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Biaxial Mechanical Properties of the Inferior Vena Cava in C57BL/6 and CB-17 SCID/bg Mice

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Abstract

Multiple murine models have proven useful in studying the natural history of neovessel development in the tissue engineering of vascular grafts. Nevertheless, to better understand longitudinal changes in the biomechanics of such neovessels, we must first quantify native tissue structure and properties. In this paper, we present the first biaxial mechanical data for, and nonlinear constitutive modeling of, the inferior vena cava from two models used in tissue engineering: wild-type C57BL/6 and immunodeficient CB-17 SCID/bg mice. Results show that inferior vena cava from the latter are significantly stiffer in the circumferential direction, both materially (as assessed by a stored energy function) and structurally (as assessed by the compliance), despite a lower intramural content of fibrillar collagen and similar wall thickness. Quantifying the natural history of neovessel development in different hosts could lead to increased insight into the mechanisms by which cells fashion and maintain extracellular matrix in order to match best the host stiffness while ensuring sufficient vascular integrity.

Keywords

Transgenic mice; Tissue engineered vascular graft; Biaxial mechanical testing; strain energy; Inferior vena cava

Introduction

Technological advances in genetics, molecular biology, in vivo imaging, and mechanical testing have resulted in the widespread use of mouse models in vascular research. In particular, mice enable the use of a vast array of molecular reagents and genetic mutations not readily available for use with other species. Mutant models, for example, have allowed investigators to begin to delineate biomechanical effects of genes that code structurally

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Conflict of interest statement

None of the authors have any professional or financial conflict of interest.

significant proteins within the arterial wall, including elastin and type I collagen (e.g., Wagenseil et al., 2005; Pfeiffer et al., 2005). Amongst the many different mouse models available, immunodeficient mice offer particular promise for research on vascular xenografts, including tissue engineered constructs. That is, these mice readily accept xeno transplants that allow roles of different human cell types to be investigated in vivo. Our group, for example, has used severe combined immunodeficiency / beige (CB-17 SCID/bg) mice to study the natural history of neovessel formation that begins with the implantation of a polymer scaffold seeded with human bone marrow derived cells and results in the formation of a neovessel that resembles a native blood vessel both structurally and functionally (Roh et al., 2010).

Given our interest in the use of tissue engineered vascular grafts (TEVGs) in congenital heart surgery, we have implanted interposition grafts in the inferior vena cava (IVC) in both C57BL/6 mice (e.g., Hibino et al., 2011; Naito et al., 2012) and CB-17 SCID/bg mice (Nelson et al., 2008; Roh et al., 2010). The C57BL/6 model faithfully recapitulates neovessel formation seen in humans, albeit over a shorter time course (Hibino et al., 2011). We submit that C57BL/6 and CB-17 SCID/bg models will continue to prove useful in elucidating cellular and molecular mechanisms underlying vascular neotissue formation (cf. Roh et al., 2010; Hibino et al., 2011), particularly given recent observations that neovessel composition and mechanics evolve toward native within 6 months (Naito et al., 2013).

To assess well the development of a neovessel, however, there is a need for detailed information on the biomechanics of the native vessels, which serve both as distal and proximal hosts for the TEVG and the long term target for mimicry. Toward this end, we collected, quantified, and compared in vitro biaxial mechanical data for the IVC from both C57BL/6 and CB-17 SCID/bg mice. Results revealed compositional and structural differences, with the IVC from the CB-17 SCID/bg mice stiffer.

Materials and Methods

Specimen Preparation

All animal care and use was approved by the IACUC at Yale University. Briefly, $n = 5$ C57BL/6 and $n = 5$ CB-17 SCID/bg female mice were euthanized at approximately 8 weeks of age via an intraperitoneal injection of Beuthanasia-D. The IVC was then isolated by gentle dissection and excised from just below the left renal vein to the distal bifurcation. Samples 5 to 7 mm in length were transferred to the laboratory in phosphate buffered saline (PBS), then cannulated on custom size-matched glass cannulae and secured with 6-0 suture. The cannulated vessels were checked for leakage by gentle inflation with air, then a small drop of glue was applied at each suture and the vessels were mounted within a custom computer-controlled biaxial testing system (Gleason et al., 2004).

