Effects of Elastin Haploinsufficiency on the Mechanical Behavior of Mouse Arteries

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Abstract

Supravalvular aortic stenosis (SVAS) is associated with decreased elastin and altered arterial mechanics. Mice with a single deletion in the elastin gene (ELN+/-) are models for SVAS. Previous studies have shown that elastin haploinsufficiency in these mice causes hypertension, decreased arterial compliance and changes in arterial wall structure. Despite these differences, ELN+/- mice have a normal lifespan, suggesting that the arteries remodel and adapt to the decreased amount of elastin. To test this hypothesis, we performed in vitro mechanical tests on abdominal aorta, ascending aorta and left common carotid arteries from ELN+/- and wildtype (C57BL/6J) mice. We compared the circumferential and longitudinal stress-stretch relationships and residual strains. The circumferential stress-stretch relationship is similar between genotypes and changes less than 3% with longitudinal stretch at lengths within 10% of the in vivo value. At mean arterial pressure, the circumferential stress in the ascending aorta is higher in ELN+/- than wildtype. Although arterial pressures are higher, the increased number of elastic lamellae in ELN+/- arteries results in similar tension/lamellae compared to wildtype. The longitudinal stress-stretch relationship is similar between genotypes for most arteries. Compared to wildtype, the in vivo longitudinal stretch is lower in ELN+/- abdominal and carotid arteries and the circumferential residual strain is higher in ELN+/- ascending aorta. The increased circumferential residual strain brings the transmural strain distribution in ELN+/- ascending aorta close to wildtype values. The mechanical behavior of ELN+/- arteries is likely due to the reduced elastin content combined with adaptive remodeling during vascular development.
Introduction

Elastin and collagen are the principal determinants of passive artery mechanics. Elastin provides reversible extensibility during cyclic loading of the cardiac cycle (22), while collagen provides strength and prevents failure at high pressure (11). It is believed that elastin bears load in both the circumferential and longitudinal directions, while collagen bears load mostly in the circumferential direction. Elastin is believed responsible for the in vivo longitudinal retraction force in arteries and consequent shortening upon excision. Decreased elastin content with aging, hypertension and artherosclerosis is accompanied by a decrease in longitudinal retraction force and circumferential compliance (change in diameter for a specified change in pressure) (8). Decreased elastin content is also found in genetic diseases such as supravalvular aortic stenosis (SVAS). SVAS is a congenital narrowing of the large arteries caused by deletions within the elastin gene that lead to functional haploinsufficiency (10). SVAS patients often exhibit hypertension (1) and have thinner and more numerous elastic lamellae (19).

Elastin is encoded by a single gene (ELN), hence the contribution of elastin to passive artery mechanics can be studied in a controlled manner with genetically modified mice. ELN +/- mice die within a few days of birth due to obstructive arterial disease (18). Unfortunately, arteries at this stage are difficult to isolate and manipulate for in vitro tests. ELN+/- mice have a normal lifespan and, similar to SVAS patients, exhibit systemic hypertension, thinner and more numerous elastic lamellae and less total elastin content. Initial studies of ELN+/- artery mechanics showed decreased compliance in large elastic vessels (10, 19). These measurements, however, were made on ex vivo arterial segments and no adjustments were made for the effect of longitudinal stretch (25). It is
known that arteries are in a stretched state in vivo and that compliance changes with increased stretch. To determine how longitudinal stretch influences compliance and the circumferential stress-stretch relationship, we conducted mechanical testing at different stretch lengths on ELN+/- and wildtype arteries.

Other mechanical properties, such as the longitudinal force-stretch and stress-stretch relationships, in vivo longitudinal stretch and circumferential residual strain, may also be affected by the decreased amount of elastin in ELN+/- arteries. In vivo longitudinal stretch is defined by the amount an artery retracts upon excision. Circumferential residual strain can be determined from the “opening angle” formed when a ring of arterial tissue is cut radially. Circumferential residual strain serves to normalize the stress and strain distribution through the artery wall (12). To assess the effects of reduced elastin on these mechanical properties, we measured the longitudinal force-stretch and stress-stretch relationships, in vivo longitudinal stretch and circumferential residual strain in ELN +/- and wildtype arteries.

**Materials and Methods**

*Animals:*

Wildtype C57BL/6J mice (ELN +/-) and mice bearing a heterozygous deletion of exon 1 in the elastin gene (ELN+/-) (18) were used for all studies. Mice were of matching age (mean 99 days) and weight (mean 30 grams) and littermates were used whenever possible. All housing, surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee.
Surgical Procedures and Blood Pressure:

Mice were anaesthetized by intraperitoneal injection of ketamine (100 mg/ml)/xylazine (20 mg/ml) cocktail. Arterial blood pressure was measured using a catheter (Millar Instruments Inc., Houston, TX) inserted into the right common carotid artery. The blood pressure was monitored for 15 minutes and the average systolic, diastolic and mean pressure were recorded. The ascending aorta, abdominal aorta and left common carotid artery were exposed and heparin (1000 unit/ml, approximately 50 units/mouse) was injected into the left ventricle to prevent blood clots during dissection. Small particles (30-90 µm diameter) of activated charcoal (Sigma, St. Louis, MO) were placed along the length of each artery on the ventral surface for measuring in vivo stretch and for calculating the longitudinal stretch ratio during mechanical testing. The carbon markers stick to the artery wall without adhesive, probably due to electrostatic effects. Images were recorded of each artery in vivo with a stereomicroscope coupled to a video camera. The arteries were removed, placed in a physiologic saline solution (130 mM NaCl, 15 mM NaHCO₃, 5.5 mM dextrose, 4.7 mM KCl, 1.2 mM MgSO₄·7H₂O, 1.2 mM KH₂PO₄, .026 mM EDTA and 1.6 mM CaCl₂, pH 7.2) and images were recorded of each artery in the ex vivo state before compliance testing. Blood pressure and mechanical test data for all three arteries was not necessarily obtained for every mouse. The sample size for each data set is given in the results.

Mechanical Testing:

The arteries were cannulated on specially designed stainless steel cannulae and mounted on a pressure and force arteriograph (Danish Myotechnology, Copenhagen,
Denmark) (9, 10). The arteries were secured with silk surgical suture (size 10-0) tied at grooves machined into the end of each cannula. The experiments were performed in physiologic saline solution at 37° C. The artery was transilluminated under an inverted microscope connected to a CCD camera and a computer. A central region along the artery length with identifiable carbon markers was chosen for diameter and length measurements. The arteriograph software continuously tracks the artery outer diameter by locating the change in pixel intensity between the illuminated artery and the dark background. The software was not designed to track the length markers, so the marker distance was determined manually from still images taken before each protocol. The distance between markers (l) was calculated using Image J software as described below. The arteriograph increases or decreases the intravascular (transmural) pressure of the artery segment by changing the fluid flow through the cannulae. The artery length was adjusted manually by turning a micrometer attached to one of the cannula. The starting length for each artery was determined during three preconditioning cycles from 0-175 mmHg and was defined as the minimum length at which the artery did not significantly buckle in the longitudinal direction at 175 mmHg. At the starting length, the intravascular pressure was then increased from 0-175 mmHg for three cycles in steps of 25 mmHg (12 sec/step). Preliminary data showed that the mechanical behavior of mouse arteries does not change with loading rate between continuous pressure increases of 7 mmHg/sec (maximum for the system) and stepwise pressure increases of 60-300 sec/25 mmHg step used in previous tests (9, 10). Twelve sec/step was chosen to minimize experiment time, while allowing operator supervision of the diameter tracking. After each protocol, the artery length was increased and the pressure was increased from 0-175 mmHg for three
more cycles. The pressure, outer diameter and longitudinal force were recorded at 1 Hz for each protocol. After mechanical testing, a narrow ring (1-2 mm long) was cut from the center of the artery and imaged to obtain the unloaded diameter and thickness. The artery boundaries were determined manually and the dimensions were measured with Image J software.

*In Vivo Lengths:*

Images were recorded of each artery in vivo with a stereomicroscope coupled to a video camera. After excision, the arteries were imaged in the ex vivo configuration floating in physiologic saline solution. Image J software was used to determine the centroid of each carbon marker and calculate the length between successive markers. The in vivo longitudinal stretch ratio ($\lambda^{iv}$) was determined by dividing the in vivo distance ($l^{iv}$) by the ex vivo (unloaded) distance ($L$) for each pair of markers and averaged for the length of the artery. Only markers visible in both in vivo and ex vivo images were measured. In some cases, the arteries looped or curved dramatically upon excision. In these cases, a line was drawn between successive markers following the curve of the artery and the length of this line was used as the distance between markers.

*Opening Angle:*

Narrow rings (1-2 mm long) were cut along the length of the artery and each ring was cut radially to measure the opening angle. In preliminary experiments, rings were cut from each artery after mechanical testing. However, it was difficult to remove arteries
from the test set-up without damage and to cut rings at the same longitudinal location in each artery. It was also difficult to cut rings from unpressurized arteries without collapsing the walls and changing the circular geometry. Consequently, opening angles were measured in arteries not used for mechanical testing. The mice were anaesthetized and the arteries were exposed as described above. Carbon particles were used to mark the ventral surface of the artery. Warm gelatin (14% in phosphate buffered saline (PBS) at 37° C) mixed with dextran blue (4 mg/ml) for contrast was injected through the left ventricle into the ascending aorta and common carotid arteries. The descending aorta was tied off with a suture to force gelatin into the carotid arteries. For the abdominal aorta, gelatin was injected directly into the inferior end at the iliac bifurcation. Ice-cold PBS was then poured on top of the arteries to set the gelatin. The arteries were removed and two or three rings were cut from the superior, central and inferior portion of the abdominal and left common carotid arteries. Two or three rings were cut from the proximal ascending aorta. The gelatin prevented the artery walls from collapsing while cutting rings and was removed from each ring before the radial cut was made. The rings were placed in room temperature PBS and cut radially at the ventral surface. After 30 minutes to equilibrate, the opened ring was imaged and the opening angle was measured using custom written scripts in Matlab software. The opening angle (OA) was defined as the angle subtended by the lines connecting the midpoint of the inner circumference with the ends of the ring (6, 11). Example OA measurements are shown in Figure 7b and c.

