Supplementary Materials for

Mechanics-driven mechanobiological mechanisms of arterial tortuosity

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Table S1. Geometrical and biaxial mechanical metrics. Pressure-dependent values were computed at systolic pressures (120 mmHg), consistent with tail-cuff measurements. Results are presented for the four study groups: two genotypes (Fbln5+/+ and Fbln5−/−) at two ages (20 and 52 weeks), as mean ± standard error of the mean (SEM).

|                      | Descending Thoracic Aorta (DTA) |
|----------------------|---------------------------------|
|                      | 52-week Fbln5+/+ | 52-week Fbln5−/− | 20-Week Fbln5+/+ | 20-Week Fbln5−/− |
| n (samples)          | n = 4             | n = 4             | n = 3             | n = 4             |
| Body Mass (g)        | 25.31 ± 1.98      | 27.61 ± 1.10      | 24.52 ± 2.45      | 23.85 ± 0.81      |
| Unloaded dimensions  |                   |                   |                   |                   |
| Wall Thickness (μm)  | 90 ± 3.8          | 93 ± 9.7          | 80 ± 0.0          | 99 ± 7.0          |
| Outer Diameter (μm)  | 870 ± 19          | 841 ± 19          | 840 ± 22          | 816 ± 19          |
| Axial Length (mm)    | 4.90 ± 0.35       | 6.48 ± 0.37       | 5.05 ± 0.36       | 7.09 ± 0.44       |
| Systolic dimensions  |                   |                   |                   |                   |
| Wall Thickness (μm)  | 39 ± 2.7          | 50 ± 5.3          | 33 ± 1.0          | 58 ± 3.6          |
| Outer Diameter (μm)  | 1323 ± 32.6       | 1152 ± 53.0       | 1326 ± 43.4       | 1100 ± 14.7       |
| Inner Radius (μm)    | 623 ± 18.3        | 526 ± 28.8        | 630 ± 22.0        | 492 ± 9.3         |
| in vivo Axial Stretch (λiv) | 1.41 ± 0.02 | 1.27 ± 0.03 | 1.43 ± 0.03 | 1.17 ± 0.02 |
| in vivo Circumferential Stretch (λv) | 1.65 ± 0.02 | 1.47 ± 0.06 | 1.70 ± 0.01 | 1.46 ± 0.02 |
| Systolic Cauchy Stresses (kPa) |             |                   |                   |                   |
| Circumferential, σv | 260 ± 22.4        | 178 ± 24.2        | 306 ± 16.9        | 139 ± 11.3        |
| Axial, σz            | 184 ± 17.8        | 118 ± 14.7        | 212 ± 7.2         | 79 ± 5.6          |
| Systolic Linearized Stiffness (MPa) |           |                   |                   |                   |
| Circumferential, C0000 | 2.04 ± 0.19       | 1.92 ± 0.14       | 2.59 ± 0.21       | 2.02 ± 0.32       |
| Axial, Czzzz         | 2.37 ± 0.22       | 1.92 ± 0.33       | 2.81 ± 0.02       | 1.22 ± 0.01       |
| Distensibility (1/MPa) | 16.72 ± 0.33     | 11.31 ± 1.72     | 15.55 ± 1.35     | 8.46 ± 1.20       |
| Systolic Stored Energy (kPa) | 51 ± 5.4         | 26 ± 5.6         | 63 ± 4.7         | 17 ± 0.9          |
Table S2. Representative values of parameters for the baseline growth and remodeling (G&R) model.
Values were obtained from biaxial biomechanical data from a non-tortuous \textit{Fbln5}^{+/+} (WT) descending thoracic aorta, and for the (partially) evolved model obtained from biaxial biomechanical data from a tortuous \textit{Fbln5}^{-/-} (KO) descending thoracic aorta. In particular, a combined degeneration of the elastic parameter $c^e$ (decreased $\sim$20\%) and deposition angle with respect to the axial direction for diagonally oriented collagen $\alpha_0$ (increased $\sim$30\%) from WT to KO proved effective in inducing marked axial growth responses and severe tortuous patterns, with other parameters showing less impact in this regard. Superscripts $e, m, c$ denote elastin, smooth muscle, and collagen; superscripts / subscripts $r, \theta, z, d$ denote radial, circumferential, axial, and symmetric diagonal directions; subscript o denotes original homeostatic values. In addition, changes in the gain parameters for rates of mass of smooth muscle and collagen with respect to baseline were assumed proportional (i.e., $\eta = K^m_o/K^c_o \cdot k^m_o/k^c_o = 1$, with $k_o$ and $K_o$ rate and intramural stress gain parameters). Flow-induced shear stress ($\tau_w$) effects for mass production were neglected (i.e., $K^m_{\tau_w} = K^c_{\tau_w} = 0$) in the present G&R simulations (cf. Latorre and Humphrey, 2020). See Materials and methods for modeling details.

