Biomechanical Phenotyping of the Murine Aorta: What Is the Best Control?

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The availability of diverse mouse models is revealing increasingly greater information on arterial mechanics, including homeostatic adaptations and pathologic maladaptations to genetic, pharmacological, and surgical manipulations. Fundamental to understanding such biomechanical changes, however, is reliable information on appropriate control vessels. In this paper, we contrast 15 different geometrical and mechanical metrics of biaxial wall mechanics for the ascending aorta across seven different types of possible control mice. We show that there is a comforting similarity across these multiple controls for most, though not all, metrics. In particular, three potential controls, namely, noninduced conditional mice, exhibit higher values of distensibility, an important clinical metric of structural stiffness, and two of these potential controls also have higher values of intrinsic circumferential material stiffness. There is motivation, therefore, to understand better the biomechanical changes that can arise with noninduced Cre-lox or similar approaches for generating mutations conditionally. In cases of germline mutations generated by breeding heterozygous +/− mice, however, the resulting homozygous +/+ mice

tend to exhibit properties similar to traditional (C57BL/6) controls. [DOI: 10.1115/1.4035551]

Keywords: fibrillin-1, fibulin-4,5, smooth muscle myosin, transforming growth factor receptor, tuberous sclerosis

Introduction

Advances in genetics and experimental methods have rendered the mouse the experimental animal of choice in many studies of vascular biology, mechanics, and medicine. Yet, the availability of many different background strains and the wide variety of genetically modified mice has led to a key question—What is the best control to which results should be compared? In many cases, mice heterozygous (+/−) for a germline mutation are bred to yield the mutant mice (−/−) of interest, with littermate “wild-type” (+/+) mice often used as controls. Similarly, conditional mutations are engineered to establish mouse strains that can be induced at desired times, with the noninduced mice often used as controls. In both cases, the mutations can also be established on different backgrounds.

In this paper, we use consistent biaxial mechanical testing and methods of data analysis to compare multiple geometrical and mechanical metrics across seven different types of possible control mice. With the ascending aorta as an illustrative vessel, we show that results are comfortably similar in most, though not all, cases considered. This study thus provides guidance for the appropriate selection of controls as well as baseline values to which diverse mouse models—genetically modified, pharmacologically treated, or surgically manipulated—of ascending aortic mechanics can be compared. The results also reveal a need for some caution, however, if one desires to use noninduced conditional mice as controls.

Methods

A custom, computer-controlled biaxial testing system [1] was used to biomechanically phenotype the ascending thoracic aorta (ATA) from seven groups of mice. Specific testing protocols and methods of data analysis have been described previously [2–4]. Briefly, we excised the ATAs from the aortic root to the brachiocephalic branch, gently removed excess perivascular tissue, cannulated the specimens on custom cannulae, and preconditioned them within a standard bathing solution (Hanks buffered saline) at 37 °C. After recording unloaded dimensions, the specimens were subjected to seven cyclic testing protocols (three pressure-diameter and four axial force-length), with diameter, pressure, axial length, and axial force recorded on-line. Associated biaxial data from the unloading path of the last cycle of testing in each of the seven protocols were then combined into a single data set and fit via nonlinear regression with a validated four-fiber family stored energy function W . Derivatives of W with respect to appropriate measures of deformation yielded information on biaxial Cauchy stress and material stiffness, typically assessed at mouse-specific values of in vivo axial stretch and systolic pressure.

Overall, we compared 15 different geometrical and mechanical metrics: unloaded outer diameter (OD), wall thickness (H), and axial length (L); systolic outer diameter (od), wall thickness (h), and inner radius (a); systolic circumferential stretch (λ_θ), Cauchy stress (σ_θ), and material stiffness ($\mathcal{E}_{\theta\theta\theta}$) as well as the associated systolic in vivo axial stretch (λ_z), Cauchy stress (σ_z), and material stiffness ($\mathcal{E}_{zzz}$); and, finally, the systolic value of stored energy (W), an energy dissipation ratio (EDR), and a measure of structural stiffness known as distensibility ($\mathcal{D}$). Again, see prior publications for specific definitions and detailed methods of measurement or calculation [2–4]. In particular, we compared results across male mice from seven different models (Table 1): C57BL/6J wild-type control, three homozygous models generated when studying effects of compromised elastic fiber integrity (fibrillin-1, $Fbn1^{+/+}$, noninduced fibulin-4, $Fbln4^{\text{loxP}/+}$, and fibulin-5, $Fbln5^{+/+}$), one homozygous model generated when studying a

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Manuscript received October 11, 2016; final manuscript received December 16, 2016; published online January 31, 2017. Assoc. Editor: Jonathan Vande Geest.

