Excessive Adventitial Remodeling Leads to Early Aortic Maladaptation in Angiotensin-Induced Hypertension

Matthew R. Bersi,* Chiara Bellini,* Jing Wu,* Kim R.C. Montaniel, David G. Harrison, Jay D. Humphrey

Abstract—The primary function of central arteries is to store elastic energy during systole and to use it to sustain blood flow during diastole. Arterial stiffening compromises this normal mechanical function and adversely affects end organs, such as the brain, heart, and kidneys. Using an angiotensin II infusion model of hypertension in wild-type mice, we show that the thoracic aorta exhibits a dramatic loss of energy storage within 2 weeks that persists for at least 4 weeks. This diminished mechanical functionality results from increased structural stiffening as a result of an excessive accumulation of adventitial collagen, not a change in the intrinsic stiffness of the wall. A detailed analysis of the transmural biaxial wall stress suggests that the exuberant production of collagen results more from an inflammatory response than from a mechano-adaptation, hence reinforcing the need to control inflammation, not just blood pressure. Although most clinical assessments of arterial stiffening focus on intimal–medial thickening, these results suggest a need to measure and control the highly active and important adventitia. (Hypertension. 2016;67:890-896. DOI: 10.1161/HYPERTENSIONAHA.115.06262.)

Key Words: arterial stiffness ■ collagen ■ elastic energy ■ hypertension ■ wall stress

Hypertension is a critical risk factor for many cardiovascular, neurovascular, and renovascular conditions.1-3 Findings over the past 2 decades suggest that large artery stiffening causes essential hypertension and associated changes in systemic hemodynamics that augment central pulse pressure.4,5 Yet, arteries also stiffen in response to changes in mechanical stress that arise from increased blood pressure, as revealed by several animal models6-8 and evidenced in humans in aortic coarctation.9 Central artery stiffening can thus be both a cause and a consequence of hypertension, and it likely involves a complex feedback between global changes in hemodynamics/physiology and local changes in wall mechanics/mechanobiology.

Another emerging concept is that inflammation is important in hypertension.10,11 We showed that T cells and cytokine interleukin-17a play important roles in the deposition of adventitial collagen in deoxycorticosterone acetate (DOCA) salt and angiotensin II (Ang II)–induced hypertension.12 The mechanical analysis performed in that study showed that these forms of hypertension cause a leftward shift in the stress–strain behavior that is often interpreted as stiffening, but did not assess the biaxial wall mechanics, did not compare changes in stiffness or energy storage, and did not consider specific contributions of the adventitia to overall stiffening. We now present the first detailed study of biaxial wall mechanics in induced hypertension based on data collected from the proximal descending thoracic aorta of male wild-type mice after 2 or 4 weeks of Ang II infusion. In particular, we quantified transmurally averaged mechanical properties that are needed to study interactions between the hemodynamics and arterial wall as well as layer-specific properties that are needed to understand differential mechanobiological responses by medial and adventitial cells. Our analyses suggest that early hypertension-induced remodeling of the aorta is mechanically maladaptive in this mouse model, which compromises the ability to store elastic energy and, by inference, to modulate the pulse wave and peripheral perfusion.

Methods

Animal Model
All animal studies were approved by the Institutional Animal Care and Use Committee of Vanderbilt University. Briefly, hypertension was induced in male wild-type mice (C57BL/6; Jackson Laboratories).
at 3 months of age via chronic infusion of Ang II (Sigma-Aldrich) at a continuous rate of 490 ng/kg per minute for 2 or 4 weeks using an osmotic mini pump (Alzet). This pump was implanted subcutaneously on the flank under sterile conditions during a brief surgery in which ketoprofen (5 mg/kg) was used for preanesthesia and ketamine (100 mg/kg) and xylazine (10 mg/kg) were used for anesthesia. Blood pressure was measured every hour over ≤4-week study period via an indwelling catheter and telemetry system (Figure S1 in the online-only Data Supplement). At the prescribed end point, the mice were euthanized by CO2 inhalation, and the descending thoracic aorta was excised from the left subclavian artery to the third pair of intercostal branches. Age-matched control vessels were obtained similarly, but after a sham procedure wherein the implanted mini pumps released normal saline rather than Ang II. Additional details can be found elsewhere.12

The online-only Data Supplement describes established methods for biaxial mechanical testing, quantitative histology, and computing wall stress, material stiffness, (an intrinsic property of the wall), structural stiffness (which depends on material stiffness and geometry), and stored energy.

Statistics
Data in the article are presented as mean±standard error of the mean. Geometric and material metrics were compared across all treatment groups (sham, 2-week Ang II, and 4-week Ang II) using a 1-way analysis of variance followed by a post hoc Bonferroni correction.

Results
Infusion of Ang II (490 ng/kg per minute) increased systemic blood pressure to 167/133 mm Hg at 2 weeks, which persisted at 4 weeks (172/129 mm Hg). Sham mice had steady state pressures of 121/99 mm Hg.12 Figure 1A and 1B shows mean in vitro pressure–diameter and axial force–length data from 2 of the 7 mechanical testing protocols for all 3 groups. Clearly, the hypertension-induced remodeling is strongly biaxial: the aortas are less distensible (ie, they exhibit a reduced range of pressure-induced diameter change; Figure 1A) and less extensible (ie, a reduced range of force-induced length change; Figure 1B). Shown, too, are stress–stretch behaviors for the pressure–diameter protocol performed at the in vivo axial length and the axial force–length protocol performed at a constant pressure of 100 mm Hg (Figure 1C and 1D). Note the leftward shift and significantly lower values of biaxial wall stress caused by hypertension. Importantly, as a result of the marked loss of distensibility and extensibility, hypertension also dramatically reduces elastic energy storage on biaxial loading (Figure 1E and 1F).

