ens} \geq 20$ kPa, corresponding to $E_{ens} \approx 0.30$ (Figs. 4 and 5). When compared to the available collagen fiber recruitment data for the medial region (Fig. 3(b)), it was observed that collagen engagement also initiated at $E \approx 0.30$. We, thus, attributed the change in total fiber

ensemble stiffness to the initiation of collagen fiber recruitment, so that E_{lb} could be directly determined from the experimental data for each specimen, removing this parameter from the fitting procedures. Moreover, this finding indicated that for fiber ensemble strains below E_{lb} , (i.e., $E_{ens} \in [0, E_{lb}]$), the S_{ens} - E_{ens} responses were completely dominated by elastin only (i.e., no collagen contribution). By taking the difference between the circumferential (S_{11}) and the longitudinal (S_{22}) components S_{Δ} from Eq. (15), with the collagen phase removed since it is assumed that does not bear load under equi-biaxial strain, we obtain

$$S_{\Delta} = S_{11} - S_{22} = d_e \phi_e K_e \int_0^{\theta} \Gamma'_e(\mu_c, \sigma_c) E_f(\cos^2 \theta - \sin^2 \theta) d\theta \quad (16)$$

following a similar procedure developed in Ref. [32]. The corresponding cost function for fitting the S_{ens} - E_{ens} for $E_{ens} \in [0, E_{lb}]$ is simply

$$SSE = \sum_i (S_{\Delta} - (\hat{S}_{11,i} - \hat{S}_{22,i}))^2 + ((S_{11}^{aniso} + S_{11}^{iso}) - \hat{S}_{11,i})^2 + ((S_{22}^{aniso} + S_{22}^{iso}) - \hat{S}_{22,i})^2 \quad (17)$$

This procedure allowed the parameters d_e and K_e to be estimated directly from the interpolated equi-biaxial data, rather than in the full model.

Next, to further facilitate fitting of the medial region data only, we approximated the measured average collagen fiber recruitment probability distribution from Ref. [20] using Eq. (10) to determine μ_r , σ_r , E_{lb} , and E_{ub} directly (Fig. 3(b)). The resulting parameters were shifted to match E_{lb} as estimated by the equi-biaxial strain interpolation described above on a per specimen basis, while keeping the span of the distribution constant. The respective parameters were then used as constants for the model. For the remaining three regions, E_{lb} was estimated directly from the equi-biaxial data using the distinct increase in tangent modulus (TM) observed when the collagen recruitment initiated as described above. The remaining recruitment parameters from the medial region were used as the initial guess during optimization with E_{lb} fixed, since it was determined directly.

A custom written MATHEMATICA program (Wolfram Research Corp.) was developed and utilized to fit the complete model (Eq. (15)) to the multiprotocol mechanical stress-strain data (Fig. 3(a)). A step-wise approach was used to reduce computational cost and increase the rate of convergence as follows. First, using the parameters obtained from the equibiaxial strain data over the full

Fig. 3 (a) Results of the bicubic Hermite surface interpolation of the 2nd Piola-Kirchhoff stress biaxial test responses to allow interpolation of an equi-biaxial strain path, shown here in red. (b) Fit of the medial region collagen fiber recruitment using a Beta cumulative distribution function for both juvenile and adult states, revealing both an excellent fit to the data along with a trend towards more rapid recruitment with strain in the adult stage.

Fig. 4 Fiber ensemble stress-strain results for the interpolated equi-biaxial strain path responses for all four regions at both age time points

measured strain region, we used three protocols (numbers 3–5; Fig. 3(a)), then five protocols (numbers 2–6; Fig. 3(a)) were fitted sequentially, with the best fit parameter from the prior step used as the initial guess for the current optimization. This approach also improved convergence to the global minimum using a gradi-

ent approach without the need of time-intensive genetic algorithms. The interior point method was chosen for its self-concordant barrier function, allowing us to easily constraint the parameters under the Karush–Kuhn–Tucker conditions. The predictive quality of the fit was examined by comparing the predicted

Fig. 5 Fiber ensemble tangent modulus-strain results for the interpolated equi-biaxial strain path responses for all four regions at both age time points. Note here the sharp increase in stiffness due to collagen fiber engagement (arrows). Also note the decreases in stiffness with age in the lateral and medial regions.

