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Critical Buckling Pressure in Mouse Carotid Arteries with Altered Elastic Fibers

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Abstract

Arteries can buckle axially under applied critical buckling pressure due to a mechanical instability. Buckling can cause arterial tortuosity leading to flow irregularities and stroke. Genetic mutations in elastic fiber proteins are associated with arterial tortuosity in humans and mice, and may be the result of alterations in critical buckling pressure. Hence, the objective of this study is to investigate how genetic defects in elastic fibers affect buckling pressure. We use mouse models of human disease with reduced amounts of elastin (*Eln*+/-) and with defects in elastic fiber assembly due to the absence of fibulin-5 (*Fbln5*-/-). We find that *Eln*+/- arteries have reduced buckling pressure compared to their wild-type controls. *Fbln5*-/- arteries have similar buckling pressure to wild-type at low axial stretch, but increased buckling pressure at high stretch. We fit material parameters to mechanical test data for *Eln*+/-, *Fbln5*-/- and wild-type arteries using Fung and four-fiber strain energy functions. Fitted parameters are used to predict theoretical buckling pressure based on equilibrium of an inflated, buckled, thick-walled cylinder. In general, the theoretical predictions underestimate the buckling pressure at low axial stretch and overestimate the buckling pressure at high stretch. The theoretical predictions with both models replicate the increased buckling pressure at high stretch for *Fbln5*-/- arteries, but the four-fiber model predictions best match the experimental trends in buckling pressure changes with axial stretch. This study provides experimental and theoretical methods for further investigating the influence of genetic mutations in elastic fibers on buckling behavior and the development of arterial tortuosity.

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Conflict of Interest Statement

There are no conflicts of interest.

Keywords

elastin; fibulin-5; mechanics; tortuosity

1. Introduction

Recent studies have demonstrated that long cylindrical arteries may lose mechanical stability and buckle into curved or tortuous shapes (Han 2008; Rachev 2009). Arterial buckling depends on the applied pressure, axial stretch, residual stress, wall geometry, and wall mechanical properties. Arterial buckling can cause tortuosity, increased resistance to flow, kinking, and even occlusion. Severely kinked and tortuous arteries are associated with aging, atherosclerosis, diabetes, hypertension and genetic diseases (Han 2012). Genetic mutations in elastic fiber proteins are associated with tortuosity of the large conducting arteries in humans (Urban and Davis 2014) and mice (Yanagisawa et al. 2002; Wagenseil et al. 2009).

Genetically-modified mice with elastic fiber defects present an opportunity to determine how combined changes in wall mechanical properties, geometry, residual stress, and in vivo axial stretch affect critical buckling pressure and the development of tortuosity in models of human disease. Decreased amounts of elastic fibers in elastin haploinsufficient mice (*Eln*^{+/-}) or disrupted elastic fibers in fibulin-5 null mice (*Fbln5*^{-/-}) cause high blood pressure, increased arterial stiffness, and decreased in vivo arterial axial stretch ratios (Yanagisawa et al. 2002; Faury et al. 2003; Wagenseil et al. 2005; Wan et al. 2010), but it is not known how these factors affect critical buckling pressure.

Using Fung (Chuong and Fung 1986) or four-fiber (Holzapfel et al. 2000; Gleason et al. 2008) strain energy functions to describe arterial mechanical behavior, theoretical models have been developed to investigate buckling in porcine arteries after collagenase treatment (Martinez and Han 2012), after elastase treatment (Lee et al. 2012), and as a function of material parameters and residual strain (Liu et al. 2014). Theoretical models have not been used to predict results in mouse arteries with defects in elastic fiber proteins that model human disease. Hence, the objectives of this study are to 1) determine the effects of reduced amounts of functional elastic fibers on the experimental critical buckling pressure in genetically-modified mouse arteries and 2) compare the experimental values to theoretical buckling pressures calculated from Fung and four-fiber strain energy functions fitted to biaxial experimental data.

2. Materials and methods

2.1 Animals

Male mice haploinsufficient for the elastin gene (*Eln*^{+/-}) and their wild-type littermates (*Eln*^{+/+}) (Li et al. 1998), and mice lacking the fibulin-5 gene (*Fbln5*^{-/-}) and their wild-type littermates (*Fbln5*^{+/+}) (Budatha et al. 2011) were used between 2 – 3 months old.