Figure 2A and 2B suggests that the lower values of wall stress in hypertension (Figure 2C and 2D) likely result from 2 primary changes: a significantly lower in vivo axial stretch (<1.35 versus 1.62; Figure 2A) and a significantly greater wall thickness (>100 versus 39 μm; Figure 2B). Interestingly, overall circumferential material stiffness is not statistically different between sham and either Ang II group (2 or 4 weeks); conversely, axial material stiffness is significantly lower in both hypertensive groups, likely because of lower axial stretches (Figure 2E and 2F). Circumferential structural stiffness is nevertheless higher in hypertension (Figure 2G; Table S1), consistent with a thicker wall, and energy storage is significantly reduced at 2 and 4 weeks (Figure 2H). Hence, despite a remarkable maintenance of overall circumferential material stiffness, hypertension induces striking morphometric (wall thickness and axial stretch), material (axial stiffness and energy storage), and structural (distensibility) changes in the thoracic aorta. For completeness, Table S1 lists all morphological and transmurally averaged mechanical findings, and Table S2 lists values of the model parameters that were used to compute stress, stiffness, and stored energy.

Figure 3A shows representative histological findings. Verhoeff Van Gieson–stained sections reveal less
undulation of elastic laminae and greater interlamellar spacing in hypertension, consistent with smooth muscle hypertrophy and intralamellar deposition of thin collagen fibers (green in dark-field picrosirius red images). Masson’s Trichrome- and picrosirius red–stained sections show that the most dramatic hypertension-induced change is adventitial thickening because of increased deposition of thick (white in picrosirius red images) fibrillar collagens. Layer-specific histological image analyses (Figure 3B) reveal that, on average, sham aortas contain 33% elastin, 33% smooth muscle, and 4% collagen in the media (70% of wall), with 1% elastin and 29% collagen in the adventitia (30% of wall). Associated with an ≈2.6-fold increase in wall cross-sectional area in hypertension, quantification reveals an ≈1.8-fold increase in smooth muscle and ≈3.3-fold increase in medial collagen, which contribute to the mild medial hypertrophy (1.7-fold increase in medial area), but a marked 4.7-fold increase in adventitial collagen that contributes to the large increase in adventitial thickness (4.6-fold increase in adventitial area) and wall percentage (on average, 55% in Ang II versus 30% in sham).

Figure 4A and 4B show calculated transmural distributions of aortic wall stress for sham and 2-week Ang II–infused mice at group-specific values of in vivo axial stretch and mean arterial pressure; associated model parameters are in Table S3. Circumferential stress is higher in the media than the adventitia in the sham group, consistent with the media bearing most of the load under physiological conditions, whereas the adventitia engages and bears more load when pressure increases acutely above normal levels, as in exercise. Circumferential stress is lower and nearly constant across the wall in hypertension, with a reduced difference between layers suggesting a greater engagement of the adventitia as expected of a protective sheath. Note the overall mean value calculated using the Laplace equation. Axial stress is nearly equally distributed between the media and the adventitia at mean arterial pressure for both groups, albeit at different values. Figure 4C and 4D shows associated values.
of material stiffness. Consistent with histological observations (Figure 3A), hypertension markedly redistributes material stiffness between layers. Despite maintaining an average value that is similar to normal, hypertension decreases circumferential stiffness in the media and increases it in the adventitia. Conversely, axial stiffness decreases more in the adventitia than in the media, thus resulting in a lower average value than normal. These results are consistent with an increase in circumferentially oriented collagen fibers in hypertension (Table S3). Finally, Figure 4E and 4F illustrate the protective mechanical role of the adventitia in a normal aorta by simulating changes in wall stress for an instantaneous change in systolic pressure from normal (sham systolic) to hypertensive (Ang II systolic) levels. Although the predicted biaxial stresses increase in the media and adventitia, the latter experiences greater increases circumferentially (80% increase) and axially (25% increase).

**Discussion**

This study demonstrates that early aortic responses to angiotensin-induced hypertension are strongly biaxial, affecting circumferential and especially axial wall properties, and markedly different between the media and adventitia. Moreover, these early responses seem to be nearly complete by 2 weeks and to persist over 4 weeks. A consequence of these differential responses is a dramatically reduced ability of the aorta to store elastic energy during systole (Figure 1F), which compromises its primary mechanical function to sustain blood flow during diastole.1,3,14

Loss of energy storage is due largely to a marked reduction in distensibility and extensibility (cf. Figure 1E and 1F), that is, adverse biaxial remodeling. Because of circumferential–axial coupling (ie, both stresses depend on circumferential and axial stretches; online-only Data Supplement), the marked decrease in axial stretch helps to protect circumferentially oriented medial smooth muscle cells from excessive stresses in hypertension (cf. Figure 2C and 2D). This reduced axial stretch also helps maintain overall circumferential material stiffness near its normal value (Figure 2E), which is mechanobiologically favorable because intramural cells appear to seek to establish and maintain wall properties near preferred values.15–17 These observations emphasize the importance of axial wall mechanics, which are seldom considered but play essential roles in the mechanobiology18 and structural integrity19 of the arterial wall and even pulse wave propagation.20 Indeed, altered axial mechanics can be among the earliest adaptations to hemodynamic changes.21,22 Nevertheless, the axial response ultimately seems maladaptive in the present case for it also contributes to a significant loss of elastic energy storage capability (Figure 1F) and presumably an inability of the aorta to augment diastolic blood flow optimally.

Another indicator of maladaptive remodeling is the reduction in wall stress well below normal (Figures 1C, 1D, 2C, and 2D). Arteries tend to adapt mechanically in many cases of altered hemodynamics and disease by maintaining circumferential stress near a homeostatic value.8,23,24 It is straightforward to show that optimal mechano-adaptations to altered blood flow and pressure result in changes in luminal radius $a$ and total wall thickness $h$ according to particular rules: $\alpha \rightarrow e^{\alpha_0}$ and $h \rightarrow \gamma e^{\beta_0}$, where $e$ denotes the fold-increase in flow and $\gamma$ the fold-increase in pressure, with
subscript 0 denoting an original (homeostatic) value. With mean arterial pressures of 144 and 106 mm Hg at 2 weeks in the angiotensin-infused and sham mice, respectively, \(\gamma = 1.36\). Although not measured in the descending thoracic aorta, changes in flow are typically small in hypertension (ie, \(\varepsilon = 1.0\)). Indeed our finding that values of luminal radius are similar across the 2 groups (Table S1) suggests little change in flow in this model of hypertension, consistent with regulation of wall shear stress.\(^{23,26}\) The measured increase in wall thickness at 2 weeks (\(h/h_0 = 1.02\mu m/39\mu m = 2.62\)) is thus well in excess of the mechano-adaptive target of \(\gamma = 1.36\), which contributes to a significant decrease in biaxial stress (Figure 1C and 1D) and mechanical functionality (Figure 1E and 1F). Similar maladaptation (\(h/h_0 = 2.79\)) is found after 4 weeks of hypertension. Although such an overcompensation could resolve over a longer period (low wall stresses typically promote atrophy\(^{27}\)), with a possible approaching of the homeostatic target,\(^{27,28}\) the early and persistent responses at 2 and 4 weeks are mechanically maladaptive due primarily to excessive collagen deposition in the adventitia.