**Data Analysis:**
Pressure, outer diameter, longitudinal force and marker length data from individual test protocols were converted to stress and stretch ratios. Although all three loading cycles were similar, the third cycle was used for further analysis. The deformed inner diameter and thickness were calculated assuming constant wall volume (9):

\[
d_i = \sqrt{d_o^2 - \frac{L(D_o^2 - D_i^2)}{l}} \quad t = \frac{d_o - d_i}{2},
\]

where \(d_i, d_o, l\) and \(t\) are the deformed inner and outer diameters, length and thickness and \(D_i, D_o, L\) and \(T\) are the unloaded inner and outer diameters, length and thickness.

Cylindrical coordinates are used in the stretch and stress notation with \(\theta, z\) and \(r\) referring to the circumferential, longitudinal and radial axes, respectively. Shear was neglected because the markers along the length of the artery did not significantly rotate out of the image plane during loading cycles. The mean stretch ratios in each direction (\(\lambda_\theta, \lambda_z\), and \(\lambda_r\)) were calculated by (20):

\[
\lambda_\theta = \frac{1}{2} \left[ \frac{d_i}{D_i} + \frac{d_o}{D_o} \right] \quad \lambda_z = \frac{l}{L} \quad \lambda_r = \frac{t}{T}.
\]

In some cases, the longitudinal stretch ratio (\(\lambda_z^*\)) was calculated with respect to the in vivo length (\(l_{iv}\)):

\[
\lambda_z^* = \frac{l}{l_{iv}} = \frac{\lambda_z}{\lambda_z^{iv}}.
\]

Assuming constant wall volume and a thin-walled tube, the mean stresses in the circumferential (\(\sigma_\theta\)) and longitudinal (\(\sigma_z\)) directions were defined by:

\[
\sigma_\theta = \frac{p_i d_i}{d_o - d_i}.
\]
\[ \sigma_z = \frac{4f + p_i \pi d_i^2}{\pi (d_o^2 - d_i^2)}, \]  

where \(p_i\) is the inner pressure and \(f\) is the longitudinal force \(^{21}\).

ANOVA or unpaired, two-tailed t-tests, assuming unequal variance were used as appropriate to determine statistical differences between groups. P < 0.05 was considered significant.

**Results**

*Pressure and Geometry*

Consistent with previous results \(^{10}\), we found that ELN+/- mice have 34-43% higher systolic, diastolic and mean arterial pressure than wildtype. Despite comparable ages and body weights, the unloaded outer diameter of each artery is 11-21% smaller in ELN+/- mice than wildtype. The unloaded thickness is smaller than wildtype in ELN+/- ascending aorta and not significantly different in abdominal and left common carotid arteries (Table 1).

*Mechanical Testing*

The mean outer diameter is smaller in ELN+/- than wildtype arteries at each pressure, except for ascending aortas at 100 mmHg (Figure 1a-c). At the in vivo longitudinal stretch ratio \(\lambda^v\), the mean force decreases slightly with increased pressure in abdominal and left common carotid arteries, with no significant differences between genotypes (Figure 1d, f). At \(\lambda^u\), the mean force decreases with increasing pressure in ascending aorta and is significantly lower in ELN+/- aortas for pressures between 75-125
mmHg (Figure 1e). Figure 2 shows representative data from one artery in each group at different longitudinal stretch ratios ($\lambda_z^*$) calculated with respect to the in vivo length. The diameter at each pressure decreases and the force increases with increasing $\lambda_z^*$. For ascending aorta, the diameter-pressure relationship shifts downward by 1-3% for each successive 10% increase in $\lambda_z^*$. For abdominal and left common carotid arteries, the diameter-pressure relationship shifts downward by 1-3% for successive 10% increases in $\lambda_z^*$ between 0.9 and 1.1. The relationship shifts more for steps farther from the in vivo length, with the downward shift increasing from 8 to 14% and 4 to 10% for successive 10% increases in $\lambda_z^*$ between 1.2 and 1.5 for abdominal and left common carotid arteries, respectively. For most arteries, the longitudinal force decreases with pressure at $\lambda_z^* \leq 1.0$, remains constant around $\lambda_z^* = 1.1$ and increases with pressure at $\lambda_z^* > 1.1$. At $\lambda_z^* < 0.8$ for abdominal and $\lambda_z^* < 1.0$ for left common carotid arteries, the pressure cannot be increased to 175 mmHg without the arteries bending longitudinally instead of stretching circumferentially. Bending during in vitro testing is caused by negative (compressive) longitudinal force at low longitudinal stretch and high pressure. Ascending aorta could not be tested at $\lambda_z^* < 0.9$ because of the limited length of the arterial segment.