Table 1 Mean physiological data for the seven groups of mice. Note that *Myh11*^{+/+}, *Fbn1*^{+/+}, and *Fbln5*^{+/+} are homozygous non-mutant littermate mice (+/+) that were generated from the breeding of heterozygous mutant mice (+/-) to produce homozygous mutant mice (-/-), that the C57BL/6J are wild-type mice, and *Fbln4*^{loxP/+}, *mT/mG.Myh11-CreER*^{T2}.*Tgfb2*^{fl/fl} + Veh, and *mT/mG.Myh11-CreER*^{T2}.*Tgfb2*^{fl/fl} + Veh are noninduced mice that maintained the potential of postnatal activation of genetic mutations upon induction. The corresponding target mutation, if any, is indicated for each group, together with the background strain of the mice. Values of systolic pressure were adjusted to conscious resting mouse conditions according to the method used for blood pressure measurement [23,24]. The top four rows indicate the four groups that are not statistically different (see below analyses) and thus constitute our pooled controls.

Potential control	Targeted mutation	Background	Age (weeks)	Body mass(g)	<i>P</i> _{sys,dias} (mmHg)	<i>P</i> _{sys,adj} (mmHg)
<i>Myh11</i> ^{+/+}	<i>Myh11</i> ^{R247C/R247C}	C57BL/6J; 129SvJ	27.3 ± 0.5	27.4 ± 1.9	124 ± 3/91 ± 3—T	125
<i>Fbn1</i> ^{+/+}	<i>Fbn1</i> ^{mgR/mgR}	C57BL/6J; Sv129; SW	8.5 ± 0.1	29.0 ± 1.2	124 ± 1/91 ± 1—C	124
<i>Fbln5</i> ^{+/+}	<i>Fbln5</i> ^{-/-}	C57BL/6J; 129/SvEv	21.2 ± 0.2	31.9 ± 1.8	120 ± 6/84 ± 6—T	120
C57BL/6J wild-type	None	C57BL/6J	15.2 ± 0.1	28.9 ± 0.7	127 ± 1/97 ± 2—T	128
<i>Fbln4</i> ^{loxP/+}	<i>Fbln4</i> ^{SMKO}	C57BL/6J; 129SvEv	13.1 ± 0.2	17.1 ± 0.6	104 ± 1/70 ± 1—S	128
<i>mT/mG.Myh11-CreER</i> ^{T2} . <i>Tgfb2</i> ^{fl/fl} + Veh	[...]. <i>Tgfb2</i> ^{fl/fl} + Tmx	backbred to C57BL/6	8.1 ± 0.1	22.6 ± 0.7	100 ± 2/77 ± 2—M	117
<i>mT/mG.Myh11-CreER</i> ^{T2} . <i>Tsc1</i> ^{fl/fl} + Veh	[...]. <i>Tsc1</i> ^{fl/fl} + Tmx	backbred to C57BL/6	12.2 ± 0.1	29.0 ± 0.6	105 ± 2/79 ± 3—M	123

Note: C = indwelling (aortic) polyethylene catheter in the conscious mouse; M = Millar ascending aortic catheter in the anesthetized mouse (1.2% isoflurane); S = Scisense ascending aortic catheter in the anesthetized mouse (1.5% isoflurane); T = noninvasive tail cuff method in the conscious mouse. [...] denotes *mT/mG.Myh11-CreER*^{T2}, Veh denotes a noninducing injectable substance that is to be contrasted with Tmx, which denotes the injectable drug tamoxifen that is used to induce the targeted mutation.

smooth muscle myosin heavy chain mutation (*Myh11*^{+/+}), and two homozygous, noninduced models otherwise used to study disrupted smooth muscle (SMC)-specific altered signaling pathways (via the transforming growth factor-beta receptor 2, *mT/mG.Myh11-CreER*^{T2}.*Tgfb2*^{fl/fl} + Veh, and tuberous sclerosis 1, *mT/mG.Myh11-CreER*^{T2}.*Tsc1*^{fl/fl} + Veh), where Veh denotes an injected non-inducing “vehicle” substance. In each case, data from *n* = 5 to 7 specimens per group were used for comparison. Note that the ages of the animals were dictated by other studies that focused on consequences of the mutations (e.g., see [4–8]). For details on the different targeted mutations and means of establishing both the mutants and controls, see the original papers [9–13].