Fig. 6 Using the results of Fig. 5, the collagen engagement strain (E_{lb}) results for all four regions at both age time points. There were in general slightly large values for the anterior and lateral regions, and minimal changes with age.

response to the data for the remaining two protocols (numbers 1 and 7, Fig. 3(a)).

3 Results

3.1 Post-Processed Structural and Mechanical Data. The averaged fiber orientation distribution data of elastin and collagen fibers were well fit by their respective modified Beta probability distribution functions (Eqs. (6) and (14), respectively), with $r^2 > 0.94$ (Fig. 2). Next, Eq. (11) (the modified Beta density function for collagen recruitment) provided an accurate representation of the mean measured collagen recruitment data of juvenile ($r^2 = 0.97$) and adult ($r^2 = 0.98$) specimens, respectively

(Fig. 3(b)). This last result provided important evidence to support the use of Eq. (11) to represent collagen fiber recruitment with ensemble strain for the ovine PA at both the juvenile and adult stages.

The interpolated equi-biaxial responses demonstrated excellent consistency within each region and age group, for both the stress (Fig. 4) and tangent moduli (Fig. 5). In all four regions and two ages, the initiation of collagen recruitment was clearly marked (Fig. 5) at $E_{ens} \sim 0.30$. Consistent with our previous study [20], the anterior and posterior regions demonstrated minimal changes in tangent moduli with age, whereas the lateral and medial regions both showed a decrease in maximum stiffness (Fig. 5). Since no fiber rotations can occur for equi-biaxial strain loading path, the observed decrease in regional tissue stiffness must be due to changes in either fiber moduli or collagen fiber recruitment. Next, from these data the corresponding E_{lb} were obtained as transition points in the tangent modulus-strain relations (Fig. 5), which demonstrated no changes with age and only significant differences between the lateral and medial groups (Fig. 6).

3.2 Overall Fit Results. When applied to the medial region data, the complete model (Eq. (15)) demonstrated an excellent quality of fit to all protocols used ($r^2 \geq 0.99$, protocols 2–6; Fig. 7). Moreover, when applied to measured strains from the “outer” protocols (numbers 1 and 7; Fig. 7), the excellent predictive capabilities were apparent (Fig. 7(b)). Interestingly, no apparent differences in fit quality or values for the k_e and k_c were observed when either the actual measured or interpolated fiber splay data (Fig. 2) were used (Fig. 8). This last result suggested that the β distribution approximation to the fiber splay is sufficient to capture the tissue response. Finally, equivalent quality fits were obtained for the remaining three regions for all mechanical data measured.

Fig. 7 (a) Constitutive model fit to the average five-protocol biaxial stress-stretch data (protocols 2–6, see inset) of juvenile and adult medial PA wall specimens. (b) Predicted fit results to protocols 1 and 7 (see inset), showing excellent agreement.

Fig. 8 Predicted collagen and elastin moduli for the medial region using the actual experimental data and the β distributions. No differences were observed, suggesting the modified Beta distribution for the fiber splay (see Fig. 2) is sufficient to capture the in-plane responses of the ovine PA.

Table 3 List of complete model parameter results (Eq. (12)), with J: juvenile; and A: adult. The collagen (k_c) and elastin (k_e) moduli are in kPa, while all other parameters are unitless

		Parameters												
		Elastin				Collagen								
		Orientation				Orientation				Recruitment				
Age		K _c	μ _c	σ _c	d _c	K _c	μ _c	σ _c	d _c	μ _r	σ _r	E _{lb}	E _{ub}	R ²
Pos	J	185.00	0.06	0.48	0.49	2443.41	0.08	0.34	0.83	0.48	0.12	0.31	0.95	0.995
	A	193.35	0.02	0.44	0.39	2302.88	1.11	0.32	0.58	0.54	0.15	0.31	1.00	0.996
Ant	J	146.78	0.03	0.41	0.45	1584.96	0.18	0.33	0.70	0.52	0.10	0.36	1.00	0.993
	A	170.96	0.21	0.37	0.49	1919.11	0.11	0.36	0.77	0.60	0.15	0.36	1.02	0.994
Lat	J	150.47	−0.06	0.44	0.58	2386.52	0.56	0.39	0.79	0.54	0.12	0.36	1.13	0.995
	A	152.28	−0.07	0.41	0.39	1961.92	0.11	0.27	0.68	0.54	0.14	0.35	1.13	0.987
Med	J	154.61	−0.49	0.61	0.75	2711.86	−0.18	0.29	0.85	0.61	0.20	0.27	1.17	0.948
	A	226.17	0.04	0.47	0.61	746.72	0.13	0.48	0.84	0.52	0.16	0.30	1.12	0.972

Complete parameter results are presented in Table 3 for the summarized mean values for all regions in both age groups, with standard errors key parameters shown in Figs. 9–12 to facilitate presentation of the statistical results.