Littermates were used as controls because *Eln* and *Fbln5* mice are in different genetic backgrounds (C57Bl6 and mixed, respectively). All protocols were approved by the Institutional Animal Care and Use Committee. Mice were sacrificed by CO₂ inhalation, then

the right and left carotid arteries were removed, placed in physiological saline solution (PSS), stored at 4° C, and used for mechanical tests within three days (Amin et al. 2011). Mechanical tests were performed with each carotid mounted in a pressure myograph system (Danish Myotechnology) in PSS at 37° C (Wagenseil et al. 2005). Separate arteries were used for experimental determination of critical buckling pressure and biaxial mechanical behavior. The in vivo stretch ratio (λ_z^{iv}) of each carotid artery used for biaxial mechanical behavior was measured by taking images of the artery before and after dissection with a digital camera connected to a dissection microscope.

2.2 Experimental measurement of critical buckling pressure

The carotid artery was set at axial stretch ratios (λ_z) from 1.0 – 1.4, then inflated under lumen pressure in increments of 5 mmHg until it buckled or until a maximum pressure of 200 mmHg was reached. Images were taken at each increment near buckling for offline determination of experimental critical buckling pressure (P_{cr}^{exp}). The pressurized images were layered on top of the unloaded image and the outer edges and the midline of the artery were outlined using custom scripts in Matlab. P_{cr}^{exp} was defined as the pressure at which the maximum lateral displacement of the midline of the pressurized artery was greater than the radius of the unloaded artery (Fig. 1).

2.3 Experimental measurement of biaxial mechanical behavior

Biaxial mechanical tests were performed as previously described (Amin et al. 2012). The carotid artery was mounted in the myograph at the unloaded length and stretched axially to λ_z^{iv} . The artery was preconditioned for three cycles in the circumferential (0 to 175 mmHg) and axial directions (λ_z^{iv} to $1.2 - 1.4 \times \lambda_z^{iv}$, with a maximum force around 8 mN). The artery was then inflated three times from 0 – 175 mmHg at constant axial stretches and stretched axially three times at constant pressures (50, 100, and 150 mmHg). The axial stretch range with respect to the unloaded length varied for each artery, but was typically between $\lambda_z = 1.2 - 1.7$. The circumferential inflation cycles were performed automatically with the myograph software (steps of 25 mmHg, 12 sec/step), while the axial stretch cycles were performed by manual rotation of the micrometer attached to one end of the carotid artery (~10 μ m/sec). Pressure, outer diameter, and axial force were recorded at 1 Hz for all cycles. The axial stretch ratio at each time point was calculated assuming a constant stretch rate. Custom scripts in Matlab software were used to isolate the third loading cycle of each protocol for further analyses. Unloaded dimensions were determined from images of cut arterial rings (200 – 300 μ m thick) taken with a digital camera connected to a dissection microscope. The loaded inner diameter was calculated by incompressibility (Faury et al. 1999).

2.4 Fitting material parameters for the strain energy functions

Assuming an incompressible, axisymmetric, thick-walled cylinder with no shear, the inflation and extension of the carotid artery can be described by the stretch ratios in each direction [circumferential (θ), axial (z), and radial (r)],

$$\lambda_\theta = \frac{r}{R} \frac{\pi}{\theta_o}, \lambda_z = \frac{l}{L}, \lambda_r = \frac{1}{\lambda_\theta \lambda_z} \quad (1)$$

where r = deformed radius, R = undeformed radius in the stress-free state, $\theta_o = 1/2$ the central angle of the arc formed in the stress-free state (Chuong and Fung 1986), l = deformed length, and L = undeformed length. The stress-free state was not explicitly measured in this study. The unloaded state was measured and related to the stress-free state according to Matsumoto and Hayashi (1996) using published opening angles ($\Phi = \pi - \theta_o$) of 60° for *Elm+/-* and *Elm+/-* (Wagenseil et al. 2005), 150° for *Fblm5-/-* and 90° for *Fblm5+/-* (Wan et al. 2010) carotid arteries.

The strain energy functions (W) used to model the biaxial mechanical behavior of the carotid arteries are the Fung exponential model (W_F) (Chuong and Fung 1986) and four-fiber model (W_4) (Holzapfel et al. 2000; Gleason et al. 2008) given by,

$$W_F = \frac{1}{2} b_0 (e^Q - 1), \text{ with } \quad (2)$$

$$Q = b_1 E_\theta^2 + b_2 E_z^2 + b_3 E_r^2 + 2b_4 E_\theta E_z + 2b_5 E_z E_r + 2b_6 E_r E_\theta, \text{ and } \quad (3)$$

