Elastin insufficiency predisposes to elevated pulmonary circulatory pressures through changes in elastic artery structure

Adrian Shifren, Anthony G. Durmowicz, Russell H. Knutsen, Gilles Faury, and Robert P. Mecham

Departments of Internal Medicine, Pediatrics, and Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri; and Laboratoire Physiopathologies Vasculaires: Interactions Cellulaires, Signalisation et Vieillissement, Université Joseph Fourier, Institut National de la Santé et de la Recherche Médicale U882, Commissariat à l’Énergie Atomique, Grenoble, France

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Elastin insufficiency predisposes to elevated pulmonary circulatory pressures through changes in elastic artery structure. J Appl Physiol 105: 1610–1619, 2008. First published September 4, 2008; doi:10.1152/japplphysiol.90563.2008.—Elastin is a major structural component of large elastic arteries and a principal determinant of arterial biomechanical properties. Elastin loss-of-function mutations in humans have been linked to the autosomal-dominant disease supravalvular aortic stenosis, which is characterized by stenotic lesions in both the systemic and pulmonary circulations.

To better understand how elastin insufficiency influences the pulmonary circulation, we evaluated pulmonary cardiovascular physiology in a unique set of transgenic and knockout mice with graded vascular elastin dosage (range 45–120% of wild type). The central pulmonary arteries of elastin-insufficient mice had smaller internal diameters (P < 0.0001), thinner walls (P = 0.002), and increased opening angles (P = 0.002) compared with wild-type controls. Pulmonary circulatory pressures, measured by right ventricular catheterization, were significantly elevated in elastin-insufficient mice (P < 0.0001) and showed an inverse correlation with elastin level. Although elastin-insufficient animals exhibited mild to moderate right ventricular hypertrophy (P = 0.0001) and intrapulmonary vascular remodeling, the changes were less than expected, given the high right ventricular pressures, and were attenuated compared with those seen in hypoxia-induced models of pulmonary arterial hypertension. The absence of extensive pathologic cardiac remodeling at the high pressures in these animals suggests a developmental adaptation designed to maintain right-sided cardiac output in a vascular system with altered elastin content.

The importance of elastin to normal vascular function was shown by linkage of elastin loss of function mutations to the disease supravalvular aortic stenosis (SVAS) [Online Mendelian Inheritance in Man (OMIM) no. 185500] (45). SVAS can occur sporadically or as a familial condition with autosomal dominant inheritance and is characterized by a congenital narrowing of the large elastic vessels, most typically the ascending aorta (31, 49). SVAS also occurs as part of Williams syndrome (OMIM no. 194050), where a microdeletion at 7q11.23 results in individuals hemizygous for the elastin gene (35). Systemic hypertension is frequently associated with SVAS, but is of variable penetrance and severity. In addition to localized narrowing of elastic arteries, nonstenotic regions are characterized by an increase in the number of elastic lamellae and smooth muscle cells (22). Elastin insufficiency also has implications for the pulmonary circulation, where pulmonary stenoses are frequently associated with SVAS and Williams syndrome (23, 36). Detailed descriptions of the vascular pathology and the existence and severity of pulmonary arterial hypertension (PAH) are, however, limited in this group of patients. Although PAH is traditionally considered a disease of the peripheral lung vasculature, recent data suggest that the proximal elastic pulmonary arteries (PAs) contribute significantly to the increased right ventricular (RV) afterload in pulmonary hypertensive states (11, 48). This occurs through acute mechanisms like strain stiffening, as well as chronic adaptations like structural remodeling of vessel walls in response to elevated pulmonary pressures (48). Furthermore, measurement of these mechanical changes allows for improved mortality prediction in patients with PAH (27).

Mice heterozygous for elastin (Eln+/−) have many traits in common with individuals with SVAS. The mice have a high penetrance of systemic cardiovascular abnormalities, including an increased number of aortic elastic lamellae and elevated systemic pressures (15, 47). In this study, we evaluate the anatomy, physiology, and biomechanical properties of the pulmonary circulation of C57BL6 mice with genetically determined graded elastin insufficiency. We show that elastin insufficiency results in mechanical and structural remodeling of the proximal PAs and elevated pulmonary circulatory pressures. Unlike existing models of pathological PAH, this model is one of congenital adaptation of the pulmonary cardiovascular system to insufficient elastin gene product dosage and is distinct from primary PAH.

