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Disparate Changes in the Mechanical Properties of Murine Carotid Arteries and Aorta in Response to Chronic Infusion of Angiotensin-II

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Abstract

Chronic infusion of angiotensin-II has proved useful for generating dissecting aortic aneurysms in atheroprone mice. These lesions preferentially form in the suprarenal abdominal aorta and sometimes in the ascending aorta, but reasons for such localization remain unknown. This study focused on why these lesions do not form in other large (central) arteries. Toward this end, we quantified and compared the geometry, composition, and biaxial material behavior (using a nonlinear constitutive relation) of common carotid arteries from three groups of mice: non-treated controls as well as mice receiving a subcutaneous infusion of angiotensin-II for 28 days that either did or did not lead to the development of a dissecting aortic aneurysm. Consistent with the mild hypertension induced by the angiotensin-II, the carotid wall thickened as expected and remodeled modestly. There was no evidence, however, of a marked loss of elastic fibers or smooth muscle cells, each of which appear to be initiating events for the development of aneurysms, and there was no evidence of intramural discontinuities that might give rise to dissections.

Keywords

stiffness; stress-strain; mechanics; constitutive properties

Introduction

Angiotensin-II (Ang-II) is a potent vasopressor having pleiotropic activity. Systemic increases of Ang-II can cause vasoconstriction throughout the arterial tree, and thus increased blood pressure, whereas local increases of Ang-II within the arterial wall can lead

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to an increased production of diverse cytokines and chemokines, and thus significant localized remodeling of the wall. For example, local increases in intramural Ang-II in response to elevated wall stress in hypertension can lead to an increased production of the chemokine monocyte chemoattractant protein-1 (Eberth et al., 2010), which recruits monocytes that differentiate into macrophages that produce cytokines and matrix metalloproteinases (Braiser et al., 2002; Higuchi et al., 2007; Mehta and Griendling, 2007). Together, the different cytokines and proteinases can induce a significant turnover of cells and extracellular matrix, thus leading to changes in wall geometry, composition, and mechanical behavior.

During the past decade, chronic infusion of Ang-II has been used both to accelerate the development of atherosclerotic plaques and to generate dissecting aortic aneurysms in mice prone to atherosclerosis (e.g., the apolipoprotein-E null, or ApoE^{-/-}, mouse). See, for example, Cha et al. (2010) and Daugherty et al. (2011), respectively. Of particular interest herein, however, is the observation that the resulting aortic aneurysms tend to occur preferentially at two locations – primarily in the suprarenal aorta, but sometimes in the ascending aorta (Daugherty et al., 2010; Daugherty et al., 2011). Consistent with the aforementioned turnover of cells and extracellular matrix, these aneurysms are characterized by dramatic localized changes in the geometry, composition, and material properties of the aortic wall. Although mechanics and mechanobiology play fundamental roles in the natural history of both atherosclerosis and aneurysms, there has been surprisingly little attention directed toward the mechanical properties of either the evolving atherosclerotic plaques or the aortic aneurysms. Regarding the latter, Danpinid et al. (2010) combined catheter-based pressure measurements and ultrasound-based geometric measurements to infer in vivo a Young's modulus for the dilating abdominal aorta. Georgen et al. (2011) used magnetic resonance imaging to infer in vivo the changes in cyclic wall strain over the cardiac cycle, which provide some insight into changes in the underlying structural stiffness of the wall. In contrast, Genovese et al. (2012) used a panoramic digital image correlation method to assess full field surface strains in pressure-distended excised abdominal aneurysms, which similarly reflected underlying increases in stiffness.

Although there is a continuing need for studies of the mechanics of atherosclerotic plaques and aneurysms that develop in response to the chronic infusion of Ang-II in mice, the goal of the present paper is different. Here, we quantify possible changes in the geometry, composition, and biaxial material behavior of common carotid arteries in Ang-II infused ApoE^{-/-} mice. Both the aorta and the common carotid arteries are classified as central (elastic) arteries, and they share many histological and cell biological features – a similarly dominant media consisting of musculo-elastic fascicles that are defined by alternating layers of elastic fibers and smooth muscle cells plus a thinner primarily collagenous adventitia. Yet, common carotid arteries seldom dissect or develop aneurysms (cf. Stevic et al., 2011) and it is of interest to quantify their response to insults that otherwise tend to promote such lesions.

Methods

Animal Model and Specimen Preparation

All animal use protocols conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23) and were approved by the Texas A&M University Institutional Animal Care and Use Committee. Specifically, male apolipoprotein null mice (ApoE^{-/-}) were maintained on a normal diet throughout the study¹. At 8 weeks of age, the mice were anesthetized with 1.5 – 2.0% isoflurane gas and an Alzet mini-osmotic pump (Durect Corp., CA) was implanted subcutaneously in the mid-scapular region. These pumps delivered Ang-II continuously at 1000 ng/kg/min for 28 days (cf. Barisione et al., 2006), after which the mice were euthanized with an intraperitoneal injection of sodium pentobarbital to allow excision of the common carotid arteries and the suprarenal abdominal aorta. Employing methods established previously in our laboratory (cf. Dye et al., 2007; Eberth et al., 2009a; Collins et al., 2011; Ferruzzi et al., 2011), the excised arteries were gently cleaned of excess perivascular tissue, cannulated on custom glass pipettes, and secured on the pipettes using 6-0 suture.