Mechanical Tests

The initial unloaded dimensions (i.e., length L and outer diameter OD) and zero-offset of the axial force transducer were recorded and the specimen was extended to its in vivo axial stretch λ_z^{iv} , which is defined as the ratio of the axial length after and before excision. Note that length was controlled by two precision stepper motors mounted at opposite ends of the testing chamber, and luminal pressure P was controlled hydrostatically based on measurements by in-line distal and proximal pressure transducers. Changes in diameter were measured on-line using a side-mounted video camera, and axial force f_T was measured using a transducer connected to the distal cannula. Following equilibration in PBS for 30 minutes with a flow of 10 ml/min and pressure of 10 mmHg, the specimen was preconditioned via 4 cycles of pressurization from 1 to 20 mmHg while maintained stretched at λ_z^{iv} . After the

unloaded dimensions were measured again, mechanical testing was performed by sequentially pressurizing the specimens cyclically from 1 to 20 mmHg at three different axial extensions, the in vivo value λ_z^{iv} as well as $\pm 5\%$ of λ_z^{iv} .

Data Analysis

Structural stiffness was quantified via the area compliance, namely

$$C(P) = \frac{\partial A(P)}{\partial P} \quad (1)$$

where $A(P)$ is the luminal cross-sectional area at each value of intraluminal pressure P . Inner radius r_i and wall thickness h were calculated from measured quantities by assuming incompressibility, namely

$$r_i = \sqrt{r_a^2 - \frac{G}{V} / \pi l}, h = r_a - r_i \quad (2)$$

where $\frac{G}{V}$ is the measured wall volume in the unloaded state, l is the current length between the sutures, and r_a is the video measured outer radius. Initial (unloaded) circumference and thickness H were also estimated from histological sections that were fixed at zero pressure.

Mean values of the intramural Cauchy stresses were estimated via (Humphrey, 2002)

$$\sigma_\theta^{exp} = \frac{P r_i}{r_a - r_i}, \sigma_z^{exp} = \frac{f_T + P \pi r_i^2}{\pi (r_a^2 - r_i^2)}, \quad (3)$$

where P is the transmural pressure (i.e., luminal pressure assuming negligible perivascular pressure) and f_T is the axial force measured by the transducer. Circumferential stretch was calculated at the mid-wall, with $r_{mid} = (r_a + r_i) / 2$, whereas axial stretch was calculated based on overall length and radial stretch was estimated using incompressibility. Hence, mean stretch ratios were

$$\lambda_\theta = \frac{r_{mid}}{r_{mid}^0}, \lambda_z = \frac{l}{L}, \lambda_r = \frac{1}{\lambda_\theta \lambda_z}, \quad (4)$$

where r_{mid}^0 and L are radius and axial length in the unloaded configuration following preconditioning.

Constitutive Modeling

To quantify the passive mechanical data, we used a structurally motivated stored energy function W that has been found to describe well the behavior of diverse murine arteries (cf. Gleason et al., 2008; Eberth et al., 2009; Wan et al., 2010). This so-called four-fiber family model is written as

$$W = \frac{c}{2} (I_c - 3) + \sum_k \frac{c_1^k}{4c_2^k} \left\{ \exp \left[c_2^k \left((\lambda^k)^2 - 1 \right)^2 \right] - 1 \right\} \quad (5)$$

where c , c_1^k and c_2^k are material parameters that must be determined via nonlinear regression; the index $k = 1, 2, 3, 4$ represents assumed axial, circumferential, and two symmetric diagonal fiber families. I_c is the first principal invariant of the right Cauchy-Green tensor, whereby $I_c = \lambda_\theta^2 + \lambda_z^2 + \lambda_r^2$, and λ^k is the stretch of the k^{th} fiber family, determined by

$$\lambda^k = \sqrt{\lambda_\theta^2 \sin^2 \alpha_0^k + \lambda_z^2 \cos^2 \alpha_0^k}, \quad (6)$$