3.3 Regional Differences. In the juvenile state, key model parameters exhibited differences between all four regions of the PA (Figs. 9–12). For the predicted fiber splay responses, the posterior region had the lowest elastin d_e value of ~ 0.5 , followed by increasing values for the remaining three regions (~ 0.5 to ~ 0.75 ; Fig. 9(a)). In contrast, the collagen d_c values did not appear to exhibit marked regional differences (all about 0.7 to 0.8; Fig. 9(b)). Similar trends were found for the actual dispersion σ_e and σ_c (Fig. 10) with only the medial region exhibiting substantial differences from the other regions, especially for elastin (Fig. 10(a)). It

is also interesting to note that the collagen and elastin fiber splays were neither similar in value nor exhibited similar regional differences, and thus, appear to be structurally decoupled. Collagen fiber recruitment followed similar trends, with the posterior region's mean recruitment being the smallest, followed by increasing values in order of anterior, lateral, and medial regions (Fig. 11(a)). In contrast, the recruitment standard deviation demonstrated that the medial region was larger and thus recruited over a larger strain range (Fig. 11(b)). Regional differences in collagen and elastin moduli in the juvenile state were generally similar, with elastin in the range of 150 to 200 kPa and collagen from ~ 1600 to 2700 kPa (Fig. 12).

3.4 Effects of Age. No major differences were found between the juvenile and adult PA in the posterior, anterior and lateral regions for most model parameters (Table 3). Some differences are found between elastin modulus for the anterior region ($p < 0.035$) (Fig. 11). However, this is only a 14% drop in stiffness than the adult anterior region, which does not produce significant difference in the tissue level biomechanics (Fig. 5). Slight differences are seen in the equi-biaxial strain data for the lateral region. This is due to the mass fraction difference (Fig. 1) and difference in the amount of oriented elastin (Fig. 9), although the difference failed to be statistically significant ($p < 0.11$). For the adult PA, we found the regional differences to be generally similar to the juvenile PA. While some trends were apparent in d_e and d_c , these were not marked (Fig. 9). For the fiber dispersion σ_e and σ_c , only the lateral and medial σ_c demonstrated regional differences in the adult state (Fig. 10(b)). Similar responses were found for the recruitment parameters (Fig. 11). The anterior region had a highest μ_r for the adult largely due to a slight increase with age ($p < 0.12$) and the drop in the medial region with age. No other major regional difference were found in the adult comparing to the juvenile.

The primary finding for age related changes was for the collagen and elastin moduli in the medial region (Fig. 12). While the elastin modulus for this region only modestly increased from ~ 150 kPa to 225 kPa, the collagen modulus dropped from 2711 kPa for the juvenile to slightly more than one quarter of its value at 747 kPa for the adult ($p < 0.022$, Fig. 12). Corresponding histological and structural measurements also demonstrated that: (1) a 47% increase in volume fraction of collagen (Figs. 1(c)) and (2) a significant drop in elastin splay ($p < 0.00002$) a 60% increase in the percentage of collagen splay (Fig. 10). Interestingly as well, both predicted μ_r and σ_r decreased with age in the medial region (Fig. 11), in agreement with the observed more rapid measured collagen fiber recruitment for this region (Fig. 3(b)). Collectively, the modeling results suggest substantial tissue remodeling in the

Fig. 9 Predicted values for (a) elastin (d_e) and (b) collagen (d_c) values for all four regions at both age time points. Here the medial region had the large values for d_e (statistically different from the anterior and posterior regions), whereas d_c exhibited no statistically significant regional or age differences.

Fig. 10 Predicted fiber splay deviation parameter σ for (a) elastin (σ_e) and (b) collagen (σ_c) fibers for all four regions at both age time points. While some modest differences with region and age occurred, the main finding was the medial region's drop in σ_e and increase in σ_c with age. Values presented as radians, and in degrees these changes are σ_c : juvenile = 17 deg to adult = 28 deg, σ_e : juvenile = 35 deg to adult = 27 deg.

medial region with age, whereas the other three measured regions were largely stable with age.