Biaxial Testing²

Cannulated carotid arteries were placed in a custom testing system (Gleason et al., 2004) within a phosphate-buffered Hanks solution and maintained at 37°C; a previous study showed that the Hanks solution renders the behavior passive (Ferruzzi et al., 2011), thus negating the need to consider contributions by active smooth muscle cells. Briefly, the glass pipettes were then connected to stages that are controlled by two stepper motors that move in opposing directions. Hence, the arteries could be cyclically pressurized while held at prescribed axial stretches or they could be cyclically extended at constant pressures, both while keeping the center of the specimen at the same location. Pressurization was accomplished by connecting the glass pipettes to a computer-controlled pump via stiff tubing.

All specimens were subjected to two cycles each of pressure-diameter protocols ($P = 10$ to 140 mmHg) at three different axial extensions (at and $\pm 5\%$ of the estimated in vivo axial stretch ratio λ_z^{iv}) and two cycles of axial force-length protocols ($f = 0$ to 9.8 mN) at three different pressures (60, 100, and 140 mmHg), each following three initial cycles that served to precondition the specimen. Luminal pressure, outer diameter in the central region, axial force, and overall axial extension were measured on-line using a custom LabView program, appropriate transducers, and a video-microscope (further details on the experimental system can be found in Gleason et al., 2004). Unloaded dimensions were measured interactively by identifying the length at which the vessel began to bend at zero pressure; circumferential and axial stretches were calculated relative to these dimensions. Note, too, that wall volume remains nearly constant during transient motions (i.e., without growth and remodeling), hence the deformed inner radius a and wall thickness h were calculated, at each applied

¹High fat diets are often used to accelerate the development of atherosclerosis in ApoE^{-/-} mice, but our goal was to determine changes in wall properties independent of atherosclerosis.

²Our results for the suprarenal aorta are found elsewhere (Collins et al., 2011; Genovese et al., 2012).

pressure and axial load, from on-line measurements of outer radius b and axial length ℓ via the incompressibility constraint (Gleason et al., 2004), namely

$$a = \sqrt{b^2 - \bar{V}/(\pi\ell)}, \quad h = b - a \quad (1,2)$$

where $\bar{V}$ is the mean wall volume, which was estimated based on 12 measurements of inner and outer radius at multiple static pressures and lengths. These measurements were facilitated by a pre-programmed, computer-controlled routine, which increased consistency from specimen to specimen.

Assuming a 2-D state of stress within the central region of the thin-walled biaxially tested arteries, the deformation gradient tensor has the form $\mathbf{F} = \text{diag}(\lambda_r, \lambda_\theta, \lambda_z)$. The axial and circumferential stretch ratios were calculated via

$$\lambda_z = \frac{\ell}{L}, \quad \lambda_\theta = \frac{r_{mid}}{R_{mid}}, \quad (3,4)$$

where ℓ (and L) and r_{mid} (and R_{mid}) represent loaded (and unloaded) axial length and mid-wall radius, respectively, with $r_{mid} = a + h/2$ and similarly for R_{mid} . Incompressibility requires $\lambda_r = 1/\lambda_z\lambda_\theta$. The experimentally inferred mean Cauchy stresses, in axial and circumferential directions, are given by

$$t_{zz}^{\text{exp}} = \frac{f_T^{\text{exp}} + \pi a^2 P^{\text{exp}}}{\pi h(2a+h)}, \quad t_{\theta\theta}^{\text{exp}} = \frac{P^{\text{exp}} a}{h}, \quad (5,6)$$

where f_T^{exp} is measured by the force transducer and P^{exp} is measured by pressure transducers placed distal and proximal to the specimen, which enables the intraluminal pressure to be well estimated despite significant pressure drops across the glass cannulae (Gleason et al., 2004). Equations 5 and 6 are obtained from equilibrium assuming the artery deforms axisymmetrically under axial extension and distension. The presence of residual stresses in arteries renders these results for mean stress particularly meaningful, except perhaps during periods of rapid growth and remodeling (Humphrey, 2002); note, too, that the thin-walled approximation can also be argued for mouse carotid arteries, which consist of 3 to 4 smooth muscle layers that would not be expected to exhibit marked radial gradients in phenotypic responses.

Constitutive Modeling

We employed a “four fiber family” hyperelastic constitutive model to quantify the measured passive biaxial mechanical behaviors. This model has proven useful in capturing biaxial mechanical responses of diverse mouse arteries (Gleason et al., 2008; Eberth et al., 2009a; Wan et al., 2010; Collins et al., 2011; Eberth et al., 2011; Ferruzzi et al., 2011). The specific form of the strain energy function is

$$W(\mathbf{C}, \mathbf{M}^i) = \frac{c}{2}(I_c - 3) + \sum_{i=1}^4 \frac{c_1^i}{4c_2^i} \left\{ \exp \left[c_2^i (I_4^i - 1)^2 \right] - 1 \right\}, \quad (7)$$

where c, c_1^i are material parameters having units of stress and c_2^i are dimensionless, $I_C = \text{tr} \mathbf{C}$ is the first invariant of the right Cauchy-Green tensor, and $I_4^i = \mathbf{M}^i \cdot \mathbf{C} \mathbf{M}^i$ is the square of the stretch of the i -th fiber family (i.e., $(\lambda^i)^2$). Fiber orientations are defined in the unloaded reference configuration by unit vectors $\mathbf{M}^i$ that depend on angles α_{θ}^i defined between fiber and axial directions. Axial and circumferential fibers were thus defined at $\alpha_{\theta}^1=0$ and $\alpha_{\theta}^2=90$ degrees, respectively, while symmetrically oriented diagonal fibers were accounted for via a single parameter, $\alpha_{\theta}^3 = -\alpha_{\theta}^4 = \alpha_{\theta}$. Note that $I_4^{i=1} = (\lambda^{i=1})^2 = \lambda_z^2$, $I_4^{i=2} = (\lambda^{i=2})^2 = \lambda_{\theta}^2$, and

$$I_4^d = (\lambda^{i=3,4})^2 = \lambda_z^2 \cos^2 \alpha_0 + \lambda_{\theta}^2 \sin^2 \alpha_0, \quad (8)$$

where the superscript d denotes “diagonal”. The two diagonal fiber families are typically regarded as mechanically equivalent, hence $c_1^3 = c_1^4 \equiv c_1^{3,4}$, $c_2^3 = c_2^4 \equiv c_2^{3,4}$. The overall constitutive model thus requires eight unknown parameters ($c, c_1^1, c_1^2, c_1^3, c_1^4, c_2^1, c_2^2, c_2^{3,4}, \alpha_0$) to be estimated to describe the passive mechanical behavior.