where α_0^k is the angle of the k^{th} fiber family with respect to the axial direction of the vessel in a reference (unloaded) configuration. Equations 5 and 6 are motivated by a possible isotropic contribution to load bearing by an amorphous elastin-dominated matrix (notwithstanding evidence of anisotropy of elastin in veins by Sokolis (2012)) and anisotropic contributions by four families of oriented collagen fibers, noting that the presence of four families also allows the model to account phenomenologically for possible lateral cross-linking or interactions amongst collagen fibers. The mean in-plane components (i.e., circumferential and axial) of the Cauchy stress can thus be computed via (Humphrey, 2002)

$$\sigma_\theta^{th} = \lambda_\theta \frac{\partial W}{\partial \lambda_\theta} - \lambda_r \frac{\partial W}{\partial \lambda_r} \quad (7)$$

$$\sigma_z^{th} = \lambda_z \frac{\partial W}{\partial \lambda_z} - \lambda_r \frac{\partial W}{\partial \lambda_r} \quad (8)$$

where λ_r and λ_z if thin-walled. We did not include possible residual stress related opening angles given our interest in descriptors of mean wall stress, which tend to represent well the stress field under normal (homeostatic) conditions (Humphrey, 2002).

Best-fit values of the eight unknown model parameters ($c, c_1^1, c_2^1, c_1^2, c_2^2, c_1^{3,4}, c_2^{3,4}, \alpha_0$) were estimated by minimizing the following nonlinear objective function,

$$e = \sum_{i=1}^N \left[\left(\frac{P^{exp} - P^{th}}{P^{exp}} \right)_i^2 + \left(\frac{f^{exp} - f^{th}}{f^{exp}} \right)_i^2 \right] \quad (7)$$

where N is the number of data points (typically ~200 points) obtained by combining pressurization data at the three different values of axial stretch. Initial guesses for the minimization were $(c, c_1^1, c_2^1, c_1^2, c_2^2, c_1^{3,4}, c_2^{3,4}, \alpha_0) = (1 \text{ kPa}, 1 \text{ kPa}, 1, 1 \text{ kPa}, 1, 1 \text{ kPa}, 1, 45 \text{ deg} - \text{prescribed in radians})$, and the convergence criterion tolerance was set at 10^{-10} .

Histological and Biochemical Analysis

Following mechanical testing, all specimens were unloaded and fixed in formalin overnight, then embedded in paraffin and sectioned at 4 microns. Sections were stained using hematoxylin-eosin (H&E) and visualized using an Olympus BX/51 microscope equipped with a DP70 digital camera. In addition, approximately 2-mm long segments of fresh IVC were homogenized and lyophilized for quantitative biochemical analysis. Elastin content was determined using a FastinTM colorimetric assay (Biocolor Assays, Inc.). Lyophilized samples were weighed and transferred to 1.5 ml microcentrifuge tubes containing 100 μ L 0.25 M oxalic acid. The tubes were then placed on a heat block for 60 minutes at 100 ° to convert insoluble elastin to water-soluble alpha-elastin. A standard curve was created using controls provided with the kit and elastin content was detected via spectrometry at 513 nm after precipitation and dye binding of alpha-elastin. Collagen content was determined by a SircolTM colorimetric assay (Biocolor Assays, Inc.). Lyophilized samples were weighed and transferred to 1.5 ml microcentrifuge tubes containing 200 μ L of pepsin (Sigma-Aldrich) and 0.1 mg/ml of 0.5M acetic acid to solubilize collagen with overnight incubation. A standard curve was created using controls provided with the kit and collagen content was

detected via spectrometry at 555 nm after precipitation and dye (Sirius Red) binding of collagen.

Statistical Analysis

Data are presented as mean $\pm$ standard deviation and were compared between C57BL/6 and CB-17 SCID/bg using a student's t-test, with $p < 0.05$ considered significant.