Statistical analyses were used, first, to determine if any differences existed across the seven groups in each metric and, second, to identify if any collection of groups could be used to define

overall mean “control” values for all of the metrics. Toward this end, a one-way analysis of variance (ANOVA) was first performed on each metric to assess statistically significant differences, if any, across the seven groups; significance was set at *p* < 0.05. After all groups were compared simultaneously, in cases where differences were found, individual groups or combinations thereof were then sequentially removed until a subset of groups was found with no differences for that particular metric. Geometrical and mechanical metrics from this identified subset were then pooled to describe the mean behavior of the ascending aorta for control adult male mice.

Results

Biaxial data from pressure-diameter and axial force-length tests revealed qualitatively similar structural behaviors across the seven different mouse models (Fig. 1(a) and 1(b)), with the former yet

Fig. 1 Mechanical data from cyclic in vitro biaxial testing reveal similar structural, (a) and (b), and material, (c) and (d), behaviors across the seven groups of potential control mice. The black symbols show homozygous nonmutant littermate mice (+/+) generated by breeding heterozygous mutant mice (+/-), the gray symbol indicates pure C57BL/6J wild-type mice, and open symbols show noninduced mice in which mutations could be induced conditionally with tamoxifen. Veh indicates treatment with vehicle rather than tamoxifen. The error bars represent the standard error of the mean (SEM).

Table 2 Best-fit values of model parameters in the four-fiber family constitutive relation that was used to characterize the ascending thoracic aorta (ATA) from the seven groups of mice. The top four rows indicate the four groups that are not statistically different based on results in Fig. 2.

	Elastic fibers	Axial collagen		Circumferential 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)	$c_1^{3,4}$	α_0 (deg)	$RMSE$
<i>Myh11</i> ^{+/+}	29.32	14.89	0.02	15.91	0.20	4.67	0.48	41.72	0.061
<i>Fbn1</i> ^{+/+}	43.99	14.98	0.03	07.59	0.23	6.48	0.43	43.76	0.048
<i>Fbln5</i> ^{+/+}	26.16	16.65	0.08	14.88	0.08	4.73	0.43	44.06	0.079
C57BL/6J WT	31.55	22.14	0.01	09.34	0.14	6.29	0.33	45.07	0.068
<i>Fbln4</i> ^{loxP/+}	34.18	17.77	0.03	08.90	0.20	3.73	0.54	41.47	0.047
<i>Tgfb2</i> ^{tf} + Veh	24.51	19.83	0.01	11.82	0.01	5.31	0.30	45.39	0.096
<i>Tsc1</i> ^{tf} + Veh	35.42	15.05	0.01	07.50	0.17	4.72	0.35	42.17	0.074

emphasizing slight differences in the overall outer diameter and the latter emphasizing slight differences in the in vivo value of the axial stretch. When converted into standard biaxial Cauchy stress—stretch results (Figs. 1(c) and 1(d)), these data also showed considerable similarity in material responses across the seven groups. One exception, however, is the circumferential behavior of the noninduced *Tgfb2*^{tf} (*mT/mG.Myh11-CreER*^{T2}.*Tgfb2*^{tf} + Veh) ATA, which appears more compliant than the other vessels.

Table 2 reports mean values of the constitutive parameters for all seven mouse models. Table 3 lists mean $\pm$ standard error of the mean (SEM) values of unloaded dimensions (measured experimentally) as well as structural, material, and energy metrics at systole (estimated computationally) for all seven groups. No statistically significant differences were found across *Myh11*^{+/+}, *Fbn1*^{+/+}, *Fbln5*^{+/+}, and C57BL/6J ATAs for any metric except the unloaded axial length (L), which was higher in the *Fbln5*^{+/+} group. The *Fbln5*^{+/+} specimens were the first ATAs tested on the biaxial device and, in retrospective, we believe that a suboptimal cannulation procedure might have included a portion of the brachiocephalic branch as part of the segment of interest, which in turn led to an overestimation of the unloaded length. Regardless, this parameter did not appear to affect normalized pointwise measures such as stress, stiffness, or energy, and all subsequent testing was consistent. Hence, the mechanical data from *Myh11*^{+/+}, *Fbn1*^{+/+}, *Fbln5*^{+/+}, and C57BL/6J specimens were combined to

define the average adult male murine control ATA, up to 27 weeks of age. Each of the other three mouse models (*Fbln4*^{loxP/+}, *mT/mG.Myh11-CreER*^{T2}.*Tgfb2*^{tf} + Veh, and *mT/mG.Myh11-CreER*^{T2}.*Tsc1*^{tf} + Veh) showed differences in some of the metrics considered, mainly outer diameter (unloaded or systolic) and circumferential behaviors (stretch, stress, stiffness, or distensibility).