Address for reprint requests and other correspondence: A. Shifren, Dept. of Internal Medicine, CB 8052, Washington Univ. School of Medicine, 660 S. Euclid, St. Louis, MO 63110 (e-mail: ashifren@cellbiology.wustl.edu).
through the internal jugular vein into the RV. Pressures were recorded (model SPR671, Millar Instruments, Houston, TX) was advanced in air to minimize vasodilatory effects. A 1.4-F Millar microcatheter rane and placed on a warming pad. Isoflurane was maintained at 1%

Table 1. Characterization of mice with altered elastin dosage

<table>
<thead>
<tr>
<th></th>
<th>hBAC-mWT</th>
<th>WT</th>
<th>hBAC-mHET</th>
<th>Eln⁺⁻⁻</th>
<th>hBAC-mNULL</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, days</td>
<td>100±4</td>
<td>99±6</td>
<td>101±3</td>
<td>98±5</td>
<td>99±3</td>
<td>0.85</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>32.4±2</td>
<td>30.4</td>
<td>31.3</td>
<td>32.2</td>
<td>31.3</td>
<td>0.66</td>
</tr>
<tr>
<td>Sex ratio (male:female)</td>
<td>52.48</td>
<td>50.50</td>
<td>49.51</td>
<td>51.49</td>
<td>51.49</td>
<td>0.74</td>
</tr>
<tr>
<td>Elastin content, pg desmosine/µg protein</td>
<td>3.13</td>
<td>2.64</td>
<td>2.25</td>
<td>1.53</td>
<td>1.19</td>
<td>0.53</td>
</tr>
<tr>
<td>Elastin content, %WT</td>
<td>119</td>
<td>100</td>
<td>85</td>
<td>59</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>RV systolic pressure, mmHg</td>
<td>19.8±2.8</td>
<td>19.8±2.8</td>
<td>32.1±4.1</td>
<td>54.6±5.1</td>
<td>77.9±9.6</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>RV diastolic pressure, mmHg</td>
<td>2.5±1.1</td>
<td>3.1±2.1</td>
<td>9.9±4.1</td>
<td>13.2±3.6</td>
<td>26.3±3.6</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

Values are means ± SE. WT, wild type; RV, right ventricle. See MATERIALS AND METHODS for definition of mouse genotypes. Equal numbers of male and female mice were used in each experiment. Elastin content determined using 4 main pulmonary arteries per genotype. RV pressures are for 6 animals per genotype analyzed.

MATERIALS AND METHODS

Animals. Study mice were 3-mo-old males and females in the C57BL/6 background to allow comparison of data with other studies and eliminate possible confounding variables caused by postnatal changes in the pulmonary circuit (9). Equal numbers of males and females were used in all experiments. Eln⁺⁻⁻ mice bearing a heterozygous deletion of exon 1 of the mouse elastin gene (Eln⁺⁻⁻) have been described (24). Wild-type (WT) (Eln⁺⁻⁻) littermates from Eln⁺⁻⁻ crosses were used as WT controls. Bacterial artificial chromosome (BAC) transgenic mice were generated using human BAC (hBAC) clone CTB-512J22 containing the complete human elastin gene (hBAC-ELN) as described (19). hBAC-mWT mice are homozygous for the human elastin transgene and heterozygous for the mouse elastin gene (ELN⁺⁻⁺, Eln⁺⁻⁻). hBAC-mHET mice were generated from hBAC-mWT × Eln⁺⁻⁻ crosses and are homozygous for the human elastin transgene and heterozygous for the mouse elastin gene (ELN⁺⁻⁺, Eln⁺⁻⁻). hBAC-mNULL mice were generated from hBAC-mHET × hBAC-mHET crosses and are homozygous for the human elastin transgene and null for the mouse elastin gene (ELN⁺⁻⁺, Eln⁻⁻⁻). All elastin-insufficient mice are fertile and have normal life spans. All animals were maintained under identical conditions in accordance with institutional guidelines, and all procedures were approved by the Animal Studies Committee of the Washington University School of Medicine. An observer blinded to genotype performed all analyses.