Results

At 8 weeks of age, the CB-17 SCID/bg mice were significantly heavier than the C57BL/6 mice: 23.0 ± 0.32 vs. 18.4 ± 0.68 g ($p < 0.001$). Consistent with this difference in body mass, the unloaded outer diameter was also greater in the CB-17 SCID/bg than the C57BL/6 mice: 810 ± 11.47 vs. 711 ± 26.84 μ m ($p < 0.01$). Neither unloaded wall thickness (21.08 ± 0.72 vs. 22.20 ± 1.26 μ m) nor the in vivo axial stretch (1.43 ± 0.03 vs. 1.39 ± 0.03) was different, however.

Figure 1 shows representative photographs of an IVC during mechanical testing. At the in-vivo stretch, diameter increased approximately two-fold when pressure increased from 0 to 20 mmHg. As it can be seen, however, the axially loaded vessels tended to collapse at 0 mmHg, hence cyclic testing was performed over the range 1 to 20 mmHg. Figure 2a compares mean pressure – diameter responses at λ_z^{iv} following preconditioning. Although outer diameter was initially greater in the CB-17 SCID/bg IVCs, it increased more in the C57BL/6 IVCs (90% for the former versus 152% for the latter, $p < 0.05$), especially above 5 mmHg. That IVCs from the C57BL/6 mice were indeed more compliant at low pressures (< 8 mmHg) is revealed by Figure 2b. The difference in compliance was greatest at 2 mmHg (0.44 ± 0.2 vs. 0.20 ± 0.05 mm²/mmHg, $p < 0.05$), but became small with increasing pressure.

Circumferential Cauchy stress-stretch plots are shown in Figure 3 for all 10 specimens (solid symbols show data from CB-17 SCID/bg and open symbols show data from C57BL/6 mice). Consistent with pressure-diameter and compliance data (which reflect structural stiffness), the IVCs from the CB-17 SCID/bg mice appeared materially stiffer than those from the C57BL/6 mice, particularly above a stress of 20 kPa. The four-fiber family strain energy function fit very well the pressure-diameter and reasonably well the associated force-pressure data for IVCs from both genotypes (not shown), which resulted in very good predictions of the circumferential stress-stretch behavior (e.g., Figure 4a) and reasonable predictions of the axial stress-stretch behavior (Figure 4b). This difference in describing circumferential versus axial behavior is comparable to that of others (cf. Sokolis, 2012). Best-fit values of the model parameters are listed in Table 1. While individual model parameter values were not statistically different between the two groups, values of both c_2^1 and c_2^2 were higher for the CB-17 SCID/bg IVCs than the C57BL/6 IVCs. Recall that these parameters are meant to reflect the collagen dominated behavior in the circumferential direction, which the data revealed to be stiffer in the CB-17 SCID/bg IVCs (Figures 3 and 4). This general finding of greater stiffness of IVCs from the immunodeficient mice was confirmed by plots of the stored energy (Figure 5).

Quantification of elastin by biochemical assay (Fastin) revealed no statistically significant difference between the two groups, consistent with a lack of significant difference in the best-fit value of the parameter c in the stored energy, which is thought to reflect the elastin dominated behavior. In contrast, the Sircol assay revealed a significantly higher collagen content in the C57BL/6 IVCs than in the CB-17 SCID/bg IVCs (12.3 ± 4.7 vs. 6.0 ± 2.9 , $p < 0.05$). Given the stiffer circumferential behavior exhibited by the CB-17 SCID/bg IVCs, and

the higher values of the associated model parameters, this finding is a good reminder that it is the organization (e.g., transmural distribution, orientation, fiber diameter, cross-linking, and proportion of type I to III), not just amount, of a constituent that manifests macroscopically as stiffness.

Discussion

In contrast to the extensive literature on arterial wall mechanics (cf. Humphrey, 2002), much less is known about the mechanical properties of veins. Although this discrepancy in attention is due in large part to the preponderance of life threatening vascular diseases in arteries (e.g., atherosclerotic lesions, aneurysms, and dissections), knowledge of mechanical properties of veins is important. Veins are widely used as autologous arterial grafts and their response in arterio-venous fistulas is fundamental to clinical success in dialysis treatment.