4 Discussion

4.1 Overall Findings. Overall, the predicted elastin moduli remained largely unchanged with age and compared well between the four regions studied, lying within a relatively narrow range of 150 to 200 kPa. Similarly, the predicted collagen moduli ranged from 1600 to 2700 kPa in the four regions studied in the juvenile state. However, major age effects were found in the medial aspect of the PA wall (Fig. 1). In this region, we observed that the elastin and collagen fiber splay underwent opposite changes, with the collagen standard deviation juvenile = 17 deg to adult = 28 deg, elastin standard deviation juvenile = 35 deg to adult = 27 deg (Fig. 10). In parallel, we observed more rapid collagen fiber strain recruitment with age similar to that observed experimentally [20], but these were not statistically significant (Fig. 11).

Clearly, the most profound changes in this region were observed for the collagen fiber moduli, which went from 2700 kPa

for the juvenile stage to 746 kPa in the adult (Fig. 12). These changes were likely due to the impingement of relatively stiff ascending aorta on the growing PA medial region, and were apparently a local effect. Intuitively, the effects of the local impingement would be to lower the local wall stress, consistent with the observed decrease in collagen modulus. While this change was in part driven by the observed increase in total tissue elastin volume fraction relative to the collagen volume fraction (Fig. 1(c)), these changes were also a direct result of the changes in local tissue structure.

The results of the present study extend these studies by utilizing a detailed meso-scale (i.e., bulk fiber level) structural model, coupled with extensive experimental measures, to obtain further insight into the PA remodeling process. This approach was taken since altered mechanical properties of arteries are critically associated with microstructural remodeling [33,34]. The mechanical loading-deformation relation of elastin and collagen fibers is fundamental to understanding the underlying microstructural mechanisms of arterial tissue behavior. Structurally motivated models, such as used herein, incorporate significant mechanical aspects of the underlying microstructure to better predict the mechanical

Fig. 11 Predicted collagen fiber recruitment for (a) mean (μ_r) and (b) standard deviation (σ_r) for all four regions at both age time points. The main observed changes were found for σ_r , which demonstrated decreases with age in the anterior, posterior, and lateral regions, but an increase in the medial region.

behavior and understand the mechanisms governing the structure-function relationship of biological tissues. The present constitutive model takes account of contributions of the collagen component, elastin or elastic fibers, ground matrix consisting of smooth muscle cells and other noncellular materials, along with detailed in-plane biaxial mechanical behaviors [20] (Figs. 1–6).

As observed in our geometric studies [18,19], the focal changes observed in the medial region are likely due to the impingement of relatively stiff ascending aorta on the growing PA. This effect is likely more pronounced since these two great vessels are held together within a connective tissue sheath (J. E. Mayer, Jr., private communication). Intuitively, the effects of the local impingement would be to lower the local wall stress. If so, then this would be consistent with the observed decrease in collagen modulus. However, it does not explain the concomitant increase in elastin modulus. Clearly, some type of compensatory mechanism is in play in here, but the nature of which remains unknown. What can be said with some certainty is that, during the postnatal somatic growth, local stresses can substantially modulate the development of regional tissue microstructure and mechanical behaviors in the PA.

In our parallel study on the corresponding mechanical properties [20] the medial and lateral locations experienced local, moderate increases in anisotropy. Moreover, the PA thickness remained constant with growth. When this fact is combined with the observed stable overall mechanical behavior and increase in vessel diameter with growth, a simple Laplace Law wall stress estimate suggests an increase in effective PA wall stress with postnatal maturation. This observation is contrary to the accepted theory of maintenance of homeostatic stress levels in the regulation of vascular function, and suggests alternative mechanisms regulate postnatal somatic growth.

4.2 Relation to Other Studies: The Role of Elastin. While there are no similar studies on postnatal somatic growth, perhaps the most closely related studies are associated with hypertension and other arterial diseases. The increased rate of collagen and

elastin synthesis in blood vessels in connection with both systemic and pulmonary hypertension has been revealed by many biological studies. Structural and degradative alterations of medial elastin is found to be a major contributing factor in physiological phenomena such as aging, and the initiation and development of cardiovascular disease, such as aortic aneurysms [16,17]. Although elastin network plays a significant role in modifying the mechanical behavior of blood vessels, the mechanical properties of elastin are not fully determined, and studies connecting microstructural changes with elastin mechanics are scarce.