The present study is the first to delineate biaxial wall stresses in the media and adventitia for an Ang II infusion model. Circumferential stresses are normally higher in the media than in the adventitia (sham; Figure 4A), consistent with the goal of storing energy in elastic fibers during systolic distensions. The highly nonlinear behavior of the normal collagen-rich adventitia allows stresses to increase more in this layer in response to a sudden increase in pressure, however, which enables it to protect medial smooth muscle cells and elastic fibers from excessive stresses (Figure 4E and 4F). It seems that Ang II–induced hypertension increases adventitial stress more than medial stress, consistent with greater remodeling of the former (Figure 3A). Importantly, adventitial remodeling is also more dramatic in other models of induced hypertension, including DOCA salt and aortic banding.\(^{12,28,29}\) There is, therefore, a pressing need to account for differential stresses in these 2 layers and associated differences in smooth muscle cell and fibroblast mechanobiology,\(^{12,30}\) as well as possible paracrine signaling among these cells.\(^{31}\) Yet, consistent with aforementioned indicators of maladaptation, adventitial thickening is far greater than needed to restore adventitial and medial stresses to normal values (Figure 4A and 4B). This overcompensation suggests either a dysfunctional mechanosensing that leads to a fibrotic response or additional contributors to the exuberant collagen production.\(^{32}\) Among other factors, inflammation is important in multiple rodent models that exhibit adventitial thickening, including induced hypertension\(^{12,33,34}\) and central artery aging.\(^{35,36}\) Indeed, the adventitia is now recognized to be a biologically complex layer that is involved in many cases of arterial remodeling and injury response.\(^{37,38}\) It not only contains resident fibroblasts that can differentiate into myofibroblasts, it is also a source of progenitor and inflammatory cells.\(^{39}\) Both pressure-induced wall stresses (which can augment local production of Ang II as well as diverse cytokines and proteases)\(^{40,41}\) and exogenous Ang II which stimulates production of other proinflammatory molecules\(^{42-44}\) can promote the recruitment and activation of inflammatory cells. Genetic manipulation studies in mice demonstrate that macrophages and T cells play particularly important roles in such cases.\(^{12,45}\) It is not surprising, therefore, that ex vivo models confirm that effects of pressure-induced stress and exogenous Ang II are synergistic in the production of matrix,\(^{46}\) consistent with in vivo studies.\(^{34}\) Cell culture and ex vivo studies similarly show that increased mechanical loading\(^{47}\) and exogenous Ang II increase the production of proteases, independent of inflammatory cells.\(^{43}\) Deposition and degradation are fundamental to overall extracellular matrix remodeling.

We recently reported complementary data wherein mouse aortic fibroblasts were subjected to cyclic stretching, interkeukin-17a, or Ang II and different combinations thereof.\(^{12}\) Cyclic stretching increased the production of collagen 1a1, 3a1, and 5a1 in a stress-dependent fashion, whereas interkeukin-17a and Ang II independently increased collagen production. Combined with results of Figure 4, these data support the hypothesis that a combination of preferential pressure-induced mechanical stresses and angiotensin-induced inflammation led to the exuberant and rapid production of matrix in the adventitia (Figure 3). Similar adventitial collagen deposition has been reported in aortic banding,\(^{31}\) DOCA salt hypertension,\(^{12}\) and a model of hypertension associated with increased vascular oxidative stress. It is therefore likely that our findings of maladaptive wall mechanics are not specific to Ang II, but instead are relevant to many models of hypertension.

**Perspectives**

The present experimental findings and computational results suggest that hypertension preferentially increases circumferential stress within the adventitial layer, the primary site of matrix accumulation and remodeling. Because the overall aortic response is mechanically maladaptive, the overexuberant production of adventitial collagen nevertheless seems to result more from an inflammatory response, hence reinforcing the need to control inflammation, not just blood pressure. Finally, although most clinical assessments of arterial stiffening focus on intimal–medial thickening, our results suggest a need to control the highly active and important adventitia that can adversely limit elastic energy storage by an otherwise competent medial layer of the aortic wall.

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**Disclosures**

None.
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**Novelty and Significance**

**What Is New?**

- This is the first analysis of how aortic axial and circumferential mechanical properties are altered differentially in the media and adventitia in hypertension.
- This analysis reveals that hypertension causes a striking decline in the ability of the aorta to store energy, which compromises its primary mechanical function.
- The exuberant deposition of adventitial collagen in hypertension is maladaptive and exceeds that necessary to normalize wall stress.

**What Is Relevant?**

- These changes in aortic compliance have striking implications for systemic perfusion.
- Loss of energy storage reduces the ability of the aorta to maintain diastolic flow, whereas the increase in biaxial stiffness enhances systolic forward flow.