Hemodynamic measurements. Mice were anesthetized using isoflurane and placed on a warming pad. Isoflurane was maintained at 1% in air to minimize vasodilatory effects. A 1.4-F Millar microwire catheter (model SPR671, Millar Instruments, Houston, TX) was advanced through the internal jugular vein into the RV. Pressures were recorded for >15 min using Chart 5 software (ADInstruments, Colorado Springs, CO). If the heart rate fell <300 beats/min, it was assumed that the level of anesthesia or trauma was inhibiting cardiac function, and the measurements were excluded from analysis. Each genotype analyzed contained six animals. After death, the hearts, main PAs (MPA), and the extrapulmonary segments of the right (RPA) and left PAs (LPA) were excised, packed on ice, and tested within 12 h of excision. Pressure measurements in each genotype were verified by using a pressure transducer attached to a 26-gauge needle introduced percutaneously into the RV, as described (25), with confirmation of RV placement at postmortem (data not shown).

Elastin content measurement. Four MPAs from each genotype were dissected free of extraneous connective tissue, minced, pooled, and hydrolyzed with 6 N HCl (41). Total protein and desmosine levels in hydrolysates were determined in triplicate using a Beckman 6300 amino acid analyzer, as described (3). Pressure-diameter testing. MPAs and LPAs were mounted on a pressure arteriograph (Danish Myotechnology, Copenhagen, Denmark) in balanced physiological saline at 37°C. Vessels were transilluminated under a microscope connected to a charged-coupled device camera and computerized measurement system, as described (14), to allow continuous recording of vessel diameters. Intravascular pressure was increased from 0 to 90 mmHg in 10-mmHg steps (30 s/step) to minimize experiment time, while allowing operator supervision of

Fig. 1. Serial pulmonary artery (PA) segments were immunostained for mouse (top) and human (bottom) elastin to demonstrate incorporation of human elastin in hBAC-mWT and hBAC-mHET PA, and humanization of hBAC-mNULL PA elastin. Not all sections are perpendicular to the vessel long axis. Magnification ×40. See MATERIALS AND METHODS for definition of mouse genotypes.
diameter tracking. Each genotype analyzed contained six RPA and six LPA. Since RPA and LPA data were statistically the same, only RPA data are shown.

Opening angle measurement. Opening angle measurements were performed as previously described (47). Rings were cut from RPA and LPA and then cut radially at the ventral surface. After equilibration, the opened ring was imaged, and the opening angle [subtended by lines connecting the midpoint of the inner circumference with the ends of the ring (6, 16)] was measured using Matlab software (The Mathworks, Natick, MA) (47). WT mouse opening angles were comparable to those measured in other rodent studies (17). Each genotype analyzed contained 12 vessel rings. Because RPA and LPA data were statistically the same, only RPA data are shown.

Vessel mechanics. Incremental elastic modulus ($E_{inc}$) was calculated as:

$$E_{inc} = \frac{\Delta P_{ave} \cdot 2ID \cdot OD^2}{\Delta ID \cdot OD^2 - ID^2} + \frac{2P_{ave} \cdot OD^2}{OD^2 - ID^2}$$

(1)

where $\Delta P_{ave}$ is incremental change in average transmural pressure; $\Delta ID$ is corresponding change in internal diameter; and ID, OD, and $P_{ave}$ are inner diameter, outer diameter, and average pressure at the beginning of the increment, respectively (5, 21). Calculated $E_{inc}$ for WT vessels was consistent with those from other studies (5, 8, 21). Each genotype analyzed contained nine vessels.

Data from test protocols were converted to stress and stretch ratios. Loaded inner diameter was calculated by assuming constant wall volume:

$$d_i = \sqrt{d_o^2 - \frac{L(D_o^2 - D_i^2)}{l}}$$

$$D_i = D_o - T$$

(2)

where $d_i$, $d_o$, and $l$ are loaded inner and outer diameters and length, respectively; and $D_i$, $D_o$, $L$, and $T$ are unloaded inner and outer diameters, length, and thickness, respectively (14). In the stress notation, $0$ refers to circumferential axis. Mean circumferential stretch ratio ($\lambda_o$) was calculated as (28):

$$\lambda_o = \frac{1}{2} \left( \frac{d_o}{D_o} + \frac{d_i}{D_i} \right)$$

(3)