Consistent with the aforementioned 2-D assumption, that is, a plane state of stress ($t_{rr} = 0$), equation (5) yields theoretically calculated Cauchy stresses of the form

$$t_{zz}^{th} = c \left(\lambda_z^2 - \frac{1}{\lambda_{\theta}^2 \lambda_z^2} \right) + c_1^1 (\lambda_z^2 - 1) \exp \left[c_2^1 (\lambda_z^2 - 1)^2 \right] \lambda_z^2 + 2c_1^{3,4} (I_4^d - 1) \exp \left[c_2^{3,4} (I_4^d - 1)^2 \right] \lambda_z^2 \cos^2 \alpha_0, \quad (9)$$

$$t_{\theta\theta}^{th} = c \left(\lambda_{\theta}^2 - \frac{1}{\lambda_z^2 \lambda_{\theta}^2} \right) + c_1^2 (\lambda_{\theta}^2 - 1) \exp \left[c_2^2 (\lambda_{\theta}^2 - 1)^2 \right] \lambda_{\theta}^2 + 2c_1^{3,4} (I_4^d - 1) \exp \left[c_2^{3,4} (I_4^d - 1)^2 \right] \lambda_{\theta}^2 \sin^2 \alpha_0, \quad (10)$$

which, in turn, enable a straightforward regression analysis of the biaxial data.

Parameter Estimation

Best-fit values of the eight unknown model parameters $c, c_1^1, c_1^2, c_1^3, c_1^4, c_2^1, c_2^2, c_2^{3,4}, \alpha_0$ in equations (9) and (10) were determined using a nonlinear least squares minimization of the error e between the theoretically predicted (th) and experimentally inferred (exp) applied loads, namely

$$e = \sum_{j=1}^N \left[\left(\frac{P^{th} - P^{exp}}{\overline{P}^{exp}} \right)^2_j + \left(\frac{f^{th} - f^{exp}}{\overline{f}^{exp}} \right)^2_j \right], \quad (11)$$

where N is the total number of data points (i.e., equilibrium configurations) and the overbar denotes values averaged over the entire data set (cf. Wan et al., 2010). Theoretical values of

axial force and pressure were calculated from theoretical Cauchy stresses in equations (9) and (10) using the relations in equations (5) and (6). Hence, f^h and P^h represent nonlinear functions depending on measured values of λ_z , λ_θ , a , and h , as well as the eight unknown parameters. The error e was minimized using the built-in function *lsqnonlin* in MATLAB , subject to physical constraints that $c, c_1^i, c_2^i \geq 0$ and $0 \leq \alpha_0 \leq \pi/2$ due to the symmetry of the diagonal fibers. Finally, the goodness-of-fit was estimated via the root mean square of the fitting error, defined as

$$RMSE = \sqrt{\frac{e}{N}}, \quad (12)$$

Histology

At the completion of mechanical testing, all vessels were fixed in 4% formalin in an unloaded state for 1 hour and subsequently stored at room temperature in 70% ethanol prior to embedding in paraffin and sectioning. Embedded samples were then sectioned at 5 microns and stained with either Verhoeff van Gieson (VVG), to identify elastin, or Masson's Trichrome (MTC), to identify collagen and cellular contents (cytoplasm). The VVG and MTC stained slides were imaged under normal light using an Olympus BX/51 microscope and associated Olympus DP70 digital camera. Analysis of the digital images included an initial global thresholding to isolate the stained cross-section and to washout the background to a uniform white value. Images were then passed through a custom MATLAB script to convert the original red, green, blue (RGB) images to the hue, saturation, lightness (HSL) color space to enable additional pixel-specific thresholding to delineate individual constituents of interest (i.e., elastin, smooth muscle, and collagen). This color space transformation is useful for it reduces the number of parameters needed to isolate specific color ranges from 3 in the Cartesian RGB space to 1 in the cylindrical HSL space. Thus, the VVG-stained sections revealed elastic fibers, primarily consisting of elastin, in black ($H = 0^\circ - 360^\circ$, $S = 0.0 - 1.0$, $L = 0.0 - 0.22$) and other tissues such as collagen, smooth muscle, and glycosaminoglycans as red-pink ($H = 300^\circ - 17^\circ$, $S = 0.01 - 1.0$, $L = 0.1 - 0.9$) (Eberth et al., 2009b). Similarly, the MTC-stained slides show fibrous tissue, consisting of primarily fibrillar collagen and associated glycoproteins, in blue ($H = 150^\circ - 260^\circ$, $S = 0.12 - 1.0$, $L = 0.14 - 1.0$) and cytoplasm, localized primarily to medial smooth muscle cells, in red ($H = 275^\circ - 10^\circ$, $S = 0.13 - 1.0$, $L = 0.14 - 1.0$).