The absence of similar crimp or undulation architecture to that of the collagen fibers in the elastin network of the arterial wall and substantially smaller elastic modulus of elastin fiber means that elastin can become readily load bearing as the arterial wall starts passively extending in the in situ configuration or under the in vitro planar biaxial tensile testing condition. In the current study we observed a linear S-E behavior. Although elastin network plays a significant role in modifying the mechanical behavior of blood vessels, the mechanical properties of elastin are not fully determined. Arteries are generally considered anisotropic, and this property is usually attributed to collagen fibers while the elastin contribution has been represented by the isotropic Neo-Hookean model [35,36]. Lillie and coworkers [37] performed uniaxial tests on purified aortic elastin and demonstrated that the elastic tissue possessed an inherent anisotropy, with the circumferential stiffness 1.4 times the axial. Their results agreed with values obtained in uniaxial tests of alkali-purified elastic tissue from canine and ovine aortas [38]. They also demonstrated that the elastic tissue behavior was nonlinear in uniaxial tests. Ogden and Saccomandi [39] proposed using a model to account for the nonlinear isotropic contribution of the elastin network. The study of biaxial tensile behavior of isolated elastin networks of bovine thoracic aorta by Zou and Zhang in 2009 [40] revealed significant mechanical characteristics of arterial elastin. Their experimental results consistently indicated that elastin network possessed strong anisotropy that was comparable to the intact arteries, with the circumferential direction being stiffer than the longitudinal direction. Based on an entropy-based non-Gaussian affine statistical model of a network of randomly oriented molecular chains [41], they determined that change in elastin fiber orientation leads to markedly different stress-strain response. A recent study by Qi and colleagues [26] assumed an oriented single fiber phase superposed on an isotropic phase for the PA. In the current study, it was demonstrated that elastin substantially contributes to the mechanical behavior of the arterial wall. When taken with the present results, it appears that any constitutive model of the arterial wall cannot determine the structure-function relationship of arterial wall without taking into account the anisotropic contribution of the elastin network, regardless of the specific approach taken.

In order to gain insight into the possible physiological modifications of elastin structural properties with growth and the prominent role of elastin in the alteration of pulmonary artery mechanics in hypertension [15], more structurally relevant elastin models need to be developed. The elastin and collagen fibers are closely associated with each other in the media and are organized into concentric rings of lamella units around the arterial lumen with smooth muscle cells lying between these lamellae [42]. In order to elucidate load-bearing mechanism of elastin and collagen constituents of the arterial wall, the effect of the cross-linking of elastin fibers with each other and their interaction with collagen fibers has to be included into the constitutive model. Thus, future models will also have to incorporate collagen-elastin interactions. We also noted the presence of residual stresses in our studies, so that the potential role of GAGs as a mechanism for residual stress must be accounted for in future studies [43].

4.3 Limitations. To obtain the collagen and elastin structures in the unloaded state, we focused our MPM measurements on thin tissue sections to avoid depth penetration issues. Furthermore, all

Fig. 12 Predicted (a) collagen (κ_c) and (b) elastin (κ_e) fiber moduli regions at both age time points. The most drastic changes were for the elastin modulus κ_e increasing by $\sim 50\%$ and the collagen modulus κ_c decreasing to only $\sim 25\%$ of the juvenile value in the medial region.

elastin and collagen multiphoton images were compared with the conventional histological stains in adjacent tissue slices of the same sample. Specifically, Masson's trichrome stain was used for collagen and Verhoeff-Van Gieson stain for elastin. Note too that the Verhoeff-Van Gieson stain for elastin also allowed clear identification of arterial layer structure (Fig. 1). Different arterial layers were identified and their thicknesses manually measured using ImageJ software. The measured volume fractions utilizing the two methods were very similar, supporting the assumption that the measured MPM data accurately captured the requisite structural data.