Assuming constant wall volume and a thin-walled tube, mean circumferential stress ($\sigma_\theta$) was defined as:

Fig. 2. A: right ventricular (RV) pressure was measured using a microcatheter introduced through the internal jugular vein. *$n = 6$ Animals per genotype. Bars represent mean systolic (solid) and diastolic (shaded) pressures ± SE. *$P < 0.00001$, §$P < 0.0001$, ¶$P = 0.96$, and †$P = 0.41$ vs. wild type (WT). B: pressure tracings from WT and hBAC-mNULL mice demonstrating measured systolic and diastolic pressure differences.
where \( \sigma_s \) is pressure in vivo \((30)\). To model transmural stress gradients, with and without residual stress, circumferential stretch ratio at the inner \((d_i/d_0)\) and outer wall \((d_o/d_0)\) of each PA was calculated using mean opening angle (to include residual stress) or an angle of zero (to exclude residual stress). All modeling was done with Matlab software (The Mathworks) \((47)\). Since RPA and LPA data were statistically the same, only RPA data are shown.

**Arterial histology.** RPs and LPs were inflated to 5-cmH\(_2\)O pressure with gelatin to preserve patency and then formalin-fixed and embedded in paraffin \((15)\). Five-micrometer-thick sections were stained with hematoxylin and eosin and Hart’s elastic stain. For counting elastic lamellae and measuring wall thickness, vessels were divided into quadrants, with number and thickness determined in each quadrant using three sections per mouse. Wall thickness measurements included all three vessel layers (intima, media, and adventitia) and were measured from the lumen to the outermost limit of the adventitia. Only sections perpendicular to the vessel long axis were used for measurements. Each genotype analyzed contained six animals.

**Vessel immunofluorescence.** Paraffin sections were blocked with 5% normal goat serum, and primary antibody was applied overnight at 4°C. Polyclonal anti-mouse recombinant tropoelastin \((1:1,000)\) was used to detect mouse elastin, and polyclonal anti-human aortic elastin \((1:500)\) was used to detect human elastin. Secondary fluorescein-conjugated goat anti-rabbit antibody \((1:500, \text{Jackson Immuno-Research, Westgrove, PA})\) was used to detect mouse elastin, and polyclonal anti-human aortic elastin \((1:500)\) was used to detect human elastin. Secondary fluorescein-conjugated goat anti-rabbit antibody \((1:500, \text{Jackson Immuno-Research, Westgrove, PA})\) was applied at room temperature for 1 h. Images were captured with a Zeiss Axioskop microscope, an inline Zeiss Axiocam camera, and Axiovision software (Carl Zeiss, Thornwood, NY).

**Peripheral artery analysis.** Lungs were inflated to 25-cmH\(_2\)O pressure with 10% buffered formalin, fixed for 24 h, and embedded in paraffin. Five-micrometer-thick sections were stained with monoclonal \(\alpha\)-smooth muscle actin antibody \((1:400, \text{Sigma-Aldrich, St. Louis, MO})\) and Vector Mouse-on-Mouse Kit (Vector Laboratories, Burlingame, CA), following manufacturer instructions. Sections were developed with Vector ABC Kit (Vector Laboratories) and diaminobenzidine and then counterstained with hematoxylin. Small vessels \((15-50\mu m \text{ external diameter})\) from three randomly chosen fields at \(x10\) magnification were identified and quantified as muscularized \((\text{actin staining } >25\% \text{ of vessel circumference})\) or nonmuscularized \(<25\%)\) \((51)\) using Image J software (version 1.37, National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/), and the percentage of muscularized arteries was calculated. Hart’s stained sections were used to count alveoli and small vessels from three randomly chosen fields at \(x10\) magnification, and average ratios of arteries to alveoli were calculated. Each genotype analyzed contained six different animals.

**Statistical analyses.** Analysis was performed using one-way ANOVA to determine differences between more than two groups of data, and Student’s \(t\)-tests (2-tailed distribution with 2-sample equal variance) were used for paired value comparisons. Results are presented as mean values \(\pm\ SE\), unless otherwise stated. \(P\) values \(<0.05\) were chosen as the threshold for statistically significant differences.