Given the inherent differences in age, body mass, and in vivo systolic pressure among the seven mouse models (Table 1), we investigated whether these factors were the source of the observed differences in geometrical and mechanical metrics. Figure 2 plots the 15 metrics of interest as a function of age. Again, there is comforting similarity in most metrics across the seven groups, which span from 8 to 27 weeks of age. A few exceptions are noted, however. Despite their young age, the noninduced *Tgfb2*^{tf} (*mT/mG.Myh11-CreER*^{T2}.*Tgfb2*^{tf} + Veh) and noninduced *Tsc1*^{tf} (*mT/mG.Myh11-CreER*^{T2}.*Tsc1*^{tf} + Veh) ATAs exhibit higher values of systolic circumferential stiffness ($\mathcal{C}_{0000}$ — Fig. 2(i)) for a systolic blood pressure that is either lower than (noninduced *Tgfb2*^{tf}) or similar to (noninduced *Tsc1*^{tf}) the reference value for the average control male mouse. Furthermore, neither age nor blood pressure appears to dictate the differences in unloaded thickness H (Fig. 2(b)) for the noninduced *Tsc1*^{tf}, circumferential stretch λ_θ (Fig. 2(g)) for the noninduced *Tgfb2*^{tf}, or circumferential stress σ_θ (Fig. 2(h)) for the noninduced *Fbln4*^{loxP/+}. Interestingly, the noninduced *Tgfb2*^{tf}, *Tsc1*^{tf}, and *Fbln4*^{loxP/+} ATAs all

Table 3 Morphological and mechanical data (mean $\pm$ SEM) for the ascending thoracic aorta from the seven groups of mice. All pressure-dependent values are computed at the adjusted systolic pressure specific for each group (cf. Table 1). The superscript b indicates significant differences from those of pooled controls, which manifested in conditional mice, consistent with results in Fig. 2.