**Summary**

Our analysis of biaxial wall mechanics reveals that hypertension not only leads to increased structural stiffness of the aorta, but also profoundly reduces the ability of the aorta to store elastic energy. This is largely because of dramatic, maladaptive remodeling of the adventitia. These alterations of aortic compliance likely contribute to end organ damage and untoward clinical outcomes. Efforts to modulate adventitial remodeling will likely have therapeutic benefit.
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Methods

Animal Model. All animal studies were approved by the Institutional Animal Care and Use Committee of Vanderbilt University. Briefly, hypertension was induced in male wild-type mice (C57BL/6; Jackson Laboratories) at 3 months of age via chronic infusion of angiotensin-II (Ang II; Sigma-Aldrich) at a continuous rate of 490 ng/kg/min for 2 or 4 weeks using an osmotic mini-pump (Alzet). Pumps were implanted subcutaneously on the flank under sterile conditions and blood pressure was measured every hour over the up to 4-week study period via an indwelling catheter and telemetry system (Figure S1). At the prescribed endpoint, the mice were euthanized and the descending thoracic aorta was excised from the left subclavian artery to the third pair of intercostal branches. Age-matched control vessels were obtained similarly, but following a Sham procedure wherein the implanted mini-pumps released normal saline rather than Ang II. Additional details can be found elsewhere. Given that the angiotensin-induced increase in blood pressure reached a near steady state by 10 to 14 days, we focused primarily on a comparison of early hypertensive remodeling (at 2 weeks) with normal aortic structure and properties (Sham controls). Nevertheless, we also quantified remodeling at 4 weeks to determine if early remodeling remained the same or progressed over the subsequent 2 weeks.

Biaxial Mechanical Testing. Following overnight shipment in an iced physiologic solution, samples were cleaned of perivascular tissue and the intercostal branches were ligated using single strands from a braided 7-0 nylon suture. Vessels were then cannulated on pulled glass micropipets, secured with proximal and distal ligatures of 6-0 silk suture, and tested mechanically using a custom computer-controlled biaxial device. Figure S2 shows a schematic drawing of the biaxial device and associated components along with images of representative samples from each group. Briefly, following equilibration for approximately 30 minutes while submerged in a Hanks buffered physiologic solution (Invitrogen Life Technologies) at 37°C, the specimens were subjected to standard mechanical preconditioning consisting of 4 cycles of pressurization from 10 to 140 mmHg while the vessel was held near its in vivo axial length. Next, the unloaded configuration (outer diameter and axial length) was recorded, the in vivo axial stretch was estimated based on the force-pressure relationship, and the vessels were subjected to a series of 3 cyclic pressure-diameter protocols (from 10 to 140 mmHg at the in vivo axial stretch and at ±5% of this stretch) and 4 cyclic axial force-length protocols (from 0 to 40 mN at constant pressures of 10, 60, 100, and 140 mmHg). Pressure, axial force, outer diameter, and axial length were recorded on-line over 2 cycles for all 7 protocols, thus generating hundreds of data points for material characterization. To facilitate the computation of stress and strain, wall thickness was measured in the unloaded configuration which, via the assumption of incompressibility, allowed the thickness to be calculated from on-line measurements at all loaded (pressure and axial force) configurations. Note that a Hanks solution yields a near passive mechanical behavior, which focused attention on expected changes in the extracellular matrix. It is currently not clear what role smooth muscle contractility plays in vivo in the function of elastic arteries such as the aorta, nor is it clear what level of smooth muscle tone should be induced in vitro to mimic in vivo properties. For these different reasons, we purposely...
focused on passive properties, noting that we have previously confirmed that these properties are not altered following overnight storage of vessels in an iced physiologic solution.

In a subset of specimens, porcine pancreatic elastase (Worthington Biochemical) was used to estimate the values of stretch at which the collagen fibers were deposited within the aortic wall, that is, their “deposition stretches” \(^5\). Briefly, the outer diameter and axial length of the cannulated samples were measured before and after intraluminal exposure of elastase (3 mL at 7.5 U/mL) for approximately 15 minutes at \(\text{in vivo}\) loading conditions (\(\text{in vivo}\) stretch and 80 mmHg at 37°C). As shown previously, this exposure degrades most of the intramural elastin \(^6\). Because aortic elastin is deposited within the wall primarily during the perinatal period and has a long half-life, it is “pre-stretched” during somatic growth. Removal of pre-stretched elastin, even in an unloaded state, thus causes the vessel to increase in dimension. These measurements, in combination with the homeostatic stretches obtained from the biaxial data, provided important insight into the level of pre-stretch of elastin, and by inference, the deposition stretches of smooth muscle and collagen, as needed in the detailed analysis of the wall stress (see below).

**Histology.** Following mechanical testing, the aortas were fixed overnight in a 10% neutral buffered formalin solution and stored in 70% EtOH at 4°C. Vessels were then embedded in paraffin, sectioned serially, and stained with Verhoeff van Giesen (VVG), Masson’s trichrome (MTC), or picrosirus red (PSR). Intact cross-sections of stained samples were composed by stitching individual images taken using an Olympus BX/51 microscope at 40x magnification (CellSens Dimension). A custom MATLAB script was then used to extract the percentage of the wall occupied by media or adventitia as well as the area fractions for elastin, collagen, and smooth muscle from the VVG- and MTC-stained cross-sections and similarly the proportions of thick (orange-red) versus thin (yellow-green) birefringent collagen fibers in the media from PSR-stained sections imaged under polarized light \(^7,8\).

We emphasize that quantifying the histology in the same samples that were tested mechanically not only provides group-specific information, it also allows one to assess whether the mechanical tests induced any damage that could compromise the results. The absence of test-induced damage was confirmed by comparing the current histological findings with prior findings for samples that had not been tested mechanically \(^1\).