**RESULTS**

**Study mice.** Mice from all five genotypes did not differ in age, weight, or sex distribution (Table 1). Desmosine content of WT MPA was defined as 100% elastin content (Table 1). By comparison, \(\text{Eln}^{+/−}\) MPA contain \(60\%\) of WT elastin levels, \(\text{hBAC-mHET}\) MPA \(\sim 85\%\) of WT levels, and \(\text{hBAC-mNULL}\) MPA \(\sim 45\%\) of WT levels. \(\text{hBAC-mWT}\) MPA have an increased elastin content of \(\sim 120\%\) of WT levels. These values are in agreement with desmosine levels measured in both systemic vessels \((19)\) and whole lung \((41)\). Immunostaining with human and mouse elastin-specific antibodies (Fig. 1) demonstrates that PA from WT and \(\text{Eln}^{+/−}\) mice contain only mouse elastin, and that human elastin incorporates into elastic fibers with mouse elastin in PA from \(\text{hBAC-mWT}\) and \(\text{hBAC-mHET}\) mice. \(\text{hBAC-mNULL}\) PA are humanized for elastin protein and contain human elastin exclusively.

**Elastin-insufficient mice have markedly elevated RV pressures.** Compared with WT animals, elastin-insufficient mice demonstrate a striking elevation in RV systolic and diastolic pressures in a manner inversely proportional to elastin content (Table 1 and Fig. 2A). By comparison, mice with supraphysiological elastin content (\(\text{hBAC-mWT}\)) show no difference in systolic \((P = 0.96)\) or diastolic \((P = 0.41)\) pressures. Representative catheter pressure tracings from WT and \(\text{hBAC-mNULL}\) mice are shown in Fig. 2B.

**Fig. 3.** A: RV hypertrophy (RVH) was assessed by calculating RV-to-body weight (BW) ratios, \(n = 6\) Hearts per genotype. Bars represent means \(\pm\ SE\). *\(P < 0.0001\) and †\(P = 0.79\) vs. WT. B: RVH correlates closely with RV systolic pressure. Circles represent mean elastin-insufficient RVH at each operating pressure \(\pm\ SE\). *\(P < 0.0001\) and †\(P = 0.79\) vs. WT. \(R^2 = 0.88\) for RVH vs. pressure.
**RVH correlates with RV systolic pressure.** Elastin-insufficient mice demonstrate RVH (Fig. 3A) as elastin content decreases and RV pressure increases. Figure 3B expresses RVH as a function of systolic pressure and demonstrates that RVH and RV systolic pressure are very closely correlated. hBAC-mWT mice again demonstrate no difference from WT controls ($P = 0.79$).

**Elastin-insufficient mice exhibit intrapulmonary arteriolar remodeling.** Muscularization of peripheral arterioles, assessed by α-smooth muscle actin staining, is increased in elastin-insufficient mice in proportion to RV systolic pressure (Fig. 4, A and B). Although mouse models traditionally demonstrate only small amounts of peripheral vascular remodeling in response to PAH, the arteriolar muscularization in our mice is markedly attenuated compared even with historic controls (12, 13, 18, 51), despite the greater pressures in our animals.

Peripheral vessel numbers per 100 alveoli also decline as RV pressures increase (WT $= 2.01 \pm 0.06$, hBAC-mHET $= 1.93 \pm 0.05$, $Eln^{+/−} = 1.84 \pm 0.04$, $P = 0.02$, Fig. 4C) and are again attenuated compared with historic controls (13, 34, 51). The only genotype where peripheral vessel number apparently increases is hBAC-mNULL mice ($2.66 \pm 0.12$). This, however, is due to alveolar enlargement, resulting from failed secondary alveolar septation (Fig. 4D) (41) and a consequent decrease in alveolar number. hBAC-mWT mice ($1.99 \pm 0.07$) are again no different from WT controls ($P = 0.78$).