Careful studies of the mechanical properties of veins date back to at least the mid-1970s (Azuma and Hasegawa, 1973; Wesley et al., 1975). Interestingly, the associated uniaxial and biaxial data on canine and human femoral, jugular, and saphenous veins revealed distensibilities / extensibilities that ranged from ~1.5 to 2.5, which is consistent with recent measurements on porcine and bovine jugular veins (Rossmann et al., 2010; Sokolis, 2012) as well as those found herein for the murine IVC (cf. Figure 3). McGilvray et al. (2010) reported data from in-plane biaxial testing of IVC from C57BL/6 mice. In contrast to the present findings, they reported circumferential extensions on the order of 1.5, but much higher stresses (e.g., 2 MPa and higher, values that are uncommon in such tests) than measured herein. McGilvray and colleagues used the fiber-dispersion model of Gasser et al. (2006) to model their data, which provided a suitable fit. Because of differences in functional forms, however, we are not able to compare directly the quantitative results or to determine reasons for the different ranges of stress.

Perhaps the most comprehensive study to date on the biaxial mechanics of veins is that of Sokolis (2012), who reported distensibilities / extensibilities in the range 1.5 to 2.5. He showed further that the present four-fiber family model described porcine jugular vein data far better than did either traditional Fung-type models or a similar two-fiber family model. Although he suggested that replacement of the one-parameter neo-Hookean contribution to W with a three-parameter quadratic term improved the fit over the present four-fiber family model, this assessment did not account for the increase in the number of parameter values (e.g., via the Akaike Information Criterion). We used the present four-fiber family model to facilitate future comparisons of the mechanics of murine arteries (cf. Gleason et al., 2008) and veins, which could be important in studying both vein graft and arterio-venous fistula models. Sokolis (2012) also noted marked differences in reported values of in vivo axial stretch, opening angle, and other mechanical metrics amongst veins from different locations and species (cf. Desch and Weizsacker, 2007). Hence, comparisons should be made appropriately. Nevertheless, our best-fit parameter values are comparable to those reported by Sokolis for porcine jugular veins, with the exception that our axial parameters tended to be higher. Given that these parameters contribute significantly to the axial behavior (cf. Fig 10 in Sokolis, 2012), and that our measured in vivo stretch (~1.4) was significantly less than that for the porcine jugular (~1.65), suggests that the four-fiber family model captured real differences between these two types of veins.

Of particular importance herein, we have previously used interposition IVC grafts in both C57BL/6 and CB-17 SCID/bg mice to study the mechanisms and natural history of the formation of a neovessel from a degradable polymer scaffold. The tacit assumption in the field of vascular tissue engineering is that one not only seeks a graft with sufficient suture retention and burst strength, one ultimately seeks a graft that matches well the compliance of

the host vessel. The present findings reveal that host IVCs in CB-17 SCID/bg and C57BL/6 mice exhibit both a different structural composition (cf. Figure 6) and stiffness (cf. Figures 2 and 3). Presumably, therefore, achieving an optimal graft in these two genotypes should result in neovessels having different structure and properties as well.

Like arteries, veins tend to consist of three layers: intima, media, and adventitia. Unlike arteries, however, the media in veins tends not to be well demarcated by elastic laminae and there tends to be an overall preponderance of collagen (Svejcar et al., 1962). Nevertheless, veins are capacitance vessels and they have greater compliance at low pressures despite becoming very stiff at supra-physiologic values of pressure (e.g., > 30 mmHg). Clearly, there is a need for increased attention to the orientation, undulation, diameter, and cross-linking of venous collagen to understand these unique properties (cf. Sokolis, 2012). Indeed, it is interesting that the CB-17 SCID/bg IVC had less collagen and yet greater stiffness than the C57BL/6 IVC.