The circumferential stretch ratio and circumferential and longitudinal stresses at $\lambda_z^{iv}$ ($\lambda_z^* = 1.0$) were averaged for each pressure step. Mean circumferential stretch ratios increase from 1.0-2.1, circumferential stresses increase from 0-370 kPa and longitudinal stresses increase from 0-210 kPa, for pressure increases from 0-175 mmHg for all arteries (Figure 3). There are no significant differences between ELN+/- and wildtype abdominal aortas for the data in Figure 3a, d or g. ELN+/- ascending aortas show no significant
difference from wildtype for circumferential or longitudinal stresses (Figure 3e, h), but have significantly higher circumferential stretch ratios at pressures between 75-125 mmHg (Figure 3b). ELN+/- left common carotid arteries show no difference from wildtype in circumferential stretch ratio or longitudinal stress (Figure 3c, i), but show significantly lower circumferential stresses at all pressures greater than 0 mmHg (Figure 3f). At mean arterial pressure (wildtype = 98 mmHg, ELN+/- = 136 mmHg), the longitudinal stress is similar for all arteries (Figure 3g, h, i) and the circumferential stretch ratio and stress are similar for abdominal and left common carotid arteries (Figure 3a, c, d, f). At mean arterial pressure, the circumferential stretch ratio in ELN+/- ascending aorta is 20% higher and the circumferential stress is 68% higher than wildtype (Figure 3b, e). The circumferential stress-stretch relationship at different $\lambda_c$ is similar between genotypes for all arteries (representative data for one artery in each group in Figure 4a-c). The longitudinal stresses at equivalent circumferential stretch ratios are also similar (representative data for one artery in each group in Figure 4d-f). The abdominal aorta shows considerable variability in the longitudinal stress values at higher $\lambda_c$ (Figure 4d) due to the extreme nonlinearity of the longitudinal stress-stretch relationship (Figure 5a).

For most arteries, the longitudinal stress-stretch relationship at zero pressure appears similar between genotypes. The relationship is nonlinear and highly variable between individual arteries, with ELN+/- arteries showing more variation than wildtype (Figure 5). The data from individual arteries were not averaged because each artery was not stretched to identical longitudinal stretch values. Abdominal aortas show the most nonlinear behavior, followed by left common carotid arteries and then ascending aortas.
For abdominal aortas, wildtype arteries become nonlinear around $\lambda_{z}^* = 1.2$, while ELN+/- arteries become nonlinear around $\lambda_{z}^* = 1.1$ (N = 4) or $\lambda_{z}^* = 1.3$ (N = 3). Ascending aortas have a gradual nonlinear transition around $\lambda_{z}^* = 1.5$, though a few ELN+/- aortas have sharp nonlinear transitions not observed in the wildtype aortas. Left common carotid arteries become nonlinear at $\lambda_{z}^* = 1.5-1.7$. The trends are similar for non-zero pressures and if the longitudinal stretch ratio ($\lambda_{z}$) is calculated with respect to the unloaded length.

**In Vivo Length**

For abdominal and left common carotid arteries, $\lambda_{z}^{iv}$ is smaller in ELN+/- than wildtype. For ascending aorta, $\lambda_{z}^{iv}$ is not significantly different between ELN+/- and wildtype (Figure 6a). For abdominal and left common carotid arteries, the ex vivo shape of the artery is different between ELN+/- and wildtype (Figure 6b and d). The ELN+/- arteries either loop (40% of left common carotids) or curve significantly (60% of left common carotids and 100% of abdominal aortas), while the wildtype arteries never loop and only curve slightly. The shape of the ascending aorta does not differ between ELN+/- and wildtype (Figure 6c).

**Opening Angle**

Compared to wildtype arteries, the mean OA of ELN+/- arteries is larger in ascending aorta and not significantly different in abdominal and left common carotid arteries (Figure 7a). The OA for abdominal and left common carotid arteries does not vary significantly with longitudinal position in either genotype ($p = 0.3-0.8$).
Representative OA measurements for wildtype and ELN+/- ascending aortas are shown in Figure 7b and c. The circumferential stretch ratio at the inner \(d_i/D_i\) and outer wall \(d_o/D_o\) of each artery type was calculated using the mean OA, unloaded dimensions and dimensions at mean arterial pressure. In wildtype and ELN+/- mice, the circumferential stretch ratio is 10-13% higher at the inner wall for the abdominal aorta, 2-3% lower at the inner wall for the ascending aorta and 15-17% higher at the inner wall for the left common carotid at mean arterial pressure. If the OA in ELN+/- ascending aorta was the same as wildtype, the circumferential stretch ratio would be 1% higher at the inner wall than the outer wall. The higher OA in ELN+/- ascending aorta makes the transmural strain distribution more similar to wildtype.