**Elastin insufficiency alters central PA architecture.** Despite comparable ages and body weights, RPA and LPA in hBAC-mNULL mice, and to lesser extent in $Eln^{+/−}$ mice, had smaller diameters in vivo compared with other genotypes (not shown). Ex vivo full wall thickness (intima, media, and adventitia) of both RPA and LPA decreases in proportion to the degree of elastin insufficiency, as does inner (luminal) diameter (Table 2, values for RPA shown). Interestingly, no discrete pulmonary stenoses were noted in any of the elastin-insufficient PA. Lamellar number increases with decreasing elastin content (Table 2), a finding consistent with systemic vessels in $Eln^{+/−}$ mice (15, 24, 47) and SVAS patients (24). As before, hBAC-
mWT mice show no differences in PA wall thickness ($P = 0.90$), inner diameter ($P = 0.98$), or lamellar number ($P = 0.91$) compared with WT mice.

$E_{inc}$ is similar over the in vivo pressure range between genotypes. LPA and RPA pressure-diameter curves demonstrate a nonlinear increase in vessel diameter with progressive pressure loading (Fig. 5, RPA data shown). Calculation of the PA $E_{inc}$ over the in vivo pressure change confirmed that vessel stiffness is equivalent in all five genotypes over their respective pulse pressure increments, despite different elastin contents (Table 3, values for RPA shown). The most interesting finding was that PA operating diameter (lumen diameter at RV systolic pressure) in all five groups is similar (Table 3), indicating that in vivo cross-sectional area during systole is the same between genotypes, despite differences in their starting geometries and blood pressures.

Elastin-insufficient PA adapt to increased mechanical stresses. Calculation of wall thickness-to-luminal cross-sectional area ratios demonstrates that changes in wall thickness and vessel diameter are proportional, with no difference in the ratios between genotypes ($P = 0.90$). This is not typical of hypertensive vessels and indicates that mechanical remodeling of the vessel walls has normalized vessel stress in each genotype in a manner different from typical models of PAH (10, 43) that involve postnatal injury.

Residual stress in large elastic arteries is thought to be a homeostatic mechanism for normalizing the circumferential stress gradient through the artery wall, thereby ensuring a uniform transmural stress distribution in vivo (6). Without residual stress, circumferential stress would vary from a maximum at the inner wall surface to a minimum at the outer wall surface (20), producing a transmural stress gradient proportional to the blood pressure within the vessel lumen. Residual stress is incorporated into biomechanical modeling using opening angle at the zero-stress state to allow for calculation of the transmural stress gradient (6, 30, 46). As PA elastin content decreases, the measured mean opening angle increases (Table 3 and Fig. 6A), indicating increased residual stress in elastin-insufficient vessels. Again, hBAC-mWT animals are no different from WT animals ($P = 0.77$). Modeling of PA transmural stress distributions under physiological conditions with inclusion and exclusion of opening angles indicates that the increased residual stress in elastin-insufficient PA effectively normalizes the transmural stress gradients in each genotype (Fig. 6B), despite significant differences in measured systolic pressures.

**DISCUSSION**

Elastin-insufficient mice demonstrate anatomic remodeling of the proximal vasculature that includes thinner vessel walls and preserved artery wall thickness-to-luminal ratios (Table 2). Our laboratory’s previous work demonstrated that blood vessels from newborn calves with hypoxia-induced pulmonary hypertension manifest with a two- to fourfold increase in pulmonary vascular elastin production (33). Other studies have reported a predisposition to pathological PAH in rats with increased endogenous elastase activity (29) and protection from PAH in mice overexpressing elastase inhibitors (51). Collectively, these data suggest that elastin modulates changes in vessel wall stresses resulting from elevated pulmonary pressures, and that elastin insufficiency may lead to a biomechanical disadvantage predisposing mice to the development of elevated pulmonary pressures.