**Quantification of Mechanical Properties.** It is well known in continuum biomechanics that a nonlinear (pseudo)elastic behavior is best described using a stored energy function \(W\) \(^9\). Essentially, this function quantifies the energy stored within a tissue, per unit volume, upon mechanical loading and, consequently, the amount of energy available to the tissue to do work on its surroundings as it is unloaded. Moreover, first and second derivatives of \(W\) with respect to an appropriate measure of strain provide information, respectively, on the stress (a second order tensor) and the material stiffness (a fourth order tensor). Hence, this single scalar function provides a comprehensive characterization of the material behavior. Full details on the nonlinear mechanics of the arterial wall can be found elsewhere \(^9\), as can details on our methods of quantifying both mean \(^3\) and layer-specific transmural \(^5\) wall properties and stresses in mice. For completeness, however, we list some of the primary equations here.
**Bulk Mechanical Properties** – Studies of the effects of wall mechanics on hemodynamics (e.g., effects of arterial stiffness on pulse wave velocity) require information on the bulk (i.e., transmurally averaged) material properties. Toward this end, the biaxial mechanical data were first quantified using a validated nonlinear stress-strain relation that was derived from a spatially homogenized, microstructurally-motivated elastic stored energy function $W^3$. Specifically, we used a “four-fiber family” form of $W$ that was motivated by histological observations of nearly isotropically distributed elastin, four predominant families of locally parallel collagen fibers (axial, circumferential, and two symmetric diagonal), and circumferentially oriented smooth muscle. It is currently not possible to delineate contributions due to smooth muscle and circumferential collagen in the media, hence their contributions were treated phenomenologically via a single composite fiber family. Similarly, it is currently not possible to delineate effects of cross-links or physical entanglements, hence the parameters characterizing $W$ (see below) were determined via nonlinear regression using the collected macroscopic data (see Table S2), thus yielding a sufficient phenomenological descriptor for studying fluid-solid-interactions. This stored energy function can be written

$$W(C, M^j) = \frac{c}{2} (I_C - 3) + \sum_{j=1}^{4} \frac{c^j}{4c_2} \left\{ \exp \left[ c^j (I_{C}^j - 1) \right] - 1 \right\}, \quad (S.1)$$

where $c$, $c^j_1$, and $c^j_2$ ($j = 1, 2, 3, 4$) are model parameters, with $c$ and $c^j_1$ having units of stress (kPa) and $c^j_2$ dimensionless, $C = F^T F$ is the right Cauchy-Green tensor, and $F$ is the deformation gradient tensor. $M^j = [0, \sin \alpha_0^j, \cos \alpha_0^j]$ is a unit vector in the direction of the $j^{th}$ fiber family, where the angle $\alpha_0^j$ is computed relative to the axial direction in a reference configuration. Thus, axial and circumferential fiber families are oriented at $\alpha_0 = 0$ and $\alpha_0 = 90$ degrees, respectively. In addition, $I_C = tr(C)$ and $I_{C}^j = M^j \cdot C M^j$ are coordinate invariant measures of deformation that can be written in terms of stretch ratios, namely

$$I_C = \lambda_\theta^2 + \lambda_z^2 + \frac{1}{\lambda_\theta^2 \lambda_z^2}, \quad I_{C}^j = \lambda_\theta^2 \sin^2 \alpha_0^j + \lambda_z^2 \cos^2 \alpha_0^j, \quad (S.2)$$

for $F = diag(\lambda_r, \lambda_\theta, \lambda_z)$, noting that incompressibility requires $\lambda_r = 1/(\lambda_\theta \lambda_z)$. The Cauchy stress tensor $\sigma$ can then be computed as

$$\sigma = -pI + 2F \frac{\partial W}{\partial C} F^T, \quad (S.3)$$

where $I$ is the second order identity tensor, the superscript $T$ indicates the transpose of the tensor, and $p$ is a Lagrange multiplier that enforces the incompressibility.

Clearly, then, the biaxial stresses could be calculated easily from $W$ for all 7 biaxial testing protocols given on-line measurements of the deformations and values of the 8 model parameters. Best-fit values of these parameters were determined from the
biaxial data using a nonlinear regression algorithm that minimized an objective function based on the sum-of-the-squares of differences between measured and predicted normalized pressures and axial forces. Toward this end, note that we adopted a 2-D formulation (i.e., we neglected the radial stress relative to the circumferential and axial stresses), hence expressions for pressure and force were obtained directly by inverting global equilibrium equations for stress, namely solving

$$\sigma_\theta = \langle \sigma_{\theta\theta} \rangle = \frac{Pr_i}{h}, \quad \sigma_z = \langle \sigma_{zz} \rangle = \frac{f}{\pi h(2r_i + h)} \quad \text{(S.4)}$$

for the transmural pressure $P$ and the total axial force on the vessel $f = f_T + \pi r_i^2 P$ (with $f_T$ representing the transducer-measured axial force), where $r_i$ is the measured inner radius and $h$ is the wall thickness, both in the loaded configurations. In this way, the best-fit model parameters assured that the resulting stress-strain relation satisfied equilibrium at each pressurized and axially loaded state. Given these parameter values, we could then compute mean wall stress, material stiffness, and overall elastic energy storage for any deformation, in vitro or in vivo, all of which were computed and compared between the Sham and Ang II treated vessels at both mean and systolic pressures (see Table S1). Note, therefore, that the components of the stiffness tensor ($\psi_{ijkl}$), linearized about any biaxial state of interest, were computed as

$$\psi_{ijkl} = 2\delta_{ik} F_{ia}^\alpha F_{jb}^\alpha \frac{\partial W}{\partial C_{AB}} + 2\delta_{jk} F_{ia}^\alpha F_{ib}^\alpha \frac{\partial W}{\partial C_{AB}} + 4 F_{ia}^\alpha F_{ib}^\alpha F_{ij}^\alpha F_{ik}^\alpha \frac{\partial^2 W}{\partial C_{AB} \partial C_{PQ}} \bigg|_{C^0}, \quad \text{(S.5)}$$

where $\delta_{ij}$ are the components of the second order identity tensor $I$, $F^\alpha$ is the deformation gradient tensor that maps the chosen reference configuration into a finitely deformed in vivo configuration, and $C^0$ is the corresponding right Cauchy-Green tensor. By linearizing about an appropriate in vivo state, one obtains directly the values of material stiffness that are needed in computational models of the effects of wall mechanics on the hemodynamics. For example, using the identified values of material stiffness, we computed a metric of structural stiffness similar to that which is fundamental to the Moens-Korteweg equation for the pulse wave velocity, which can be written as $PWV = \sqrt{Eh/2\rho a}$, where $E$ is the Young’s modulus (i.e., isotropic material stiffness for a linear response), $\rho$ is the mass density of the blood, and $h$ and $a$ are wall thickness and luminal radius. Namely, we calculated a structural stiffness (in N/m) as $h/\psi_{\theta\theta\theta\theta}$, where $h$ is the wall thickness and $\psi_{\theta\theta\theta\theta}$ is the linearized circumferential material stiffness, each evaluated at a specified pressure (i.e., systole or diastole).