| Parameter                                | \textit{Fbln5}^{+/+} DTA | \textit{Fbln5}^{-/-} DTA |
|------------------------------------------|--------------------------|-------------------------|
| Radius, thickness                        | $a_o, h_o$               | 0.568mm, 0.034mm        | 0.560mm, 0.042mm        |
| Mass fractions                           | $\phi^e_o, \phi^m_o, \phi^c_o$ | 0.331, 0.265, 0.404      | 0.344, 0.148, 0.508     |
| Collagen fractions                       | $\beta^\theta_o, \beta^\theta, \beta^d$ | 0.045, 0.062, 0.893     | 0.062, 0.083, 0.855     |
| Collagen orientation                     | $\alpha_o$               | 38.1°                   | 50.3°                   |
| Elastic parameters                       | $c^e, c^m, c^c, c^\theta, c^\psi$ | 88.5kPa, 419.1kPa, 0.105, 1278kPa, 11.1 | 69.9kPa, 807.6kPa, 2.64, 310.4kPa, 31.8 |
| Deposition stretches                     | $G^e_o, G^e, G^m, G^c$   | 2.15, 1.57, 1/(G^e_oG^e), 1.20, 1.09 | 1.89, 1.49, 1/(G^e_oG^e), 1.17, 1.07 |
Table S3. Summary of statistical comparisons. Statistical pairwise comparisons between convex and concave regions at proximal and distal ends of the eight tortuous Fbln5−/− samples as well as for the computational simulations (last row) for multiple material parameters. The magnitude of the effect size is denoted by g (Hedges test), with negative values of g describing an opposite trend (concave region higher than the matching convex one). Note that the order of the samples (1 to 4) for each age group is similar to that in Figure 3 (left-to-right for each panel). For the adjusted p-values derived from Sidak multiple comparisons (following the one-way ANOVA test), *, **, and *** denote p<0.05, p<0.01, and p<0.001, respectively, and ns indicates non-significant comparison.