PAH induces a marked change in the opening angles of central pulmonary vessels (17), suggesting that changes in vascular circumferential residual stress may effectively normalize transmural stress distribution through elastin-insufficient vessel walls. Our data show that opening angle does increase with decreasing elastin content (Table 3), indicating that circumferential residual stress increases to compensate for the higher transmural stresses experienced at higher physiological operating pressures in elastin-insufficient mice. Mathematical modeling that includes circumferential residual stress results in a more uniform distribution of transmural stress (Fig. 6), noticeably decreasing the stress at the inner wall in all five genotypes compared with modeling where residual stress is excluded.
The unloaded internal PA diameter (and hence vessel cross-sectional area) decreases with decreasing elastin content (Table 2). The smaller unloaded dimensions of elastin-insufficient vessels result in downward displacement of the elastin-insufficient pressure-diameter curves and hence smaller dimensions at each measured pressure. Since decreased luminal area increases resistance to blood flow, our data suggest that the increased RV pressures associated with elastin insufficiency function to expand the proximal vascular cross-sectional area to accommodate cardiac output by overcoming the increased RV afterload imposed by the smaller starting dimensions of elastin-insufficient vessels. The result is similar operating diameters at measured systolic pressures in all five genotypes, allowing for maintenance of cardiac output and perfusion pressure. The larger circumferential stretch ratio of elastin-insufficient PA at measured operating pressures (Table 3) indicates a greater degree of stretch from the zero-stress state in elastin-insufficient animals, suggesting that this phenomenon is indeed occurring.

| Table 3. Biomechanical properties of elastin-insufficient right PA |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | hBAC-mWT         | WT              | hBAC-mHET        | Eln+/−           | hBAC-mNULL       |  P (ANOVA)       |
| Opening angle, ° | 122±10           | 120±11           | 131±8            | 143±8            | 158±9            | 0.0001           |
| \(E_{inc}\), kPa | 138±7.8          | 141±9.2          | 134±7.8          | 130±7.0          | 137±8.3          | 0.87             |
| \(\lambda_0\)   | 1.85±0.12        | 1.84±0.16        | 2.25±0.16        | 2.52±0.15        | 2.55±0.17        | 0.007            |
| \(\sigma_0\), kPa| 95±13            | 92±12            | 222±30           | 473±64           | 741±126          | <0.0001          |

Values are means ± SE. \(E_{inc}\), elastic modulus; \(\lambda_0\), mean circumferential stretch ratio; \(\sigma_0\), mean circumferential stress. Both right and left PA segments were evaluated. Differences between the two sides were minimal and not significant. Data are representative of both right and left PA measurements. Opening angle measurements are for 6 right PA per genotype analyzed.

Fig. 6. A: arterial rings spring open when cut radially. The angle subtended by the resulting segment is the opening angle (OA), an indicator of circumferential residual strain. Bars represent mean OA (in degrees) ± SE; \(n = 12\) rings per genotype. *\(P = 0.0001\) and †\(P = 0.77\) vs. WT. B: modeling of transmural stress distribution of vessels from each genotype under physiological conditions with inclusion and exclusion of OAs. a, hBAC-mNULL; b, Eln+/−; c, hBAC-mHET; d, WT; e, hBAC-mWT. Models are based on measurements from 6 vessels per genotype.
In contrast to elastin-insufficient PA, hBAC-mWT vessels display no architectural or biomechanical differences from WT PA. This suggests that evolution has optimized the elastin content of PA with respect to mechanical function, and that, while elastin insufficiency negatively affects tissue mechanics, elastin excess, at least to 120% of WT levels, has no structural or mechanical impact. Whether greater amounts of elastin (>120% of WT levels) will alter vessel mechanics is unknown.

Our findings differ from those seen in traditional models of adult-onset PAH (40, 52). Our mice experience elastin insufficiency throughout embryonic development, and the attenuated cardiovascular remodeling in elastin-insufficient mice may represent physiological adaptation of the pulmonary circulatory system to congenital elastin insufficiency rather than a pathological response to elevated pulmonary pressures. Three lines of evidence support this hypothesis.

First, chronic PAH in normal conditions leads to elevation of RV pressures and subsequent RVH. In our model, RV pressures increase in proportion to decreased vascular elastin content, with concomitant RVH. However, despite a strong correlation between RV systolic pressure and RVH, direct comparison to historic WT controls developing PAH following hypoxic exposure (44, 51) indicate that the degree of hyper-tension to historic WT controls developing PAH following exposure to chronic hypoxia (51) demonstrate muscularization and distal vessel loss occur in proportion to elevated pulmonary pressures. Three lines of evidence suggest this hypothesis.

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41. Weinberg CE, Hertzberg JR, Ivy DD, Kirby KS, Chan KC, Valdes-Cruz I, Shandas R. Extraction of pulmonary vascular compliance, pulmonary vascular resistance, and right ventricular work from single-pressure and Doppler flow measurements in children with pulmonary