Finally, we also computed and compared the often used structural parameter called distensibility

$$D = [d_{sys} - d_{diat}]/[(d_{diat})(P_{sys} - P_{diat})], \quad \text{(S.6)}$$
where \( d \) is the outer diameter, and \( \text{sys} \) and \( \text{dias} \) denote, respectively, the systolic and diastolic values of diameter and pressure. The schematic diagram shown in Figure S3 summarizes the data needed to quantify the bulk mechanical properties.

**Layer-Specific Properties and Transmural Stress Distributions** – Studies of arterial mechanobiology require separate information on the properties of and stresses within the media and adventitia\(^5\). Hence, the biaxial data were further analyzed using a novel bi-layered model of the arterial wall. Briefly, this model is based on layer-specific stored energy functions that are similar to that used to model the mean behavior of the wall, though with some subtle differences. Whereas classical analyses of wall stress use an unloaded configuration as a reference\(^9\), our structurally-motivated, layer-specific relations accounted for the different constituents (e.g., elastic fibers or multiple families of collagen fibers) having different “pre-stresses” in a homeostatic in vivo configuration, which was used as a computational reference\(^5\). These pre-stresses result from constituent-specific deposition stretches, which were estimated by recording changes in dimensions before and after elastase exposure (see Biaxial Mechanical Testing). While the 8 constituent-specific model parameters were prescribed to be the same in the two layers of the arterial wall, differences in their relative abundance (\( \phi^i \), area fractions as estimated from the histological image analysis) endowed the media and adventitia with different mechanical responses. In particular, we assumed a mass-averaged stored energy function of the form:

\[
W = \phi^e W^e(F^e) + \phi^m W^m(\lambda^m) + \sum_{j=1}^{4} \phi^c W^c(\lambda^c_j) \quad (S.7)
\]

where the superscripts \( i = e, m \) and \( c \) refer to elastic fibers, smooth muscle, and each of four families of collagen fibers (\( j = 1,2,3,4 \)), \( \phi^i \) and \( W^i \) are the mass fractions and the stored energy functions for the constituents that constitute the mixture, \( F^e \) is the deformation gradient tensor experienced by the elastic fibers, and \( \lambda^m \) and \( \lambda^c_j \) are the stretches experienced by the smooth muscle and the \( j \)th family of collagen fibers. Similar to Equation S.1, we let the behavior of the elastic fibers be described by a neo-Hookean stored energy function:

\[
W^e = \frac{c^e}{2} (I_{C^e} - 3), \quad (S.8)
\]

where \( c^e \) is a material parameter with the dimension of a stress (kPa), \( I_{C^e} = tr(C^e) \), \( C^e = F^e \text{T} F^e \) and \( F^e = F G^e_h \), with \( G^e_h \) the deposition stretch tensor between the natural (stress-free) configuration of the elastic fibers and the homeostatic reference configuration, with \( F \) depending on the specific deformation of the wall. Furthermore, \( G^e_h \) was assumed to be principal, with circumferential deposition stretches within the interval [1.94, 2.05] for the Sham and [1.39, 1.84] for the Ang II treated vessels, axial deposition stretches within the interval [1.55, 1.60] for the Sham and [1.26, 1.52] for the Ang II treated vessels, and the radial deposition stretch computed based on incompressibility. The nonlinear response of collagen fibers, resulting from the progressive engagement of undulated fibers, was modeled using a Fung-type exponential relationship. Again, because it is not possible to
delineate the behavior of circumferentially oriented collagen fibers in the media and associated smooth muscle, their combined contributions were similarly modeled using a Fung exponential. Hence,

\[ W^m = \frac{e_1^m}{4c_2^m} \left[ e^{e_2^m (IV^m - 1)^2} - 1 \right], \quad (S.9) \]

\[ W^c_j = \frac{c_1^c}{4c_2^c} \left[ e^{e_2^c (IV^c - 1)^2} - 1 \right], \quad (S.10) \]

where \( e_1^m \) and \( c_1^c \) are model parameters with the dimension of a stress (kPa), while \( e_2^m \) and \( c_2^c \) are dimensionless. Neither the smooth muscle nor the collagen fibers were assumed to have any radial orientation. The stretch experienced by smooth muscle was thus obtained by projecting \( \mathbf{C} \) along the cell axis,

\[ \lambda^m = \sqrt{IV^m} = G^m_h \sqrt{\mathbf{C} : (\mathbf{M}^m \otimes \mathbf{M}^m)}, \quad (S.11) \]

where \( G^m_h \) is the deposition stretch between the natural (stress-free) and homeostatic (reference) configurations, and \( \mathbf{M}^m = [0, \sin \alpha_0^m, \cos \alpha_0^m] \) is a unit vector representing smooth muscle cell orientation in the reference configuration. \( G^m_h \) was assigned within the range [1.10, 1.12] for both experimental groups, Sham and Ang II infused. Similarly, for the stretch in the direction of the collagen fibers,

\[ \lambda^c_j = \sqrt{IV^c_j} = G^c_j_h \sqrt{\mathbf{C} : (\mathbf{M}^c_j \otimes \mathbf{M}^c_j)}, \quad (S.12) \]

where \( G^c_j_h \) is the deposition stretch and \( \mathbf{M}^c_j = [0, \sin \alpha_0^c_j, \cos \alpha_0^c_j] \) is a unit vector that identifies the dominant orientation of the \( j^{th} \) family of collagen fibers. Values of \( G^c_j_h \) within the interval [1.04, 1.06] were assigned to each family of collagen fibers for both the Sham and the Ang II infused mice. Two additional parameters, \( \beta_\theta \) and \( \beta_\zeta \), which describe the portion of collagen fibers oriented circumferentially and axially, respectively, were also estimated from experimental data, bringing the total count of estimated tensile parameters to 8 (see Table S3).