There has been increasing discussion in the literature regarding roles of inflammatory cells in arterial stiffening, including that which manifests in aging and hypertension (cf. McEniery and Wilkenson, 2005; Harrison et al., 2012; Park and Lakatta, 2012). For example, patients with rheumatoid arthritis exhibit increased arterial pulse wave velocity, an indicator of arterial stiffness, and treatment with anti-tissue necrosis factor- reduces arterial stiffening. Similarly, depletion of neutrophils and macrophages in angiotension-II infusion models of hypertension lessens the elevation of blood pressure (Wenzel et al., 2011). There has been less attention to the effects of inflammatory cells on the mechanical properties of veins, however. Interestingly, the present results revealed that the IVCs from immunodeficient mice (CB-17 SCID/bg) exhibited greater stiffness compared with C57BL/6. Given the similar values of unloaded wall thickness, this difference likely reflects underlying differences in microstructure (i.e., material, not structural, stiffness). There is clearly a need to study further the underlying reasons for differences in wild-type and immunodeficient IVCs. In particular, although we previously showed the importance of monocytes / macrophages in neovessel formation (Roh et al., 2010), there is a need to investigate possible roles of other inflammatory cells.

In conclusion, we present the first biaxial mechanical data and nonlinear constitutive descriptor to compare the biomechanics of the inferior vena cava in wild-type (C57BL/6) and immunodeficient (CB-17 SCID/bg) mice. The fit of the model to biaxial data was similar to recent findings for other veins (Sokolis, 2012). Such results promise to aid in the interpretation of data on the natural history of tissue engineered neovessel development in common mouse models (cf. Naito et al., 2013) as well as in the development of computational models of such development (cf. Niklason et al., 2010).

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References

- Azuma T, Hasegawa M. Distensibility of the vein: From the architectural point of view. *Biorheology*. 1973; 10:469–479. [PubMed: 4772019]
- Desch GW, Weizsacker HW. A model for passive elastic properties of rat vena cava. *J Biomech*. 2007; 40:3130–3145. [PubMed: 17512529]
- Eberth JF, Taucer AI, Wilson E, Humphrey JD. Mechanics of carotid arteries in a mouse model of Marfan Syndrome. *Ann Biomed Eng*. 2009; 37(6):1093–104. 2009. [PubMed: 19350391]