**Discussion**

*Characteristics of ELN+/- Mice*

ELN+/- mice exhibit many of the traits observed in human diseases associated with elastin haploinsufficiency. Like SVAS patients, ELN+/- mice are hypertensive, have reduced arterial compliance and an increased number of lamellar units (10). ELN+/- arteries are useful for studying the effects of reduced elastin on arterial mechanics. However, we believe that the mechanical behavior of ELN+/- arteries is caused not only by reduced elastin, but by complex remodeling of the arterial wall in response to mechanical stimuli during development.

*Effects of Longitudinal Stretch on Arterial Compliance*
It is known that arterial compliance depends on longitudinal stretch and that arteries are in a stretched state in vivo (25). Despite these observations, the diameter-pressure relationship at the in vivo longitudinal stretch ratio ($\lambda_{iv}^{\ell}$) for both ELN+/- and wildtype arteries is similar to previous results where longitudinal stretch was not considered (10, 19); ELN+/- arteries consistently have smaller diameters than wildtype. We determined that the diameter-pressure relationship changes less than 3% within 10% of $\lambda_{iv}^{\ell}$ and 2-8% (depending on the artery type) within 20% of $\lambda_{iv}^{\ell}$. Arteries cannot be inflated without buckling when held 10-20% below $\lambda_{iv}^{\ell}$. When stretched more than 20% above $\lambda_{iv}^{\ell}$, the high longitudinal forces cause arteries on glass cannulae, like those used in previous studies (10, 19), to slip off at the ends. The metal cannulae used in this study were designed with grooves at the end to hold the securing sutures and prevent artery slippage at high longitudinal stretch. As long as the artery is not buckling or slipping longitudinally during loading cycles, the diameter-pressure relationship will be close to that observed at $\lambda_{iv}^{\ell}$. If desired, $\lambda_{iv}^{\ell}$ can be estimated by monitoring the slope of the force-pressure relationship. The longitudinal force-pressure relationship for both ELN+/- and wildtype arteries remains relatively constant or decreases slightly near $\lambda_{iv}^{\ell}$. This property has been previously documented in rat tail arteries (24), rat carotid arteries (25) and dog carotid arteries (7). Although measuring the longitudinal stretch ratio ($\lambda_{iv}$) is not necessary for obtaining physiologically relevant outer diameter-pressure relationships, $\lambda_{iv}$ is necessary to calculate the deformed inner diameter (Equation 1) and consequently the stretch ratios and stresses (Equations 2, 4 and 5). The inner diameter must be calculated
because tracking it throughout the loading cycle in large, relatively thick-walled arteries is often not possible.

Elastin Production in Vascular Development

The smaller diameter in ELN+/− arteries may be the result of decreased elastin production during development. In mice, elastin production begins as pulsatile flow develops and peaks just before birth (17). Soon after birth, there are sharp increases in body weight, blood pressure, artery length, diameter and thickness and elastin and collagen content in mammalian arteries (3, 4, 13). In a newborn rat aorta, 65% of the medial volume is occupied by smooth muscle and 13% is occupied by elastin. At four weeks of age, smooth muscle volume decreases to 40% and elastin volume increases to 48% (13). The elastin content in ELN+/− arteries is approximately 30% less than wildtype when normalized to total protein, while the collagen content is unchanged (10). The cross-sectional area of most ELN+/− arteries is also about 30% less than wildtype (calculated from Table 1). If the length of each ELN+/− artery is maintained and there is less elastin available to increase the artery cross-sectional area during growth, then the artery may develop with a smaller diameter. Hypertension in ELN+/− mice may be an adaptive mechanism to increase arterial diameter and consequently maintain cardiac output and perfusion pressure (10, 19).

Arterial Remodeling

The diameter is smaller in ELN+/− arteries than wildtype, but the circumferential stretch ratio is the same or larger for the pressure range used in this study. For pressures
between 75-125 mmHg, the circumferential stretch ratio is larger in ELN+/- ascending aorta than wildtype. This may be an adaptive mechanism, similar to hypertension, to increase the arterial diameter. Hypertension has limited potential for increasing the diameter because of arterial stiffening at high pressures (less change in diameter with the same increase in pressure). Increasing arterial diameter may be more important in the ascending aorta than other arteries to avoid left ventricular overload.

Increased pressure may increase arterial diameter, but it also increases the circumferential stress. In typical hypertension, the arterial geometry is subsequently remodeled (inner diameter decreases and thickness increases) to reduce the circumferential wall stress back to normal values (5). The diameter is decreased in ELN+/- arteries, but the thickness is not increased, and the thickness/lumen ratio (calculated from Table 1) is approximately the same in wildtype and ELN+/- arteries. Therefore, the remodeling response of ELN+/- arteries to hypertension is fundamentally different than wildtype arteries with induced hypertension. For both abdominal and left common carotid arteries, differences in mechanical behavior between wildtype and ELN+/- arteries lead to similar circumferential stresses at mean arterial pressure. In contrast, ELN+/- ascending aorta shows higher circumferential stress than wildtype at mean arterial pressure.