Because stress and stiffness are defined pointwise, the components of Cauchy stress \( (\sigma_{ij}) \) and linearized stiffness \( (\epsilon_{ijk}) \) were again computed using Equations S.3 and S.5, where the contribution of each constituent was modulated by the associated mass fractions \( \phi^l \), and specific deformations experienced by the elastic fibers, smooth muscle, and collagen fibers were accounted for through their unique deposition stretches \( G^l_h \). Once the material properties were known, classical relations were used to enforce equilibrium under the different pressure – axial load conditions, which in turn allowed calculation of transmural distributions of wall stress. It is noted that equation (S.4)_1 is the well-known Laplace equation; it describes the mean value of circumferential stress in a pressurized tube independent of the specific constitutive relation and thus is universal. For this reason, the Laplace equation also served as a convenient check of the goodness of the calculation of circumferential stress across the aortic wall based on our mixture-
based bi-layered model of the wall. The schematic drawing shown in Figure S4 summarizes the data needed to quantify the layer-specific transmural mechanical properties.

**Statistics.** Data in the manuscript are presented as mean ± standard error of the mean (SEM). Geometric and material metrics were compared across all treatment groups (Sham, 2wk Ang II, and 4wk Ang II) using a one-way ANOVA followed by a post-hoc Bonferroni correction. Several levels of significance were considered (P < 0.05, P < 0.01 and P < 0.001) and differences are indicated in figures and tables as appropriate.

**Results**

As revealed by the results in Tables S1 and S2, there was no significant difference in geometry or mechanical properties between the 2-week (n = 5) and 4-week (n = 5) Ang II treated proximal descending thoracic aortas at systolic or mean arterial pressures. For this reason, we performed the bi-layered stress analysis only for the Sham and 2-week Ang II aortas. In contrast, there were many significant differences between Sham and Ang II treated, as discussed at length in the parent paper. Of particular note, these findings suggest that the dramatic remodeling of the wall, in response to a progressive increase in blood pressure over the initial 10 to 14 days of 490 ng/kg/min angiotensin infusion, was nearly complete by 2 weeks, but persisted at least to 4 weeks. Given our focus on early remodeling, we did not examine changes over longer periods and we did not assess potential long-term recovery following the cessation of angiotensin infusion. Both issues would be of interest, but were beyond the present scope, which revealed a strongly biaxial and layer-specific early remodeling that was mechanically maladaptive.

It is suggested that our bi-layered, multi-constituent, nonlinear model of the aorta provides the most detailed assessment of wall mechanics in induced hypertension to date. One limitation, however, is that the model cannot discriminate within the media the circumferentially oriented collagen and circumferentially oriented passive smooth muscle. That the primary change in stiffness was seen in the axial direction in the adventitia suggests, however, that it was the collagen in the adventitia, not the passive smooth muscle in the media, that changed the most with induced hypertension.

Consistent with results in the parent paper, these experimental and computational findings collectively suggest that an early and exuberant deposition of collagen, particularly in the adventitia, results in a persistent, mechanically maladaptive response that is likely driven in large part by inflammation. Given the potential positive feedback loops that relate the associated increase in structural stiffness with hypertension\(^2\), there is clearly a need for early intervention.
References


Table S1 – Morphological and mechanical data (mean ± SEM) for the proximal descending thoracic aorta from Sham and both 2-week and 4-week Ang II treated mice. Pressure-dependent values were calculated at group-specific mean arterial pressures (MAP) and systolic pressures when specified. *P<0.05, **P<0.01, ***P<0.001 vs. Sham. †P<0.05, ††P<0.01 vs. 2wk Ang II.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>2wk Ang II</th>
<th>4wk Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Unloaded Dimensions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer Diameter (μm)</td>
<td>888 ± 9</td>
<td>1142 ± 34***</td>
<td>1149 ± 33***</td>
</tr>
<tr>
<td>Wall Thickness (μm)</td>
<td>112 ± 3</td>
<td>191 ± 5***</td>
<td>210 ± 2***,††</td>
</tr>
<tr>
<td><strong>In-vitro</strong> Axial Length (mm)</td>
<td>4.9 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>6.3 ± 0.2***,†</td>
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<td><strong>Systolic Dimensions</strong></td>
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<td>Outer Diameter (μm)</td>
<td>1412 ± 13</td>
<td>1438 ± 39</td>
<td>1452 ± 23</td>
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<tr>
<td>Inner Radius (μm)</td>
<td>667 ± 7</td>
<td>617 ± 23</td>
<td>617 ± 10</td>
</tr>
<tr>
<td>Wall Thickness (μm)</td>
<td>39 ± 1</td>
<td>102 ± 7***</td>
<td>109 ± 4***</td>
</tr>
<tr>
<td><strong>In-vivo</strong> Axial Stretch</td>
<td>1.62 ± 0.01</td>
<td>1.34 ± 0.02***</td>
<td>1.34 ± 0.02***</td>
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<tr>
<td><strong>Systolic Cauchy Stresses (kPa)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Circumferential</td>
<td>276.8 ± 5.9</td>
<td>138.0 ± 13.2***</td>
<td>129.8 ± 5.3***</td>
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<tr>
<td>Axial</td>
<td>289.9 ± 9.4</td>
<td>112.4 ± 12.8***</td>
<td>106.7 ± 7.6***</td>
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<tr>
<td><strong>Systolic Stiffness (MPa)</strong></td>
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<tr>
<td>Circumferential</td>
<td>1.89 ± 0.07</td>
<td>1.72 ± 0.06</td>
<td>1.70 ± 0.11</td>
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<tr>
<td>Axial</td>
<td>3.84 ± 0.14</td>
<td>2.39 ± 0.17***</td>
<td>2.42 ± 0.16***</td>
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<tr>
<td><strong>Systolic Structural Stiffness (N/m)</strong></td>
<td>73.5 ± 2.1</td>
<td>177.4 ± 18.6**</td>
<td>188.1 ± 18.9***</td>
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<tr>
<td><strong>Systolic Stored Energy (kPa)</strong></td>
<td>73.6 ± 2.9</td>
<td>19.4 ± 3.4***</td>
<td>18.7 ± 1.9***</td>
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<td></td>
<td>MAP Dimensions</td>
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<td>-----------------------</td>
</tr>
<tr>
<td><strong>Outer Diameter (μm)</strong></td>
<td>1374 ± 12</td>
<td>1419 ± 37</td>
<td>1430 ± 24</td>
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<tr>
<td><strong>Inner Radius (μm)</strong></td>
<td>647 ± 6</td>
<td>606 ± 22</td>
<td>603 ± 11</td>
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<tr>
<td><strong>Wall Thickness (μm)</strong></td>
<td>40 ± 1</td>
<td>104 ± 7***</td>
<td>111 ± 4***</td>
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<tr>
<td><strong>In-vivo Axial Stretch</strong></td>
<td>1.62 ± 0.01</td>
<td>1.34 ± 0.02***</td>
<td>1.34 ± 0.02***</td>
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<tr>
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<th>MAP Cauchy Stresses (kPa)</th>
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<tr>
<td><strong>Circumferential</strong></td>
<td>228.4 ± 4.9</td>
<td>114.9 ± 10.5***</td>
<td>103.7 ± 3.9***</td>
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</tr>
<tr>
<td><strong>Axial</strong></td>
<td>263.1 ± 9.1</td>
<td>98.1 ± 10.9***</td>
<td>91.9 ± 8***</td>
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<th>MAP Stiffness (MPa)</th>
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<tr>
<td><strong>Circumferential</strong></td>
<td>1.47 ± 0.05</td>
<td>1.33 ± 0.05</td>
<td>1.22 ± 0.08*</td>
<td></td>
</tr>
<tr>
<td><strong>Axial</strong></td>
<td>3.16 ± 0.11</td>
<td>1.94 ± 0.14***</td>
<td>1.91 ± 0.17***</td>
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<table>
<thead>
<tr>
<th></th>
<th>MAP Structural Stiffness (N/m)</th>
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<tbody>
<tr>
<td>58.7 ± 1.6</td>
<td>139.1 ± 14.8**</td>
<td>136.5 ± 13.7**</td>
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<table>
<thead>
<tr>
<th></th>
<th>MAP Stored Energy (kPa)</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>66.2 ± 2.6</td>
<td>17.3 ± 3***</td>
<td>16.5 ± 1.6***</td>
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</table>