Associated area fractions for elastin (ϕ^e), smooth muscle (ϕ^m), and collagen (ϕ^c) were calculated based on the total number of pixels that satisfied the HSL thresholding requirements for each constituent, divided by the total number of pixels identified as having any HSL parameter values other than those for the color white ($H = 360^\circ$, $S = 0.0-1.0$, $L = 1$). See Figure 1 for an illustrative example. Taking advantage of the normalized lightness parameter in the HSL color space (i.e., $L = 0$ is black and $L = 1$ is white), the average lightness value for all identified constituent pixels in a given image was also computed and interpreted as a qualitative measure of constituent density. For example, average lightness values closer to 0 qualitatively imply a denser organization of the constituent of interest.

Statistical Analysis

All data are presented as mean $\pm$ standard deviation and were evaluated using a two-tailed Student's *t*-test relative to the untreated control. Specifically, differences in unloaded length, unloaded outer diameter, and in-vivo stretch before and after Ang-II treatment were evaluated using unpaired *t*-tests while assuming unequal variances. A value $p < 0.05$ was considered significant.

Results

Table 1 lists the age, body mass, unloaded outer diameter, wall thickness, and axial length as well as the in vivo axial stretch λ_z^{iv} and inner diameter and wall thickness at near in-vivo conditions (i.e., at the in vivo stretch and pressure of 80 mmHg) for three groups: Group 1 – untreated controls ($n = 5$), Group 2 – Ang-II infused animals that did not develop a suprarenal aortic aneurysm ($n = 5$), and Group 3 – Ang-II infused animals that developed a suprarenal aortic aneurysm ($n = 4$), that is, those developing an aortic dilatation greater than 1.5x the normal diameter. There was a trend, though not statistical significance, toward an increase in the in vivo inner radius and wall thickness and a decrease in the in vivo axial stretch in the Ang-II infused mice (Groups 2 and 3), with the latter values differing the most between Group 1 and Group 3 (Figure 2). Table 2 lists best-fit model parameters for the constitutive relation (equation 7) whereas Figure 3 shows that these best-fit values provided reasonable fits to the biaxial data from all three Groups and multiple protocols. More importantly, Figure 4 shows that these values of the parameters provided good predictions of the biaxial stress – stretch behavior, which were not fit directly in the regression (cf. equation 11). Note from Table 2 that, on average, there was a tendency for the elastin-related parameter c to decrease slightly from Groups 1 to 3 and conversely a tendency for the dimensioned collagen-related parameters ($c_1^i, i=1, 2, 3, 4$) to increase slightly from Groups 1 to 3. Notwithstanding the good predictions of the biaxial stress – stretch behaviors and the modest trends in the parameter values, it is difficult to assess Group-to-Group differences in overall passive material properties based on tabulated values of eight best-fit model parameters. Figure 5 thus shows calculated mean values of strain energy W stored at the Group-specific in vivo axial stretches (Table 1) for multiple values of pressure (from 60 to 120 mmHg). Note that error bars at each pressure value have been omitted for clarity. As it can be seen, differences amongst the three groups were modest, that is, not statistically significant, which suggests that either the 4-week Ang-II infusion did not cause significant remodeling of the common carotid arteries or that remodeling maintained or restored overall mechanical properties.

Histological sections from the three groups confirmed the modest increase in wall thickness due to Ang-II infusion (cf. Table 1), but revealed further that this increase resulted in large part from an increase in medial thickness (Figure 6). Indeed, there also appeared to be an increased inter-lamellar spacing in the media of the Ang-II infused mice that accommodated both an increase in smooth muscle and collagen. There was no observed change in the measured area fraction of smooth muscle across the three groups (Table 3), however, probably because the Ang-II treated vessels also experienced an increase in the overall cross-sectional area (i.e., wall thickness and outer diameter; Table 1). On average, Groups 2

and 3 exhibited increases in the total cross sectional area of 21.12% and 33.75%, respectively, as compared to the measured cross-sectional area for Group 1. There was also an unexpected increase in the elastin area fraction in the Ang-II treated vessels (Table 3). Figure 7 compares higher magnification images of vessels from Group 1 and Group 3 and suggests that although the elastic lamellae were thicker in Ang-II treated vessels, they may have contained localized regions with slight discontinuities in staining (see the arrows). Exploiting properties of the HSL color space, average lightness values were thus calculated for each constituent (Table 4). Qualitatively, this value can be interpreted as a measure of constituent density, with values closer to 0 suggesting denser material. Table 4 reveals higher values of the lightness parameter for elastin in Groups 2 and 3 compared with Group 1, which implied a less dense packing of elastin following Ang-II infusion. Such a change in the packing of elastin could explain the computed increase in elastin area fraction and observed increase in lamellar thicknesses in Group 3. Although gross histological staining cannot elucidate specific mechanisms underlying localized decreases in elastic fiber density, images such as those in Figure 7 suggested that there may have been localized fraying of the lamellae. Whereas fraying could be an early precursor to fragmentation, as would be expected in aneurysms, there was no evidence of fragmented elastic fibers. Indeed, the increases in smooth muscle and collagen content appeared to result from adaptive growth and remodeling without any evidence of intramural defects (material discontinuities) due to the Ang-II infusion.