	<i>Myh11</i> ^{+/+}	<i>Fbn1</i> ^{+/+}	<i>Fbln5</i> ^{+/+}	C57BL/6J WT	<i>Fbln4</i> ^{loxP/+}	<i>Tgfb2</i> ^{tf} + Veh	<i>Tsc1</i> ^{tf} + Veh
n	*5*	5	5	5*	5	7	5*
Unloaded dimensions							
Outer diameter (μ m)	1033 $\pm$ 29	1100 $\pm$ 38	1090 $\pm$ 22	1133 $\pm$ 32	965 $\pm$.40 ^b	1071 $\pm$ 27	1226 $\pm$ 28 ^b
Wall thickness (μ m)	105 $\pm$ 5	112 $\pm$ 4	105 $\pm$ 1	110 $\pm$ 5	104 $\pm$ 4	106 $\pm$ 3	119 $\pm$ 3 ^b
Axial length (mm)	2.50 $\pm$ 0.13	2.45 $\pm$ 0.12	3.37 $\pm$ 0.13 ^a	2.54 $\pm$ 0.12	2.48 $\pm$ 0.10	2.84 $\pm$ 0.12	2.88 $\pm$ 0.10
Systolic dimensions							
Outer diameter (μ m)	1692 $\pm$ 60	1781 $\pm$ 23	1783 $\pm$ 38	1883 $\pm$ 74	1526 $\pm$ 58 ^b	1886 $\pm$ 7 ^b	2065 $\pm$ 45 ^b
Wall thickness (μ m)	34 $\pm$ 2	38 $\pm$ 3	35 $\pm$ 1	36 $\pm$ 2	36 $\pm$ 2	32 $\pm$ 2	37 $\pm$ 1
Circ. Stretch	1.78 $\pm$ 0.01	1.77 $\pm$ 0.06	1.78 $\pm$ 0.02	1.79 $\pm$ 0.05	1.74 $\pm$ 0.04	1.93 $\pm$ 0.05 ^b	1.83 $\pm$ 0.03
Axial stretch	1.73 $\pm$ 0.02	1.70 $\pm$ 0.04	1.71 $\pm$ 0.02	1.69 $\pm$ 0.05	1.69 $\pm$ 0.04	1.74 $\pm$ 0.04	1.77 $\pm$ 0.06
Systolic Cauchy stress (kPa)							
Circumferential	404 $\pm$ 28	382 $\pm$ 28	396 $\pm$ 6	433 $\pm$ 46	352 $\pm$ 16 ^b	450 $\pm$ 31	446 $\pm$ 20
Axial	378 $\pm$ 18	395 $\pm$ 35	378 $\pm$ 5	412 $\pm$ 18	357 $\pm$ 15	406 $\pm$ 30	412 $\pm$ 18
Systolic stiffness (MP ^a)							
Circumferential	2.66 $\pm$ 0.16	2.27 $\pm$ 0.14	2.57 $\pm$ 0.10	2.76 $\pm$ 0.11	2.38 $\pm$ 0.14	3.59 $\pm$ 0.28 ^b	3.22 $\pm$ 0.34 ^b
Axial	2.49 $\pm$ 0.14	2.33 $\pm$ 0.20	2.53 $\pm$ 0.05	2.26 $\pm$ 0.05	2.37 $\pm$ 0.08	2.29 $\pm$ 0.18	2.51 $\pm$ 0.12
Distensibility	18.54 $\pm$ 0.39	22.99 $\pm$ 1.51	21.36 $\pm$ 1.15	18.89 $\pm$ 1.77	31.06 $\pm$ 2.25 ^b	28.05 $\pm$ 1.06 ^b	25.65 $\pm$ 2.36 ^b
Systolic stored energy (kPa)	107 $\pm$ 7	119 $\pm$ 12	109 $\pm$ 2	130 $\pm$ 8	101 $\pm$ 6	127 $\pm$ 11	128 $\pm$ 6
Energy dissipation ratio (%)	2.13 $\pm$ 0.51	2.05 $\pm$ 0.40	1.89 $\pm$ 0.56	2.59 $\pm$ 0.07	1.96 $\pm$ 0.60	2.58 $\pm$ 0.50	3.11 $\pm$ 0.76

^a $p < 0.05$ compared to other controls.

^b $p < 0.05$ compared to pooled controls.

had higher values of distensibility $\mathcal{D}$ (Fig. 2(o)). Finally, Fig. 3 shows results for the only three metrics (out of the 15 considered) that appeared to correlate with body mass: OD, od, and $\mathcal{D}$.

Discussion

Modern biomechanics arose in the mid-1960s and much of the work on arterial mechanics from that time through the end of the

20th century depended primarily on data collected from vessels excised from the rat, dog, rabbit, and pig [14]. Given the more recent extensive availability of antibodies for and methods to manipulate the genetics of the mouse, however, it has become the animal model of choice in arterial mechanics over the past 15 years. In light of the accumulating database for murine arterial mechanics, there is clearly a need for consistent methods of experimental testing, data analysis, and comparison that can enable

Fig. 2 Geometrical and mechanical metrics are mostly similar across the seven groups of potential controls and do not appear to depend on age over the range from 8 to 27 weeks. Greyscale conventions for symbols follow Fig. 1 and error bars again represent the SEM. The gray shaded region in each plot defines ranges of values for the average adult control male mice obtained by pooling the four groups that were not statistically different (i.e., *Myh11*^{+/+}, *Fbn1*^{+/+}, *Fbln5*^{+/+}, and C57BL/6J). Noninduced mice (*Fbln4*^{loxP/+}, *mT/mG.Myh11-CreERT2.Tgfbf2*^{f/f} + Veh, and *mT/mG.Myh11-CreERT2.Tsc1*^{f/f} + Veh) show slight differences in multiple metrics (unloaded thickness, outer/inner diameter, stretch, stress, stiffness, and distensibility), which include circumferential properties.

Fig. 3 Unloaded outer diameter (OD), outer diameter at systole (od), and distensibility ($\mathcal{D}$) are the only metrics that seem to correlate with the body mass of the mice. Color conventions for symbols follow Figs. 1 and 2. Again, the error bars represent the SEM and the gray shaded area in each plot identifies the expected behavior of an average adult control male mouse. It is noted that two of the three noninduced mice had lower body mass than average.

broader conclusions to be gleaned from complementary studies (cf. [2] and [3]).

