A Hidden Structural Vulnerability in the Thrombospondin-2 Deficient Aorta Increases the Propensity to Intramural Delamination

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SUPPLEMENTARY MATERIAL

Table S1. Mean best-fit values of the model parameters used to characterize the transmurally homogenized (i.e., radially averaged) mechanical properties of the proximal descending thoracic aorta from *WT* and *Thbs2*$^{-/-}$ mice at 20 and 40 weeks of age (cf. Equation 1). Although it is tempting to compare values of individual parameters as a function of genotype or age, it is important to remember that this constitutive model is microstructurally motivated, yet phenomenological since values are determined by fitting biaxial mechanical data via nonlinear regression. Indeed, microstructural details such as cross-linking and physical entanglements are not modeled explicitly. It is best, therefore, to compare integrated mechanical metrics derived from the constitutive model, including material stiffness and energy storage, which are tabulated in Table S2.

	Elastic Fibers	Axial Collagen		Circ. Collagen + SMC		Symmetric Diagonal Collagen			Error
	c (kPa)	c_1^{1} (kPa)	c_2^{1}	c_1^{2} (kPa)	c_2^{2}	$c_1^{3,4}$ (kPa)	$c_2^{3,4}$	α_0 (deg)	*RMSE*
20 weeks									
WT	34.22	9.27	0.29	13.04	0.14	0.17	1.60	28	0.063
Thbs2$^{-/-}$	42.31	6.29	0.35	10.87	0.41	0.79	1.28	36	0.055
40 weeks									
WT	39.87	2.81	0.86	9.83	0.31	0.31	1.97	31	0.048
Thbs2$^{-/-}$	49.27	4.08	0.78	14.85	0.45	0.37	2.15	31	0.047

Table S2. Morphological and mechanical data (mean ± SEM) for the proximal descending thoracic aorta from *WT* and *Thbs2*$^{-/-}$ mice at 20 and 40 weeks of age. MAP denotes the group-specific mean arterial pressure at which the metric is evaluated. Note that, with few exceptions, there is little difference because of the mutation to the thrombospondin-2 gene, which reveals that the cells are able to respond well mechanobiologically under normal physiologic conditions. Interestingly, there are more differences due to modest aging (from 20 to 40 weeks of age), though energy storage (a key indicator of mechanical functionality of an elastic artery) was statistically the same across all groups at normal MAP.

	20 weeks		40 weeks	
	WT	*Thbs2*$^{-/-}$	*WT*	*Thbs2*$^{-/-}$
n	5	5	5	5
Unloaded Dimensions				
Outer Diameter (μm)	902 ± 12	950 ± 33	972 ± 21	1020 ± 27
Wall Thickness (μm)	104 ± 1	92 ± 3	107 ± 5	91 ± 1 *
In-vitro Axial Length (mm)	5.81 ± 0.28	5.72 ± 0.55	5.20 ± 0.44	6.23 ± 0.27
Dimensions @ MAP				
Outer Diameter (μm)	1399 ± 47	1364 ± 62	1492 ± 34	1498 ± 24
Wall Thickness (μm)	38 ± 1	37 ± 1	41 ± 2	38 ± 1
Circumferential Stretch	1.70 ± 0.05	1.55 ± 0.02 *	1.68 ± 0.02	1.58 ± 0.03
In-vivo Axial Stretch	1.61 ± 0.02	1.62 ± 0.02	1.54 ± 0.02	1.54 ± 0.02 †
Cauchy Stress (kPa) @ MAP				
Circumferential	251 ± 16	223 ± 10	303 ± 16	311 ± 9 †
Axial	271 ± 16	268 ± 12	267 ± 18	304 ± 20
Stiffness (MPa) @ MAP				
Circumferential	1.47 ± 0.02	1.55 ± 0.06	2.32 ± 0.08 †	2.54 ± 0.15 †
Axial	3.47 ± 0.24	2.53 ± 0.10	4.01 ± 0.66	4.31 ± 0.15 †
Stored Energy (kPa) @ MAP	73 ± 6	68 ± 3	71 ± 4	73 ± 5
Energy Dissipation Ratio (%)	3.44 ± 0.41	3.86 ± 0.79	4.46 ± 0.87	6.51 ± 1.08

* $p < 0.05$ compared to age-matched WT mice (genotype effect)
† $p < 0.05$ compared to same-genotype mice (age effect)

Table S3. Percent composition (mean ± SEM) of the three primary constituents of the proximal descending thoracic aorta from *WT* and *Thbs2*$^{-/-}$ mice at 20 and 40 weeks of age. Note that the mass fractions in each column add up to ~ 100% of the wall for each experimental group and that these results were inferred from histological images based on Verhoeff Van Giesen and Masson's Trichrome stains. See Figure 1 in the main text for illustrative histological images.

	20 weeks		40 weeks	
	WT	*Thbs2*$^{-/-}$	*WT*	*Thbs2*$^{-/-}$
Media				
Elastin	35 ± 0.7	33 ± 2.1	33 ± 2.3	30 ± 2.5
Collagen	6 ± 0.1	5 ± 0.2 *	8 ± 0.5	5 ± 0.1 *
Smooth muscle	25 ± 0.6	31 ± 0.5 *	34 ± 0.8 †	33 ± 0.4 *
Adventitia				
Elastin	5 ± 0.1	3 ± 0.1	4 ± 0.1	2 ± 0.1
Collagen	29 ± 0.5	28 ± 0.5	21 ± 0.1 †	30 ± 0.4 *

* $p < 0.05$ compared to age-matched *WT* mice (genotype effect)
† $p < 0.05$ compared to same-genotype mice (age effect)

Table S4. Mean best-fit values of the model parameters used to characterize the layer-specific mechanical properties of the proximal descending thoracic aorta from *WT* and *Thbs2*$^{-/-}$ mice at 20 and 40 weeks of age (cf. Equations 4-7). Similar to the transmurally homogenized model (Table S1), it is best to compare integrative metrics such as material stiffness and energy storage rather than individual values of the parameters. See, for example, Figure 2 in the main text.

	Elastic Fibers	SMCs + Collagen		Collagen Fibers					Error
	$c^e\ (kPa)$	${c_1}^m\ (kPa)$	${c_2}^m$	${c_1}^c\ (kPa)$	${c_2}^c$	$\alpha_0\ (deg)$	β_ϑ	β_z	*RMSE*
20 wks									
WT	220.48	271.67	7.20	4106.22	23.14	33	0.21	0.07	0.038
Thbs2$^{-/-}$	275.48	229.85	16.6	5783.98	16.56	37	0.12	0.10	0.050
40 wks									
WT	235.57	801.85	2.71	5152.17	33.04	35	0.41	0.04	0.029
Thbs2$^{-/-}$	359.24	1405.84	1.00	5422.40	31.27	32	0.23	0.03	0.038