Circumferential stress is related to the tension (T) in the arterial wall (T = pressure x inner radius). Tension in ELN+/- arteries is 42% higher than wildtype in ascending aorta and 21% higher in abdominal and left common carotid arteries. If the tension is divided by the observed number of lamellae (estimated for ascending and abdominal aorta from (19) and measured from unpublished data in this study), then the
tension/lamellae in ELN+/- arteries is 14% higher than wildtype in the ascending aorta and approximately 10% lower in the abdominal and left common carotid arteries. The tension/lamellae in all arteries for both genotypes is 0.9-1.3 Pa, which is within the range of 1-3 Pa found in most mammals (26). For the ascending aorta, differences in circumferential stress may be less important than maintaining diameter and tension/lamellae for arterial remodeling.

**Relating Mechanical Properties to Artery Wall Composition**

Theoretically, the mechanical behavior of each artery can be related to the protein composition of the wall. It is believed that elastin behaves linearly and contributes to the circumferential and longitudinal stress-stretch relationship at low stretch, while collagen behaves nonlinearly and contributes at higher stretch (8). The wildtype aorta has the highest total elastin amount and elastin/collagen ratio (10), so it would be expected to have the most linear behavior and perhaps higher stresses at low stretch ratios. In the circumferential direction, the wildtype aorta shows the expected behavior; the stress-stretch relationship is more linear than the other arteries, with a higher slope at low stretch ratios and lower slope at high stretch ratios. In the longitudinal direction, both wildtype and ELN+/- ascending aortas show gradual increases in stress with increased stretch, compared to the more nonlinear behavior (gradual increase in stress, followed by a sharp increase past a certain stretch ratio) in abdominal and left common carotid arteries. The ELN+/- ascending aorta behaves like wildtype ascending aorta in the longitudinal direction, even though the elastin amount and elastin/collagen ratio are closer to values for wildtype abdominal and left common carotid arteries (10).
explanation for this data is that the ascending aorta has a greater proportion of elastin contributing mechanically in the longitudinal direction than the other arteries and that this property is maintained despite the reduced total elastin in the ELN+/- ascending aorta. The circumferential and longitudinal stress-stretch relationships at $\lambda_z^{iv}$ for the wildtype arteries are qualitatively similar to the relationships measured by Guo and Kassab (14) in situ along the length of the aorta in C57BL/6 mice.

Differentiating between the circumferential and longitudinal stress-stretch relationship of the other artery types based on wall composition is not possible. Because ELN+/- arteries have decreased elastin throughout development, adaptive remodeling may lessen the differences in mechanical behavior that would be expected if the elastin in a mature wildtype artery was suddenly reduced by 30%. The resulting small differences in mechanical behavior may be masked by measurement errors and inter-animal variability. Other differences between ELN+/- and wildtype arteries, such as the smaller diameter, more lamellae, and increased pressure, suggest that ELN+/- arteries are being remodeled in response to the mechanical stimuli during development. Several investigators have developed microstructural models to predict the mechanical behavior of arteries based on relative amounts of elastin and collagen (2, 27). Our data show that changes in the relative amounts of elastin and collagen may not change the mechanical properties if the amounts of matrix proteins are altered from the start of development. Our data also show that changes in the mechanical properties may be different in the circumferential and longitudinal directions, possibly due to the arrangement and/or distribution of elastin and collagen in each artery type. These observations complicate any correlations between elastin and collagen amounts and mechanical behavior.
Elastin and Residual Strain

In vivo longitudinal stretch is lower in ELN+/- abdominal and left common carotid arteries, suggesting that reduced elastin does correlate with reduced longitudinal retraction (8). In vivo longitudinal stretch was similar between genotypes for ascending aorta, but the ascending aorta does not retract much upon excision and differences between genotypes may have been too small to measure in this study. Measuring the distance between arterial length markers was often complicated by the curvature of the artery, obscuring pieces of fat and occasional shifting of the markers during artery handling. In addition to the differences in the in vivo longitudinal stretch, it was observed that ELN +/- arteries appeared “loose” in vivo and may be longer overall. The in vivo longitudinal stretch measured in wildtype arteries is consistent with other measurements in the same mouse strain (14, 15).