| #sample | Axial Stiffness | Circ. Stiffness | Axial Stretch | Circ. Stretch | Energy Difference | g |
|---------|----------------|----------------|---------------|---------------|------------------|---|
|         | p g             | p g            | p g           | p g           | p g              |   |
|         |                 |                |               |               |                  |   |
| Proximal |                 |                |               |               |                  |   |
| 1       | ns -0.07 * 1.43 | ns 85 ns 3.03  | ns            |               |                  |   |
| 2       | ns 2.71 *** 3.10 | ** 20 *** -96 *** | *** 1.75 |               |                  |   |
| 3       | *** 3.40 * 0.97 | *** 204 *** -69 *** | 2.21 |               |                  |   |
| 4       | ns 0.55 ** 1.34 | *** 147 ns 77 *** | 0.75 |               |                  |   |
| Distal  |                 |                |               |               |                  |   |
| 1       | ** 0.55 *** 0.58 | ns 21 ** -45 ns | 0.07 |               |                  |   |
| 2       | ns -0.31 ** 4.15 | ** 76 *** -115 ns | -0.40 |               |                  |   |
| 3       | ns -0.46 ** 2.03 | * 23 *** -60 *** | -0.03 |               |                  |   |
| 4       | ns -0.07 *** 2.50 | ** 221 ns -115 ns | -0.29 |               |                  |   |
| Proximal |                 |                |               |               |                  |   |
| 1       | ns 0.54 ns 0.51 | *** 24 *** -64 *** | 4.64 |               |                  |   |
| 2       | * 1.86 ns 0.41 | *** 45 *** -105 *** | 0.51 |               |                  |   |
| 3       | * 0.24 *** 0.85 | ** 245 * -18 *** | 3.24 |               |                  |   |
| 4       | ns 0.38 * 1.42 | *** 182 ns -50 ns | -0.37 |               |                  |   |
| Distal  |                 |                |               |               |                  |   |
| 1       | *** 4.96 ** 1.23 | *** 23 *** -146 *** | 1.02 |               |                  |   |
| 2       | ns -1.52 ns 2.08 | ** 58 ns 4.40 * | 0.27 |               |                  |   |
| 3       | * 1.17 *** 3.22 | * 274 *** 45 ns | 0.26 |               |                  |   |
| 4       | ns 0.16 *** 2.29 | ns 38 ns 23 ns | 0.10 |               |                  |   |
| Comp. simulations | * -3.40 ** 3.63 | *** 570 *** -756 ns | 1.07 |               |                  |   |
Figure S1. MicroCT reconstructed aortic geometries from adult female Fbln5+/+ (left) and Fbln5−/− (right) mice. Notice the marked tortuosity and elongation in the fibulin-5 null aorta, reminiscent of an aging phenotype. Given that increased pressures tend to exaggerate arterial bending and buckling, note that the microCT images were acquired while the mice were anesthetized, which reduces blood pressure about 20-25%. Images collected at the Yale Translational Research Imaging Center, Dr. Albert Sinusas Director, and segmented using the open-source software CRIMSON, developed and maintained at the University of Michigan by Professor C. Alberto Figueroa. Finally, we note that the computational simulations herein were motivated by data – panoramic digital image correlation (pDIC), optical coherence tomography (OCT), multi-photon microscopy (SHG), and microCT – collected on the descending thoracic aorta from mice. Other vessels, including carotids, coronaries, femorals, vertebrals, and so forth, can exhibit different degrees of tortuosity, both amplitude and frequency, but we focused herein on the murine aorta alone.
Figure S2. Sample preparation and panoramic digital image correlation (pDIC) system. (A) The descending thoracic aortic (DTA) segment was mounted on and secured to a custom triple-needle assembly, with the proximal end tied to a fixed 21G needle and the distal end to a 22G needle that can slide on a central 30G needle to allow axial extension ($\lambda_z$); note the black suture ligatures on the inter-costal branches. Each sample is then (B) soaked in Evans blue to create a dark background, with (C) a unique speckle pattern achieved using an air-brush to apply white India-ink. The sample is placed coaxially within a 45-deg conical mirror (G, top-side view), which is (F) submerged in HBSS-filled bath. Pressurization (through the 21G needle) from 10 to 140 mmHg, in 10 mmHg increments, and axial stretching to 3 different axial lengths yielded 52 quasi-statically loaded configurations, each captured by the digital camera from 8 different viewpoints and each resulting in an image at each state (H) for data analysis to yield the reconstructed 3-D surface (D). For inverse characterization, (E) the surface of each configuration was meshed with a set of 40 circumferential by 25 axial patches. Photos credits: Panel F- Matthew Bersi, Washington University in St. Louis, panels A-C and G-Dar Weiss, Yale University.
Figure S3. Spatial distributions of various mechanical metrics. Metrics are shown for representative 20-week old Fbn5−/− aortas based on panoramic digital image correlation (pDIC) and optical coherence tomographic (OCT) data. Distributions of key mechanical metrics, identified at a distending pressure of 120 mmHg and resulting from the virtual field-based inverse characterization, are superimposed on the reconstructed reference configuration geometry (at the in vivo axial stretch and 80 mmHg), and parametrized into θ-Z planes around the vessel circumference (θϵ[-180,180]) and along its (Z) axial extent; the right-most edge of each 3-D rendering is at about 0 degrees whereas the left-most edge is at about ±180 degrees; see the middle panel of Figure 3 in the main paper for further clarification of the mapping from 3-D to 2-D. Ends of the samples were not analyzed to avoid end-effects due to the sutures that secured the samples on the needle assembly. Note that the elastically stored energy tends to be an excellent metric for elastic fiber integrity, often more reliable than standard histological inferences. Note, too, that the 1st stress invariant (right center panel) is the trace of the Cauchy stress tensor, though we neglected the stress in the radial direction (approximately zero). The thickness distribution (right bottom panel) was preprocessed using OCT-derived 3-D scans.
Figure S4. Tortuosity index (TI) at systolic pressure as a function of the in vivo circumferential (left) and axial (right) material stiffness. Each point represents the mean distribution of one sample from pDIC calculations (cf. Figure 1). Correlations, calculated using the Pearson correlation coefficient, $r$, were not significant, $p > 0.05$. The lack of correlation with the circumferential stiffness suggests that the intramural cells were yet able to mechano-sense and mechano-regulate the matrix, thus supporting the hypothesis that the development of (persistent) tortuosity results from a progressive, cell-mediated mechano-biological process driven by the evolving mechanics.
Figure S5. Regional differences in key mechanical metrics. Upper row. Local variations in elastically stored energy (difference between systole, defined at 120 mmHg, and diastole, at 80 mmHg) between convex (green and orange) and concave (yellow and blue) regions at proximal (left) and distal (right) ends of the vessel. Calculated stored energy differences were significantly higher in convex regions at the proximal end (for 6/8 samples) regardless of age, and less evident on the distal end. Computational simulations (far right column at each panel) did not reveal any differences between regions. Further details on the compared regions are in Figure 3 in the main text.