- Gasser TC, Ogden RW, Holzapfel GA. Hyperelastic modelling of arterial layers with distributed collagen fibre orientations. *J R Soc Interface*. 2006; 3(6):15–35. [PubMed: 16849214]
- Gleason RL, Dye WW, Wilson E, Humphrey JD. Quantification of the mechanical behavior of carotid arteries from wild-type, dystrophin-deficient, and sarcoglycan-delta knockout mice. *J Biomech*. 2008; 41(15):3213–3218. [PubMed: 18842267]
- Gleason RL, Grey SP, Wilson E, Humphrey JD. A multiaxial computer-controlled organ culture and biomechanical device for mouse carotid arteries. *ASME J Biomech Engr*. 2004; 126:787–795.
- Harrison DG, Marvar PJ, Titze JM. Vascular inflammatory cells in hypertension. *Front Physiol*. 2012; 3(128):1–8. [PubMed: 22275902]
- Hibino N, Yi T, Duncan DR, Rathore A, Dean E, Naito Y, Dardik A, Kyriakides T, Madri J, Pober JS, Shinoka T, Breuer CK. A critical role for macrophages in neovessel formation and the development of stenosis in tissue-engineered vascular grafts. *FASEB J*. 2011; 25:4253–4263. [PubMed: 21865316]
- Humphrey, JD. *Cardiovascular Solid Mechanics: Cells, Tissues, and Organs*. NY: Springer-Verlag; 2002.
- McEniery CM, Wilkinson IB. Large artery stiffness and inflammation. *J Hum Hyperten*. 2005; 19:507–509.
- McGilvray KC, Sarkar R, Nguyen K, Puttlitz CM. A biomechanical analysis of venous tissue in its normal and post-phlebotic conditions. *J Biomech*. 2010; 43:2941–2947. [PubMed: 20864110]
- Naito Y, Williams-Fritze M, Duncan DR, Church SN, Hibino N, Madri JA, Humphrey JD, Shinoka T, Breuer CK. Characterization of the natural history of extracellular matrix production in tissue-engineered vascular grafts during neo-vessel formation. *Cell, Tiss, Organs*. 2012; 195:60–72.
- Naito Y, Lee YU, Yi T, Church SN, Solomon D, Humphrey JD, Shinoka T, Breuer CK. Beyond Burst Pressure: Initial Evaluation of the Natural History of the Biaxial Mechanical Properties of Tissue Engineered Vascular Grafts in the Venous Circulation Using a Murine Model. *Tissue Eng Part A*. 2013 Aug 19. [Epub ahead of print].
- Nelson GN, Mirensky T, Brennan MP, Roh JD, Yi T, Wang Y, Breuer CK. Functional small diameter human tissue-engineered arterial grafts in an immunodeficient mouse model: preliminary findings. *Arch Surg*. 2008; 143:488–494. [PubMed: 18490559]
- Niklason LE, Yeh AT, Calle E, Bai Y, Valentin A, Humphrey JD. Enabling tools for engineered collagenous tissues, bioreactor design, intravital imaging, and biomechanical modeling. *Proc Nat Acad Sci*. 2010; 107:3335–3339. [PubMed: 19955446]
- Park S, Lakatta EG. Role of inflammation in the pathogenesis of arterial stiffness. *Yonsei Med J*. 2012; 53:258–261. [PubMed: 22318811]
- Pfeiffer BJ, Franklin CL, Hsieh F-H, Bank RA, Phillips CL. Alpha 2(I) collagen deficient oim mice have altered biomechanical integrity, collagen content, and collagen crosslinking of their thoracic aorta. *Mat Biol*. 2005; 24:451–458.
- Roh JD, Sawh-Martinez R, Brennan MP, Jay SM, Devine L, Rao DA, Yi T, Mirensky TL, Nalbandian A, Udelsman B, Hibino N, Shinoka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. *Proc Nat Acad Sci*. 2010; 07:4669–4674. [PubMed: 20207947]
- Rossmann JS. Elastomechanical properties of bovine veins. *JMBBM*. 2010; 3:210–215.
- Sokolis DP. Experimental investigation and constitutive modeling of the 3D histomechanical properties of vein tissue. *Biomech Model Mechanobiol*. 2012 (in press).
- Svejcar J, Prerovsky I, Linhart J, Kruml J. Content of collagen, elastin, and water in walls of the internal saphenous vein in man. *Circ Res*. 1962; 11:296–300. [PubMed: 13918679]
- Wagenseil JE, Nerurkar NL, Knutsen RH, Okamoto RJ, Mecham RP. Effects of elastin haploinsufficiency on the mechanical behavior of mouse arteries. *Am J Physiol*. 2005; 289:H1209–H1217.
- Wan W, Yanagisawa H, Gleason RL Jr. Biomechanical and microstructural properties of common carotid arteries from fibulin-5 null mice. *Ann Biomed Eng*. 2010; 38(12):3605–3617. [PubMed: 20614245]

- Wenzel P, Knorr M, Kossmann S, Stratmann J, et al. Lysozyme M-positive monocytes mediate angiotensin-II induced arterial hypertension and vascular dysfunction. *Circulation*. 2011; 124:1370–1381. [PubMed: 21875910]
- Wesley RLR, Vaishnav RN, Fuchs JCA, Patel DJ, Greenfield JC. Static linear and nonlinear elastic properties of normal and arterialized venous tissue in dog and man. *Circ Res*. 1975; 37:509–520. [PubMed: 1182942]

Figure 1.

Video images of a murine inferior vena cava (IVC) mounted in the biaxial test system for mechanical testing. Panel A shows the vessel at 0 mmHg pressure and the in vivo axial stretch (~1.4), which caused some wrinkling. Hence, tests were performed over the range 1 to 20 mmHg. Panel B shows the vessel at 20 mmHg, which reveals the marked distensibility and thus “capacitance property” of veins. The vertical red line shows the on-line edge detection used to infer outer diameter during testing.

Figure 2.