The direct measurement of the collagen recruitment behavior in Ref. [20] suggest that the collagen fibers are not fully recruited within the normal physiological deformation region of the PA wall based on the obtained biaxial stress-stretch data ($P_{\max} \sim 35$ kPa, Fig 7). In order to correctly characterize the collagen fiber properties, the artery has to be loaded above the physiological loading level. In the study by Hill et al. [44], through the combined uniaxial mechanical testing-multiphoton microscopy system, it was revealed that collagen contribution becomes dominant after about 500 kPa stress in the circumferential (or preferred) direction in the rabbit carotid artery. Thus, one probably cannot determine the effective collagen modulus from fitting of the model to the current biaxial data to obtain completely reliable estimate of the collagen fiber properties. Therefore, to account for the nonlinear anisotropic behavior of the PA wall in the juvenile and adult growth stages, it was necessary to separate out the contribution of the elastin network from the relatively linear isotropic response of the ground matrix. It was also observed that when the

recruitment function parameters were set as variables in the optimization process, the faster collagen recruitment resulted in smaller collagen effective modulus values and vice versa. Therefore, the direct measurement of collagen recruitment behavior is necessary to prevent this covariance between the collagen model parameters skewing the determination of collagen fiber property through the constitutive model. Finally, we note that constitutive models are required to describe the mechanical behavior of each layer of the arterial wall and gain insights into the mechanisms behind the mechanical response of the arterial wall. In the present study we homogenized the PA wall as a single layer. Clearly, more detailed layer studies are necessary to delineate layer specific changes.

With respect to parameter estimation, we were able break up the total model parameter estimation effort into the effective fiber properties part, followed by the entire tissue. This approach avoided having to fit the entire dataset at once with its concomitant parameter estimation problems. Moreover, once the effective fiber model parameters were obtained, we used them along with the measured splay information and all biaxial mechanical data protocols to obtain a fit to the complete model. It is important to note that for the medial region we only had to fit the collagen and elastin moduli and the ratio of unmeasured anisotropy elastin (i.e., only three parameters which are necessarily uniquely determined by the post transition stiffness, the lower strain region stiffness and anisotropy), which is a significant advantage of our approach. This methodical approach allowed us to develop a parameter set that fit the data without the ambiguities inherent in those methods that only use the mechanical data (including the associated local

minimum issues). We note too that we were able to use the medial region parameters as initial estimates to the other three regions (which had less complete data), with the assumption that regional variations, while present, were relatively modest and that all material parameters were qualitatively similar. In addition, our choice of algorithm guarantees that the local gradients are necessarily small enough that the difference between current iteration and the previous is very small (0.1 to 0.01 of the current sum of squared error). This is substantiated by the fact that our r^2 values were consistently very good ($r^2 > 0.95$; Table 3). Furthermore, our parameters are constrained based on experimental data (i.e., the mean of the recruitment distributions (μ_r) is unlikely to be greater than 1.0 when it was measured to be approximately 0.6 or less). We found no cases for which the parameters approached the constraints (with exception to when the collagen splay has no baseline isotropic response). It is, thus, reasonable to assume that that minimum is a global minimum or at least the minimum of interest (i.e., the realistic or even physically possible one). Also, given that we are using structural parameters, the similarity and agreement with the structural measurements in the medial region further corroborates with our assertion. Lastly, the structural parameters are the minimum number required to parameterize measureable structural quantities of the tissue (e.g., fiber splay and recruitment), given that such measurements are necessarily unique, it is mostly like that the solution to the optimization problem is also unique in our range of interest. Collectively, we believe this was an accurate approach and the resultant parameters accurate.

4.4 Conclusions. The present study extends our previous investigations into postnatal PA growth. As observed in our geometric studies [18,19], the focal changes in the medial region are likely due to the impingement of relatively stiff ascending aorta on the growing PA. Intuitively, the effects of the local impingement would be to lower the local wall stress. If so, then this would be consistent with the observed decrease in collagen modulus. However, it does not explain the concomitant increase in elastin modulus. Clearly, some type of compensatory mechanism is in play, but the nature of which remains unknown. What can be said with some certainty is that, during the postnatal somatic growth, local stresses can substantially modulate the development of regional tissue microstructure and mechanical behavior in the PA. However, this finding should be taken in light that we observed relatively stable overall mechanical behavior over most of the PA with growth. As the PA pressure and thickness remained constant with growth, a simple Laplace Law wall stress estimate suggests an increase changes in effective PA wall stress with by 40% [18] with postnatal maturation. This observation is contrary to the accepted theory of maintenance of homeostatic stress in the regulation of vascular function, and suggests alternative mechanisms regulate postnatal somatic growth. Understanding the underlying mechanisms, including incorporating important structural features during growth, will help to improve our understanding of congenital defects of the PA and lay the basis for functional duplication in their repair and replacement.

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