Bottom row. Mean thickness of the aortic wall (left) and adventitia alone (middle) obtained from multiple 3-D multi-photon microscopic images from 20 and 52-week-old Fbln5−/− aortas at three different physiologic pressures and sample-specific in vivo axial stretch. Adventitial thicknesses were normalized by, and thus independent of, overall wall thickness. Wall thickness appeared to increase with aging while the adventitial fraction showed limited differences with aging. Density of smooth muscle cells (right) was not found to be significantly different between the concave and convex regions (density was evaluated as cell nuclei number normalized by a unit of volume). Significant statistical differences between groups are denoted by * p<0.05 and ** p<0.001.
Figure S6. Transient elastic tortuosity manifests on vulnerable vessels under elevated physiological pressure. Simulated pressurization test for a computationally predicted partially tortuous artery shown in the second-from-the-right column in Figure 6 ($\Delta \theta = 0.9 \cdot \Delta \theta_{K,0}$) performed at the in vivo value of axial stretch and systolic pressure of 120 mmHg (central column). Also shown are associated elastic distensions for a transient increase (right column, $P = 160$ mmHg) or decrease (left column, $P = 80$ mmHg) in pressure with superimposed regional values of biaxial wall stress (first and second rows), biaxial material stiffness (third and four rows), and invariant wall volume (bottom row), the last revealing the essentially isochoric character of the simulated elastic response with locally frozen growth and remodeling and associated material insults. Note that marked changes in pressure induce only moderate elastically induced changes in geometry when computed on an otherwise vulnerable vessel.
Figure S7. Sustained hypertension induces extreme tortuosity that may not resolve during aging. First row: Simulated pressurization test for the partially tortuous artery in the second-from-the-left column in Figure 6 ($\Delta \theta = 0.8 \cdot \Delta \theta_{KO}$) performed at the in vivo value of axial stretch and systolic pressure of 120 mmHg (central column). Also shown are associated elastic distensions for a transient increase (right column, $P = 160 \text{ mmHg}$) or decrease (left column, $P = 80 \text{ mmHg}$) in pressure with superimposed invariant wall volume (mass) ratio (cf. Figure S6, where $\Delta \theta = 0.9 \cdot \Delta \theta_{KO}$).

Second row: Gradual (G&R-induced) development of severe persistent tortuosity for the same partially tortuous artery (top-central panel, $\Delta \theta = 0.8 \cdot \Delta \theta_{KO}$) when held at the in vivo value of axial stretch and subjected to a sustained increase in systolic pressure from normal ($P = 120 \text{ mmHg}$) to moderately hypertensive (far-right, $P = 135 \text{ mmHg}$). Shown is the regional increase in local wall volume resulting from a combination of axial (lengthening, major effect), radial (thickening), and circumferential (dilation) anisotropic growth (cf. last row in Figure 6, where growth is mainly longitudinal; note the different scale).

Third row: Modest differential G&R-induced decrease of tortuosity for a markedly tortuous vessel (Figure 5, top-right) afforded by a progressive change in the maximal regional orientation of diagonal fibers from that found in the convex region (left, $\hat{\theta}_1 = \pi$ in Eq. (8)) toward that found in the concave regions (right, $\hat{\theta}_1 = 0$), simulating the observed modest regional switch for the dispersion of collagen fibers between 20- and 52-week old $Fbln5^{-/-}$ samples. Shown are the resulting modified distributions $\alpha_0(r_o, \theta_o, z_o)$ via Eq. (8). Taken together, these simulations further support the hypothesis that persistent tortuosity requires complex, regional cell-mediated growth and remodeling responses to multiple insults rather than simply an instantaneous elastic buckling instability.