Discussion

Chronic subcutaneous delivery of Ang-II to ApoE^{-/-} mice has become a common procedure for studying the development and progression of dissecting aortic aneurysms (e.g., Saraff et al., 2003; Barisone et al., 2006; Cao et al., 2010; Danpinid et al., 2010; Trachet et al., 2011; Daugherty et al., 2011) and similarly for hastening the development of atherosclerosis in ApoE^{-/-} mice fed a high fat diet (Weiss et al., 2001; Choudhury et al., 2003; Zhou et al., 2005; Cha et al., 2010). Although the aneurysms appear to develop in response to the Ang-II independent of increases in blood pressure (Cassis et al., 2009), the typical levels of Ang-II infusion raise the systolic blood pressure from 18 to 54% (Deng et al., 2003; Saraf et al., 2003; Zhou et al., 2005; Rateri et al., 2011). Humphrey (2008) previously observed that an extensive literature on vascular remodeling suggests that changes in arterial caliber and wall thickness in response to modest, sustained changes in mean blood flow and pressure appear to restore mean wall shear (τ_w) and circumferential (σ_θ) stresses toward original (o) values. Invoking this hypothesis, one can predict expected geometric changes based on fold-changes in flow (parameterized by ε) and pressure (parameterized by γ), namely

$$\text{If } \tau_w = \frac{4\mu(\varepsilon Q_o)}{\pi a^3} \text{ (perturbed) and } \tau_w^o = \frac{4\mu Q_o}{\pi a_o^3} \text{ (original),} \\ \text{then } \tau_w \rightarrow \tau_w^o \text{ requires } \frac{4\mu(\varepsilon Q_o)}{\pi a^3} = \frac{4\mu Q_o}{\pi a_o^3}, \text{ or } a = \varepsilon^{1/3} a_o; \quad (13)$$

$$\text{If } \sigma_\theta = \frac{(\gamma P_\theta)(\varepsilon^{1/3} a_\theta)}{h} \text{ (perturbed) and } \sigma_\theta^0 = \frac{P_\theta a_\theta}{h_0} \text{ (original),} \\ \text{then } \sigma_\theta \rightarrow \sigma_\theta^0 \text{ requires } \frac{(\gamma P_\theta)(\varepsilon^{1/3} a_\theta)}{h} = \frac{P_\theta a_\theta}{h_0}, \text{ or } h = \varepsilon^{1/3} \gamma h_0. \quad (14)$$

Comparison of results from Groups 3 and 1 in Table 1 (e.g., $a/a_0 = 1.03$ and $h/h_0 = 1.21$) suggest a $\gamma = 1.17$, which is consistent with but on the low end of the prior reports of increases in blood pressure due to Ang-II infusion. Whether the trend toward modest increases in caliber noted for Group 3 relative to Group 1 was real (it was not statistically significant, which would imply a $\gamma = 1.21$, close to the reported 24% increase in blood pressure by Cassis et al. (2009) who used the same Ang-II dose that we used) or was due either to a modest increase in flow ($\sim 9\%$ based on calculated values of ε) or a diminished smooth muscle contractility (e.g., due to a phenotypic modulation from contractile toward synthetic) is not clear, yet any of these possibilities would be consistent with the data. Albeit based on a different model of hypertension in wild-type mice, Eberth et al. (2010) showed that smooth muscle contractility decreased in response to increased pulse pressure. Using a similar hypertension model in the mini-pig, Hu et al. (2008) showed that the smooth muscle cells exhibited a slow decrease in contractile markers that would be consistent with a loss of contractile function and increased synthetic activity. Our histological findings suggest that the increased thickening of the wall was due, in part, to modest increases in both smooth muscle (noting that a phenotypic modulation towards a synthetic phenotype is typically consistent with increased proliferation, which can be induced directly by Ang-II – see Mehta and Grinding, 2007) and collagen within the media but also adventitia, noting that the medial smooth muscle area fraction remained constant across groups due to equivalent increases in total wall area. That the changes in wall composition and model parameters reported herein were modest is perhaps best reflected by the modest change in the in vivo axial stretch ratio (from 1.64 in Group 1 to 1.55 in Group 3, a 5% reduction). Eberth et al. (2010) reported that one of the most dramatic changes in the common carotid artery in response to marked increases in pulse pressure was a reduction in the axial stretch (from 1.72 in normotension to 1.27 in hypertension, a 26% reduction), consistent with the suggestion by Humphrey et al. (2009) that the axial direction is perhaps the first to be modified mechanically in many examples of adaptive arterial growth and remodeling.

Albeit for the descending aorta in the mouse, which also does not develop aneurysms in the Ang-II infusion model, Maiellaro-Rafferty et al. (2011) reported a similarly modest reduction in the in vivo axial stretch (from 1.48 to 1.38, a 7% reduction) due to a 7-day infusion of a lower dose of Ang-II (~ 500 ng/kg/min). Also consistent with our findings for the common carotids, they reported no significant difference in the pressure – diameter behavior of the descending aortas following Ang-II infusion. In contrast, Tham et al. (2002) reported that both the thoracic and the suprarenal abdominal aorta stiffened significantly following 30 days of infusion of 1000 ng/kg/min Ang-II. Their conclusions were based on observed decreases in functional elastin and increases in intramural collagen, particularly in the adventitia, and uniaxial stress – stretch tests performed on excised rings, which unfortunately do not yield rigorous information on material properties (Humphrey, 2002). It should be noted, however, that Tham et al. used 6-month old male mice and the systolic blood pressure increased from 124 to 160 mmHg (i.e., $\gamma = 1.29$). It may be that the effects of

exogenous Ang-II are more pronounced in an older vasculature that has been compromised by the normal aging process. Indeed, Danpinid et al. (2010) also used older mice (7-10 months) in their Ang-II infusion study (1000 ng/kg/min) and they similarly found a significant increase in stiffness in the abdominal aorta even when aneurysms did not develop. The possibility that aging renders large arteries more susceptible to maladaptive growth and remodeling is supported further by the observation by Bode-Janisch et al. (2012) that there is little qualitative difference in aged thoracic aortas and those that experience dissection; the main difference is more quantitative, with dissected aortas showing greater fragmentation of elastin, loss of smooth muscle, and accumulation of glycosaminoglycans. It thus seems important when contrasting results from different mouse models to include age as a key parameter.