Comparison of mean structural properties of the IVC from C57BL/6 (wild-type) and CB-17 SCID/bg (immunodeficient) mice. Panel A shows pressure – diameter responses and Panel B shows compliance, both at the individual in vivo axial stretches. As it can be seen, IVCs from the immunodeficient mice were structurally stiffer at pressures of 5 mmHg and higher (* denotes $p < 0.05$) despite similar values of unloaded wall thickness (22.2 vs. 21.1 microns). 3.

Figure 3. Circumferential (Panel A) and axial (Panel B) Cauchy stress – stretch data for all ten specimens following preconditioning, plotted as the last cycle of loading at the individual in vivo axial stretch. Results at this axial stretch are the most relevant to the in vivo condition. As it can be seen, the IVCs from CB-17 SCID/bg mice appeared materially stiffer than those from the C57BL/6 mice, consistent with results from Figure 2.

Figure 4.

Illustrative prediction by the four-fiber family model of the Cauchy stress – stretch response at three different levels of axial stretch for one representative IVC each from the C57BL/6 and CB-17 SCID/bg mice: circumferential (Panel A) and axial (Panel B). Recall that the best-fit parameter estimation was based on pressure-diameter-force-length data, not stress-stretch data. We show the prediction, rather than fit, for this is a more stringent test of the model. Albeit shown for only one specimen per group, all results were similar. That the model fit the circumferential data better than the axial data is consistent with Sokolis (2012); note, however, that the fit to axial data was best at the in vivo length, which is most important physiologically.

Figure 5.

Plot of the predicted energy W stored in extended and distended IVCs at myriad pairs of circumferential and axial stretch for one representative specimen for each genotype based on best-fit model parameters in Table 1. Consistent with Figures 3 and 4, note the higher material stiffness exhibited by vessels from the CB-17 SCID/bg mice (Panel A) relative to those from the C57BL/6 mice (Panel B).

Figure 6. Comparison between genotypes of intramural elastin (panel A) and collagen (panel B). Values shown as mean and standard deviation, with * denoting a significant difference at $p < 0.05$.

Best-fit values of model parameters (equation 5) for all mice studied, including mean and standard deviation. These values resulted from fits to combined data sets consisting of pressure –diameter – force data at each of the three individual values of axial stretch. When comparing these values to those reported by others (e.g., Sokolis, 2012), note that despite using the same four-fiber family model, definitions of parameters vary from paper to paper (e.g., whether the angle is measured from axial or circumferential). The large standard deviations appeared to reflect the considerable inter-specimen variability seen in Figure 3.

Table 1

	Elastin		Collagen					
	c (kPa)	c_1^I (kPa)	(axial)		(circumferential)		(diagonal)	
			c_2^I	c_2^I (kPa)	c_2^2	$c_1^{3,4}$ (kPa)	$c_2^{3,4}$	$\sim$ (deg)
SCID/hg								
050412	1.524	1.713	1.199	0.889	1.160	1.046	1.632	42.9
050712	0.011	14.227	4.30E-14	0.001	2.646	3.499	1.020	44.0
050912	2.450	4.400	1.057	2.003	0.3035	0.120	2.468	41.7
051012	0.153	22.067	0.2180	0.008	3.176	2.141	1.787	41.2
051112	0.510	8.702	5.26E-11	0.001	2.561	1.378	1.303	49.2
mean	0.930	10.222	0.495	0.580	1.969	1.637	1.642	43.8
SD	1.035	8.139	0.587	0.883	1.193	1.269	0.549	3.2
C57BL/6								
070812	2.30E-14	10.281	1.596	0.292	0.073	0.273	1.395	30.9
071112	3.149	8.040	1.140	0.0006	0.438	2.988	0.689	30.8
071312	2.30E-14	4.486	2.646	0.7148	0.191	2.052	1.156	36.6
071414	2.070	4.598	0.316	9.61E-05	0.854	1.408	0.448	45.3
071712	0.620	13.548	2.30E-14	2.14E-05	0.963	1.573	0.611	39.3
mean	1.168	8.191	1.140	0.201	0.504	1.659	0.860	36.6
SD	1.394	3.864	1.055	0.314	0.394	0.989	0.398	6.1