ELN+/- abdominal and left common carotid arteries loop or curve dramatically upon excision, while wildtype arteries never loop and only curve slightly. The change in shape upon excision may be caused by residual shear in the longitudinal-circumferential direction. Shear in this direction can be thought of as twist or torsion of the artery along its length, as if the ends of were rotated in opposite directions. When markers were placed in a straight line down the artery length, they shifted into a diagonal line upon excision in both ELN+/- and wildtype abdominal aortas and left common carotid arteries. The shift was most obvious in ELN+/- left common carotid arteries with the markers changing to the opposite side of the artery as it looped. Residual shear has been observed in the left ventricle of wildtype and tight-skin mice and was quantified by the out of plane
warp angle in OA rings (23). We attempted to measure out of plane warp angle, but the specimens were too small for accurate measurements. Residual shear may be an adaptation to increased longitudinal stresses during development. Arterial geometry, retraction force and pressure all contribute to longitudinal stress. While arterial diameter and thickness can be reduced in response to mechanical stimuli, arterial length cannot be reduced in a similar manner (16). In hypertensive and/or older patients with reduced elastin, arteries often lengthen and may become tortuous (8). ELN+/- arteries may twist and possibly lengthen during growth in response to increased pressure and decreased retraction force caused by reduced elastin content. A combination of residual shear and decreased retraction force in the longitudinal direction will cause a cylindrical segment to form a loop when shortened.

The opening angle is larger in ELN+/- ascending aorta than wildtype, but not significantly different in abdominal and left common carotid arteries. Opening angle normalizes the circumferential strain through the artery wall (6), but sensitivity of the transmural strain distribution to opening angle is dependent on the arterial geometry. The geometry of the ascending aorta is more sensitive to changes in opening angle than the other arteries, therefore increasing the opening angle in ELN+/- ascending aorta is an effective method to bring the transmural strain distribution near wildtype levels. The wildtype opening angles are consistent with other measurements in the same mouse strain (14, 15).

Conclusion
This study presents detailed mechanical test data comparing wildtype and ELN+-/ mouse arteries. ELN+-/ arteries have a smaller diameter than wildtype at all pressures, but the circumferential stretch ratio is the same or greater. The circumferential mechanical behavior is relatively insensitive to longitudinal stretch near in vivo values, hence in vivo stretch does not have to be explicitly measured to obtain physiologically relevant outer diameter-pressure relationships. ELN+-/ abdominal and left common carotid arteries have an altered circumferential stress-pressure relationship that provides equivalent circumferential stresses at mean arterial pressure. ELN+-/ ascending aortas have higher circumferential stresses at mean arterial pressure, but similar tension/lamellae. ELN+-/ abdominal and left common carotid arteries have lower in vivo longitudinal stretch and ELN+-/ ascending aortas have higher circumferential residual strains that normalize the transmural strain distribution at mean arterial pressure. The mechanical behavior of ELN+-/ arteries is likely due to the decreased amount of elastin combined with adaptive remodeling during vascular development. This remodeling process is an important consideration for human diseases, such as SVAS, in which alterations in matrix proteins are present at birth.

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References


Figure Legends

Table 1
Age, body weight, arterial pressure and unloaded dimensions of wildtype and ELN+/- mice. Mean (SD), n = 6-13 for all measurements. Unpaired, two-tailed t-tests, assuming unequal variance were used to compare data. P < 0.05 was considered significant.

Figure 1
Outer diameter and force versus pressure for arteries from wildtype and ELN+/- mice. Arteries were held at the in vivo longitudinal stretch ratio during testing (\( \lambda_z^* = 1 \)). Outer diameter for (a) abdominal, (b) ascending and (c) left common carotid arteries. Significant difference (p < 0.05) for all data except ascending aorta at 100 mmHg (p = 0.06). Longitudinal force for (d) abdominal, (e) ascending and (f) left common carotid arteries. Significant difference for ascending aorta between 75-125 mmHg (p = 0.03, 0.01, 0.04). Mean (SD), n = 6-10 for each artery type. Black solid lines, filled circles = wildtype; grey dashed lines, open squares = ELN+/-.

Figure 2
Representative outer diameter and force versus pressure for one artery in each group held at different longitudinal stretch ratios (\( \lambda_z^* \)) calculated with respect to the in vivo length. \( \lambda_z^* = 0.9, 1.0, 1.2 \) (◇, □, ○, respectively) for abdominal aorta. \( \lambda_z^* = 1.0, 1.1, 1.3 \) (◇, □, ○, respectively) for ascending and left common carotid arteries. The intermediate
values of $\lambda_c^* = 1.1$ or 1.2 are omitted for clarity. Outer diameter for (a) abdominal, (b) ascending and (c) left common carotid arteries. Curves shift down with increasing $\lambda_c^*$ (indicated with arrows and labeled in b). Longitudinal force for (d) abdominal, (e) ascending and (f) left common carotid arteries. Curves shift up with increasing $\lambda_c^*$ (indicated with arrows and labeled in e). Black lines, filled symbols = wildtype; grey lines, open symbols = ELN+/-.