AAAs develop almost exclusively in the infrarenal aorta in humans. Among others, Amirbekian et al (2009) suggest that the vulnerability of this region of the aorta in humans results, in part, from a marked oscillatory flow (i.e., decreased mean wall shear stress and increased oscillatory wall shear stress). Using phase contrast Magnetic Resonance Imaging, they suggested that neither the infrarenal nor the suprarenal aorta exhibit such flows in the mouse. Consequently, they could not find a hemodynamic reason for the exclusive development of Ang-II induced aneurysms in the suprarenal mouse aorta. Trachet et al. (2011) used computational fluid dynamics to study the hemodynamics in the mouse aorta and similarly concluded that a hemodynamic reason for localization of aneurysms in the suprarenal aorta remains unclear. Collins et al. (2011) reported that the biaxial material properties of the non-diseased mouse suprarenal and infrarenal mouse aortic wall are not significantly different, again failing to provide a purely mechanical clue as to why aneurysms develop in the suprarenal region in the Ang-II infusion mouse model. One wonders, therefore, if the particular subcutaneous location of the infusion pump plays a role in localizing the most dramatic arterial responses to the suprarenal aorta, though recent studies have also reported aneurysms in the ascending aorta as well (Daugherty et al., 2010). The ascending aorta exhibits unique mechanics, of course, and thus might otherwise be the most vulnerable to non-atherosclerotic lesions resulting from the Ang-II infusion.

Changes in the geometry, composition, and material properties of the suprarenal aorta are dramatic during the development of an Ang-II induced aneurysm. For example, these lesions dilate significantly (e.g., from an ~ 0.8 mm unloaded outer diameter to well over 2 mm) and the stress – stretch behavior reveals a marked loss of extensibility (the in vivo axial stretch decreases from ~ 1.5 to 1.05) and distensibility (Genovese et al., 2012), which reflects a significant increase in circumferential stiffness and accumulation of intramural collagen (Deguchi et al., 2009). Rather than focusing on reasons why the suprarenal aorta and ascending aorta are particularly vulnerable to the formation of aneurysms in this mouse model, however, we considered the complementary question of why other large central arteries are protected. That is, given expected systemic effects of the Ang-II, including increases in systemic blood pressure, we sought to quantify possible changes in material (using a nonlinear constitutive relation) and structural (that is, geometric and compositional) properties in another representative large artery, the common carotid, which has not been studied previously in the ApoE^{-/-} Ang-II mouse model.

Towards this end, it should be emphasized that the four fiber family model (equation 7) is structurally motivated, not structurally based. That is, the functional form of this model is motivated by the assumptions that the elastin-dominated amorphous matrix can be modeled by a neo-Hookean relation whereas the collagen fiber-dominated response can be modeled by Fungexponentials. Clearly, however, the simplicity of this model, including additive stored energies and thus neglect of constituent-to-constituent interactions, reminds us that one must be cautious when interpreting estimated changes in model parameters. For example, although the four fiber families were originally motivated by multiphoton images of transmural distributions of fibrillar collagen in a cerebral artery (Wicker et al., 2008), they can collectively capture responses due to both the extension of differently oriented collagen fibers and possible transverse stiffnesses due to collagen cross-links, which are never modeled directly. Nevertheless, estimated changes in the parameter c have been shown to be consistent with the expected loss of elastin integrity in a mouse model of Marfan syndrome (Eberth et al., 2009a) and the expected decrease in the contribution of elastin to wall mechanics in hypertension (Eberth et al., 2011). Similarly, dramatic decreases in the best-fit values of this elastin associated parameter have captured the loss of elastin integrity in elastase-treated arteries (Ferruzzi et al., 2011; Collins et al., 2012). Hence, despite the need for caution, it appears that some results stemming from the parameter estimation do reflect underlying changes in microstructure.

Although the constitutive model revealed a slight decrease in the elastin-associated parameter c following infusion with Ang-II (cf. Table 2), these changes ($\sim 21\%$ on average) were not as marked as the $\sim 62\%$ decrease reported in the aforementioned study of hypertension by Eberth et al. (2011). Because the area fraction of elastin increased in Group 3 (cf. Arribas et al. (2008), who show decreased fenestration areas in the elastin lamellae of hypertensive rat aorta), this decrease in the phenomenological value of c may have reflected the histological indications of fraying or less densely packed elastin within the lamellae. We emphasize, however, that there was no evidence of Ang II induced fragmentation of elastic fibers (cf. Figures 6 and 7), which is often thought to be a first step in the development of an aneurysm. Thus, coupled with the other findings reported herein, it is thus not surprising that the common carotid artery does not develop aneurysms in young mice in response to chronic infusion of Ang-II over a 28-day period. All structural and mechanical changes appeared to be consistent with primarily an adaptive, not maladaptive, growth and remodeling response to mild hypertension. In conclusion, the dosages of Ang-II typically used to induce aneurysms in the mouse aorta (500 to 1000 ng/kg/min over 28 days) do not appear to induce maladaptive remodeling of large arteries such as the common carotids, at least not in younger mice. Continued research should be directed towards elucidation of reasons both for the lack of susceptibility of most large arteries to the development of aneurysms in Ang-II infused mice and the high vulnerability of the suprarenal and ascending aortas.

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Figure 1.