We previously showed [15] that the in vivo value of axial stretch, a key determinant of arterial properties, differs from ~ 1.71 to 1.88 in common carotid arteries across three particular types of possible control mice (C57BL/6J, C57BL/6;129Sv, and FVB/N). Nevertheless, the present study is the first to our knowledge to compare a broader set of geometrical and mechanical metrics across a larger set of potential control groups. As revealed by Figs. 1 and 2, there is considerable similarity in ATA biomechanics across the seven groups, despite noted differences in age (8–27 weeks), body mass (17–32 g), and resting systolic blood pressure (117–128 mmHg), as seen in Table 1. Nevertheless, it was somewhat surprising that the two metrics that tended to differ the most (in noninduced $Tgfbf2^{f/f}$, $Tsc1^{f/f}$, or $Fbln4^{loxP/+}$ mice) were the circumferential material stiffness and the distensibility (Figs. 2(i) and 2(o)). Studies both across [16] and within [2] species suggest that intramural cells seek to maintain the circumferential material stiffness of an elastic artery nearly constant (see also [17]), which implies that cells seek to preserve a favorable local mechanobiological environment. Distensibility, on the other hand, is a commonly used clinical metric of structural stiffness that influences the hemodynamics; it can change dramatically in hypertension and aging [18]. While we measured the resting systolic peripheral arterial pressure in each mouse, we did not compare local hemodynamics which otherwise would be expected to be similar across potential controls.

Interestingly, it was the three conditionally inducible models that exhibited (albeit slight) differences in the two metrics of circumferential stiffness, one material and one structural. This observation suggests that additional attention should be focused on potential changes in biomechanical or mechanobiological properties that result from Cre-lox or similar approaches to induce altered genetics. Among other considerations, altered protein expression may explain the observed differences in these conditional deletion lines. First, background or leaky Cre expression that is tamoxifen independent may result in aberrant recombinase activity and rare medial cells have been noted as reporter positive in the thoracic aorta of mice with *Myh11-CreER* or *Acta2-CreER* SMC-specific constructs [19]. Second, double reporter systems with ubiquitous expression of a marker prior to recombination, as, for example, the membrane-targeted tandem dimer Tomato in our noninduced $Tgfbf2^{f/f}$ and $Tsc1^{f/f}$ strains, may have subtle pathological effects even when not overtly toxic as other red fluorescent proteins [20]. Clearly, there is a need for attention to these and other possible causes of altered wall properties.

Of course, regional variations exist in passive structural and material properties along the aorta within the same mouse strain

[3,4]. Likewise, the contractile ability of SMCs varies with region in response to different agonists [8]. We focused on the passive ATA as an illustrative example, but potential differences across candidate controls should be checked for other arterial segments under passive as well as active conditions. Age can also be a strong determinant of biomechanical properties [7]; hence, comparisons across controls should also be extended to include much younger (< 8 –10 weeks) and older (> 18 months) mice for other differences could manifest with aging. We focused on male mice given the availability of data. Although we previously showed that central arteries from both $Fbln5^{+/+}$ and $Fbln5^{-/-}$ mice tend to be biomechanically similar in males and females [4], a more extensive study of sex as a biological variable is warranted. Finally, both normal biomechanical properties [15] and responses to disease and injury [21,22] could differ across other strains. Potential control mice having different backgrounds (i.e., other than the three considered) should thus be added to the list of comparisons to screen for a possibly broader set of reliable controls.

Nevertheless, the current results suggest that homozygous non-mutant littermate mice (+/+) generated when breeding heterozygous mutant mice (+/−) to produce homozygous germline mutations (−/−) appear to be reliable controls. Likewise, noninduced mice, which retain the potential of presenting conditional mutations upon induction, tend to be reliable controls for most geometrical and mechanical metrics of interest. The obvious caveat, however, is that the noninduced controls that were included in our study showed modest differences in circumferential stiffness (both material and structural), which is increasingly recognized as an important determinant of local mechanobiological and global hemodynamical responses to any perturbation from normalcy. Clearly, there is a need to determine how genetic manipulations that enable inducible mutations affect cellular behavior and/or matrix properties prior to induction. Until this issue is understood better, using other or at least additional controls (e.g., nongenetically modified background mice) appears prudent.

Acknowledgment

This work was supported, in part, by NIH Grant Nos. R01 HL105297 and R03 EB021430.

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