**Figure 3**

Circumferential stretch ratio, circumferential stress and longitudinal stress versus pressure for wildtype and ELN+/- arteries held at the in vivo longitudinal stretch ratio ($\lambda_c^*=1$). Circumferential stretch for (a) abdominal, (b) ascending and (c) left common carotid arteries. Significant difference for ascending aorta between 75-125 mmHg ($p = 0.04, 0.003, 0.01$). Circumferential stress for (d) abdominal, (e) ascending and (f) left common carotid arteries. Significant difference for left common carotid artery at pressures > 0 mmHg ($p = 0.002-0.009$). Longitudinal stress for (g) abdominal, (h) ascending and (i) left common carotid arteries. No significant difference ($p > 0.05$) for all data. Mean values (SD), n = 6-10 for each artery type. Arrows (black, solid = wildtype and grey, dashed = ELN+/-) have been added at the mean arterial pressure for each genotype to show the physiologic stretch ratios and stresses. Black solid lines, filled circles = wildtype; grey dashed lines, open squares = ELN+/-.

**Figure 4**

Representative circumferential and longitudinal stress versus circumferential stretch ratio for one artery in each group held at different longitudinal stretch ratios ($\lambda_c^*$) calculated
with respect to the in vivo length. \( \lambda_z^* = 0.9, 1.0, 1.2 \) (○, □, ◦, respectively) for abdominal aorta. \( \lambda_z^* = 1.0, 1.1, 1.3 \) (○, □, ◦, respectively) for ascending and left common carotid arteries. The intermediate values of \( \lambda_z^* = 1.1 \) or 1.2 are omitted for clarity. Circumferential stress for (a) abdominal, (b) ascending and (c) left common carotid arteries. Curves shift left with increasing \( \lambda_z^* \) (indicated with arrows and labeled in b. Longitudinal stress for (d) abdominal, (e) ascending and (f) left common carotid arteries. Curves shift up with increasing \( \lambda_z^* \) (indicated with arrows and labeled in b. Black lines, filled symbols = wildtype; grey lines, open symbols = ELN+/-.

**Figure 5**

Longitudinal stress at zero pressure versus longitudinal stretch ratio (\( \lambda_z^* \)) calculated with respect to in vivo length for (a) abdominal, (b) ascending and (c) left common carotid arteries. Individual curves are shown for each specimen, \( n = 6-10 \) for each artery type. Relationships are similar for higher pressures and if the longitudinal stretch ratio (\( \lambda_z \)) is calculated with respect to the unloaded length. Black solid lines, filled circles = wildtype; grey dashed lines, open squares = ELN+/-.

**Figure 6**

In vivo longitudinal stretch in arterial segments. (a) Mean in vivo stretch ratio. Black bars = wildtype; grey bars = ELN+/- Mean values (SD), \( n = 8-10 \) for each artery type. Significant difference for abdominal (\( p = 0.01 \)) and left common carotid (\( p = 0.007 \)) arteries, but not for ascending aorta (\( p = 0.9 \)). Representative ex vivo images for (b)
abdominal, (c) ascending and (d) left common carotid arteries. Left = wildtype, right = ELN+/-.
Scale bars = 1 mm.

**Figure 7**

Circumferential residual strain in arterial segments. (a) Mean opening angle (OA).
Black bars = wildtype; grey bars = ELN+/-.
Mean values (SD), n = 7 for each artery type.
Significant difference for ascending aorta (p = 0.004), but not abdominal (p = 0.2) or left common carotid (p = 0.7) arteries.
Representative images of ascending aorta segments are shown for (b) wildtype and (c) ELN+/-.
<table>
<thead>
<tr>
<th></th>
<th>Wildtype</th>
<th>ELN +/-</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (days)</strong></td>
<td>100 (4)</td>
<td>98 (4)</td>
<td>6.0E-2</td>
</tr>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>30 (3)</td>
<td>30 (4)</td>
<td>7.0E-1</td>
</tr>
<tr>
<td><strong>Art. press. (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>122 (28)</td>
<td>175 (19)</td>
<td>2.0E-5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80 (20)</td>
<td>107 (9)</td>
<td>5.0E-4</td>
</tr>
<tr>
<td>Mean</td>
<td>98 (23)</td>
<td>136 (10)</td>
<td>6.0E-5</td>
</tr>
<tr>
<td><strong>Unl. dimensions</strong></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Outer diam. (μm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal</td>
<td>568 (11)</td>
<td>469 (8)</td>
<td>2.0E-5</td>
</tr>
<tr>
<td>Ascending</td>
<td>1000 (12)</td>
<td>786 (12)</td>
<td>2.0E-5</td>
</tr>
<tr>
<td>Carotid</td>
<td>402 (10)</td>
<td>358 (7)</td>
<td>5.0E-3</td>
</tr>
<tr>
<td><strong>Thickness (μm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal</td>
<td>83 (4)</td>
<td>72 (4)</td>
<td>4.0E-2</td>
</tr>
<tr>
<td>Ascending</td>
<td>109 (6)</td>
<td>97 (7)</td>
<td>1.0E-2</td>
</tr>
<tr>
<td>Carotid</td>
<td>59 (4)</td>
<td>59 (4)</td>
<td>7.0E-1</td>
</tr>
</tbody>
</table>

**Table 1**
Figure 1
Abdominal

Figure 2

Ascending

Carotid

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Figure 3
Figure 4
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Figure 6
Figure 7