Method for quantifying constituent area fractions illustrated for a representative untreated control vessel. (a) VVG images were used to identify elastin ($H = 0^\circ - 360^\circ$, $S = 0.0 - 1.0$, $L = 0.0 - 0.22$). (b) MTC images were used to quantify both smooth muscle cell (SMC) cytoplasm ($H = 275^\circ - 10^\circ$, $S = 0.13 - 1.0$, $L = 0.14 - 1.0$) and collagen ($H = 150^\circ - 260^\circ$, $S = 0.12 - 1.0$, $L = 0.14 - 1.0$). In each case, we show both the original image and the extracted constituent area fractions. All images are at the same scale and can be overlaid to verify the results of the pixel isolation and subsequent quantification.

Figure 2.

Passive axial force-stretch behavior during cyclic extension tests at three fixed pressures. The vertical line represents the cross-over point, which indicates the in-vivo value of axial stretch λ_z^{iv} (Humphrey et al., 2009). (a) Representative data from the untreated control group, vessel #4. (b) Representative data from the Ang-II infusion without AAA group, vessel #1. (c) Representative data from the Ang-II infusion with AAA group, vessel #1. Despite the trend toward axial stiffening and decrease in the in-vivo axial stretch, there was

no significant difference between the two Ang-II groups and the untreated control group (cf. Table 1).

Figure 3.

Representative plots of the theoretical fits (solid lines) to experimental data ($\circ$) from passive pressure-diameter and pressure-force responses during cyclic pressurization tests at three fixed axial stretches (i.e., at λ_z^{iv} and $\pm 5\%$ this value). (a),(d) Representative results from the untreated control group, vessel #4. (b),(e) Representative results from the Ang-II infusion without AAA group, vessel #1. (c),(f) Representative results from the Ang-II infusion with AAA group, vessel #1. The associated best-fit parameters are in Table 2.

Figure 4.

Plots of the theoretically predicted (solid lines) and experimentally inferred (○) passive circumferential stress-stretch behavior during cyclic pressurization tests at three fixed axial stretches (at λ_z^{iv} and $\pm 5\%$ this value) for all three groups. (a) Representative data from the untreated control group, vessel #4. (b) Representative data from the Ang-II infusion without AAA group, vessel #1. (c) Representative data from the Ang-II infusion with AAA group, vessel #1.

Figure 5.

Mean values of the strain energy function W (equation 7) at the respective in vivo axial stretch and over a physiological pressure range (60 – 120 mmHg), shown here in kPa. Associated specimen-specific circumferential and axial stretches were used at each pressure in conjunction with the best-fit parameters for each vessel to compute the strain energy. Error bars representing the standard deviation at each pressure value were omitted for clarity. All comparisons across groups and pressures were not significantly different at a confidence level of $p < 0.05$.

Figure 6.

Histological images of representative vessels from the untreated control group (row 1), the Ang-II infusion without AAA group (row 2), and the Ang-II infusion with AAA group (row 3). The leftmost images show a Verhoeff van Gieson (VVG) stain with elastic fibers in black and collagen in red-pink; the rightmost images show a Masson's Trichrome (MTC) stain with collagen in blue and smooth muscle cells (SMC) in red. Note the increase in medial thickness in response to Ang-II infusion (rows 2 and 3), as indicated both by the increased distance between elastic lamellae in the VVG stain and the SMC hypertrophy/hyperplasia in the MTC stain. Note, too, the increased collagen deposition in the adventitia as shown by the darker blue staining in the Ang-II infused mice as compared to the control group. Thus, Ang-II infusion appeared to cause a modest adaptive growth and remodeling consistent with mild hypertension. Finally, note that the outermost images were acquired using a 20X objective and the innermost images using a 60X objective. The black scale bar represents 100 μm in each image.

Figure 7.

Histological images of a section of (a) an untreated control vessel and (b) an Ang-II treated vessel with AAA, both imaged using a 60X objective and zoomed-in further for observation. Visual comparisons suggested that the lamellae in the Ang-II treated vessels were thicker than those in the control vessels, with increased inter-lamellar distance and overall greater medial thickness. The yellow arrows in (b) indicate locations of discontinuity in staining for elastin, potentially revealing some initial fraying or less dense packing of elastin in the lamellae. The black scale bar represents 100 μm .

Mean $\pm$ standard deviations for age, body mass, and vessel geometry for the untreated control (n = 5), Ang-II treated without AAA (n = 5), and Ang-II treated with AAA (n = 4) groups. The outer diameter (OD), thickness (H) and length (L) are presented in the unloaded configuration, while the inner diameter (id) and thickness (h) are presented at near in-vivo conditions (i.e., at the estimated in-vivo stretch (λ_z^{iv}) and a pressure of 80 mmHg). (*) Indicates a significant difference at $p < 0.05$ for the measured value as compared to the untreated control.

Table 1

Vessel #	Age (wks)	Body Mass (g)	OD (μ m)	H (μ m)	L (mm)	λ_z^{iv}	id (μ m) @ 80 mmHg	h (μ m) @ 80 mmHg
Untreated	10.7 $\pm$ 0.8	26.3 $\pm$ 4.7	401 $\pm$ 12	100 $\pm$ 20	3.24 $\pm$ 0.77	1.64 $\pm$ 0.14	514 $\pm$ 20	33 $\pm$ 5
Ang-II without AAA	12.2 $\pm$ 0.2*	28.3 $\pm$ 3.2	436 $\pm$ 22*	104 $\pm$ 18	4.16 $\pm$ 0.67	1.62 $\pm$ 0.12	519 $\pm$ 28	38 $\pm$ 5
Ang-II with AAA	12.1 $\pm$ 0.1*	30.5 $\pm$ 4.3	424 $\pm$ 15*	110 $\pm$ 13	4.66 $\pm$ 0.90*	1.55 $\pm$ 0.17	527 $\pm$ 36	40 $\pm$ 6

Table 2

Best-fit values of parameters in the four fiber family constitutive model for ApoE^{-/-}-carotids under biaxial testing with and without subcutaneous Ang-II infusion. Overall mean and standard deviations (SD) are shown for convenience.