|                      | Distensibility (MPa⁻¹)       | 16.8 ± 0.7            | 6.3 ± 0.8***          | 6.3 ± 0.6***          |
Table S2 – Best-fit values of the model parameters used to characterize the mean (i.e., bulk) mechanical properties of the proximal descending thoracic aorta from Sham and the 2-week and 4-week Ang II treated groups based on the four-fiber-family form of the elastic energy function $W$ (Equation S.1).

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Elastic Fibers</th>
<th>Axial Collagen</th>
<th>Circumferential Collagen + SMC</th>
<th>Symmetric Diagonal Collagen</th>
<th>Error</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$c$ (kPa)</td>
<td>$c_1^x$ (kPa)</td>
<td>$c_2^x$ (kPa)</td>
<td>$c_1^y$ (kPa)</td>
<td>$c_2^y$ (kPa)</td>
</tr>
<tr>
<td>Sham</td>
<td>18.536</td>
<td>25.370</td>
<td>0.036</td>
<td>16.593</td>
<td>0.078</td>
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<tr>
<td>2wk Ang II</td>
<td>18.912</td>
<td>2.008</td>
<td>2.988</td>
<td>12.320</td>
<td>0.138</td>
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<tr>
<td>4wk Ang II</td>
<td>20.652</td>
<td>1.074</td>
<td>3.821</td>
<td>7.593</td>
<td>0.109</td>
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Table S3 – Best-fit material parameters used to characterize the layer-specific mechanical properties of the descending thoracic aorta from Sham and 2-week Ang II treated groups based on a mass-averaged form of the elastic energy function $W$ (Equation S.7).

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Elastic Fibers</th>
<th>SMCs</th>
<th>Collagen Fibers</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$c^e$ (kPa)</td>
<td>$c_1^m$ (kPa)</td>
<td>$c_2^m$ (kPa)</td>
<td>$c_1^c$ (kPa)</td>
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<tr>
<td>Sham</td>
<td>203.837</td>
<td>36.564</td>
<td>20.251</td>
<td>5481.533</td>
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<tr>
<td>2wk Ang II</td>
<td>259.304</td>
<td>66.348</td>
<td>6.671</td>
<td>2695.967</td>
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</table>
Figure S1: Telemetry recordings of blood pressure in mice at baseline, and during 2 or 4 weeks of either angiotensin II (490 ng/kg/min) or vehicle (sham) infusion.
Figure S2: A. Schematic drawing of the custom biaxial mechanical testing device. The primary components of the system include a set of linear actuators (1) and tri-axis translational stages (2), an in-line axial force transducer (3) attached to a set of pulled glass micropipets (4), a mounting stage (5), and the isolated vessel segment (6) to be tested in a temperature regulated bath containing physiologic media (7). B. Representative video-images acquired during mechanical testing are shown for samples from each of the three groups. For comparison images are taken at the estimated group-specific *in vivo* axial stretch and pressure of 140 mmHg.
Figure S3: Schematic drawing illustrating the pressurization and axial stretching of an artery (cross-section shown above and side-view shown below) from its intact, traction-free, unloaded reference configuration. Using the deformation gradient tensor $\mathbf{F}$ and the associated geometric changes in radius and thickness, the equilibrium equations (bottom right) can be used with an appropriate constitutive equation $W$ (cf. Eq. S.1) to quantify the bulk mechanical properties of the specimen based on the experimentally measurable quantities shown.
Figure S4: Schematic drawing illustrating the pressurization of an artery (cross-section above, side-view below) from its loaded, homeostatic reference configuration. The deposition stretch tensor between the natural (stress-free) configuration (far left) and the homeostatic reference configuration (middle) is given by $G^i$ for each constituent. Using the deformation gradient tensor $F$ associated with a change in pressure (e.g., $F^{140}$ or $F^{80}$) to obtain current configurations (far right), along with the associated geometric changes in radius and thickness, the integral form of the equilibrium equations (bottom right) can be used with an appropriate constitutive equation $W$ (cf. Eq. S.7) to quantify the layer-specific, transmural mechanical properties of the sample.