Vessel #	Elastin c (kPa)	Axial		Circumferential		Diagonal		Angle α_0 (deg)	Error RMSE
		c_1^1 (kPa)	c_2^1	c_1^2 (kPa)	c_2^2	$c_1^{3,4}$ (kPa)	$c_2^{3,4}$		
Untreated									
1	5.5020	21.4296	0.0208	0.9864	0.1320	0.0167	1.5081	33.7366	0.0931
2	11.3565	14.1724	2.3373E-14	2.7786	0.1702	0.0466	0.8617	37.3644	0.0805
3	14.6571	8.3214	0.0426	3.0781	0.2374	0.0506	1.0928	39.1308	0.0871
4	6.3036	27.7971	0.0181	2.4119	0.0419	0.0033	1.4915	35.1041	0.0685
5	12.6621	36.5033	2.3373E-14	1.7661	0.2347	0.0345	2.1888	33.9564	0.0822
Mean	10.0963	21.6448	0.0163	2.2042	0.1632	0.0303	1.4286	35.8585	0.0823
SD	4.0145	11.0908	0.0177	0.8384	0.0812	0.0200	0.5055	2.3273	0.0091
Ang-II without AAA									
1	12.0034	26.9620	2.4255E-14	5.1186	0.1164	0.0638	1.6385	31.2237	0.0480
2	11.1767	13.0610	0.0360	3.6163	0.2065	0.0995	0.8370	35.9730	0.0752
3	16.7034	33.1134	2.3374E-14	3.3684	0.2675	0.1953	1.4662	33.4048	0.0656
4	9.2451	30.8792	0.0741	5.1867	0.1152	0.1668	2.0022	31.3870	0.0578
5	4.5750	18.4120	0.0148	3.8433	0.0352	0.0332	1.0504	30.2029	0.0633
Mean	10.7407	24.4855	0.0250	4.2266	0.1482	0.1117	1.3989	32.4383	0.0620
SD	4.4043	8.4979	0.0312	0.8622	0.0902	0.0682	0.4645	2.2916	0.0100
Ang-II with AAA									
1	9.7500	59.4100	2.3373E-14	2.6373	0.0901	0.9297	1.4589	27.0323	0.0601
2	6.1742	25.9761	2.3373E-14	3.5448	0.0248	0.0214	0.8957	31.2585	0.0566
3	9.1999	17.7373	0.0116	2.8118	0.1023	0.0225	1.3295	35.8973	0.0582
4	6.6558	33.5882	0.0144	5.6927	0.0406	0.0549	3.2970	24.1951	0.0523
Mean	7.9450	34.1779	0.0065	3.6717	0.0644	0.2571	1.7453	29.5958	0.0568
SD	1.7917	18.0238	0.0076	1.4036	0.0376	0.4486	1.0622	5.1059	0.0033

Constituent area fractions and percentage of the wall for the media and adventitia for each of the three groups. Note that the elastin area fraction (ϕ^E) represents elastin and the other microfibrillar proteins that constitute the elastic fibers, the smooth muscle area fraction (ϕ^M) represents cell cytoplasm that is highly localized to the smooth muscle cells, and the collagen area fraction (ϕ^C) represents fibrillar collagen and associated glycoproteins. Each measurement has a sample size of $n = 12$, with values presented as means $\pm$ standard deviation. The superscript (*) denotes a statistically significant difference, compared to the untreated control group, at a value of $p < 0.05$.

Table 3

Group	Elastin (ϕ^E)	Smooth Muscle (ϕ^M)	Collagen (ϕ^C)	% Media	% Adventitia
Untreated	0.258 ± 0.020	0.205 ± 0.045	0.500 ± 0.106	$37.71 \pm 3.48 \%$	$62.29 \pm 3.24 \%$
ANG-II without AAA	0.242 ± 0.023	0.207 ± 0.011	0.567 ± 0.053	$42.82 \pm 3.08 \%$ *	$57.18 \pm 3.08 \%$ *
ANG-II with AAA	0.286 ± 0.029 *	0.209 ± 0.024	0.556 ± 0.086	$44.18 \pm 2.56 \%$ *	$55.82 \pm 2.56 \%$ *

Table 4

Mean lightness values measured for each constituent, with normalized parameters ranging from 0 (black) to 1 (white) in the HSL color space. Thus, mean values for a particular constituent can be interpreted as an overall constituent density (e.g., a mean value closer to 0 indicates a denser organization). Note the trends towards a less dense packing of elastin and a denser packing of collagen in response to Ang-II infusion (Groups 2 and 3). The sample sizes were $n = 12$, with values presented as means $\pm$ standard deviations. The superscript (*) denotes a statistically significant difference, compared to the untreated control group, at a value of $p < 0.05$.

	Elastin	Smooth Muscle	Collagen
Untreated	0.089 ± 0.006	0.298 ± 0.033	0.430 ± 0.044
ANG-II without AAA	$0.106 \pm 0.004^*$	0.299 ± 0.033	0.408 ± 0.029
ANG-II with AAA	$0.096 \pm 0.005^*$	0.275 ± 0.066	$0.383 \pm 0.038^*$