$$W_4 = b_0 (E_\theta + E_z + E_r) + \frac{b_{11}}{4b_{12}} \left\{ \exp \left[b_{12} (I_\theta)^2 \right] - 1 \right\} + \frac{b_{21}}{4b_{22}} \left\{ \exp \left[b_{22} (I_z)^2 \right] - 1 \right\} + \frac{b_{31}}{2b_{32}} \left\{ \exp \left[b_{32} (I_d)^2 \right] - 1 \right\}, \quad (4)$$

where E is the Greens strain in each direction [$E_i = 1/2(\lambda_i^2 - 1)$], $I_k = 2E_\theta \sin^2 \alpha_k + 2E_z \cos^2 \alpha_k$ for each k th fiber family where the subscripts represent fiber families oriented in the θ ($\alpha_1 = 90^\circ$), z ($\alpha_2 = 0^\circ$), or two identical families in the d (diagonal) ($\alpha_3 = -\alpha_4 = \alpha_0$) direction and $b_0 - b_{32}$ are material parameters.

Accordingly, based on equilibrium, the lumen pressure (P) and axial force (N) of the artery are,

$$P = \int_{r_i}^{r_e} \left(\lambda^2 \frac{\partial W}{\partial E} - \lambda_r^2 \frac{\partial W}{\partial E_r} \right) \frac{d\xi}{\xi}, \text{ and } \quad (5)$$

$$N = \pi \int_{r_i}^{r_e} \left(2\lambda_z^2 \frac{\partial W}{\partial E_z} - \lambda_r^2 \frac{\partial W}{\partial E_r} - \lambda^2 \frac{\partial W}{\partial E} \right) r dr + \pi r_i^2 P, \quad (6)$$

where r_i and r_e are the deformed inner and outer radius, respectively. The integral term in Eqn. 6 is equal to the reduced axial force, $F = N - \pi r_i^2 P$, which is measured experimentally by the inline force transducer (Holzapfel et al. 2000).

Material parameters for the strain energy functions were estimated by minimizing the error between the experimental and theoretical pressures and reduced axial forces,

$$error = \sum_{i=1}^n \left(\frac{P_{\text{exp}(i)} - P_{\text{theor}(i)}}{P_{\text{exp,mean}}} \right)^2 + \sum_{i=1}^n \left(\frac{F_{\text{exp}(i)} - F_{\text{theor}(i)}}{F_{\text{exp,mean}}} \right)^2 \quad (7)$$

Fitting error was calculated by dividing the error function by the total number of data points ($2n$) (Wan et al. 2010). Six random starting values were used to ensure a global minimum was determined. The starting values were determined by a random number generator between 0 – 1 and then scaled for the expected order of magnitude of the parameter [$\times 1000$ Pa for b_0 (Fung), b_{1l} , b_{2l} , and b_{3l} (four-fiber) and $\times 90^\circ$ for α (four-fiber)].

2.5 Theoretical analysis of critical buckling pressures

The theoretical critical buckling pressure (P_{cr}^{theor}) can be determined by force and moment equilibrium of the buckled artery as derived previously using the Fung (Lee et al. 2012) and four-fiber strain energy functions (Liu et al. 2014),

$$P_{cr}^{\text{theor}} = \frac{N + \left(\frac{n\pi}{l_e}\right)^2 H}{\pi r_i^2}, \quad (8)$$

where l_e is the equivalent length of the artery (equal to half the length for fixed ends), N is the axial force (Eqn. 6), and H is the “bending force” defined by

$$H = \pi \int_{r_i}^{r_e} \left(J_z - \frac{1}{3} J_\theta \right) r^3 dr + \frac{\pi r_i^3}{3} \int_{r_i}^{r_e} J_\theta dr, \quad (9)$$

with J_z and J_θ depending on the strain energy function. For the Fung (F) strain energy function,

$$J_z^F = \{2(b_4 E_\theta + b_2 E_z + b_5 E_r) [(1+2E_z)(b_4 E_\theta + b_2 E_z + b_5 E_r) - (1+2E_r)(b_6 E_\theta + b_5 E_z + b_3 E_r)] + (1+2E_z)b_2 - (1+2E_r), \text{ and} \quad (10)$$

$$J_\theta^F = \{2(b_4 E_\theta + b_2 E_z + b_5 E_r) [(1+2E_\theta)(b_1 E_\theta + b_4 E_z + b_6 E_r) - (1+2E_r)(b_6 E_\theta + b_5 E_z + b_3 E_r)] + (1+2E_\theta)b_4 - (1+2E_r), \quad (11)$$

and for the four-fiber (4) strain energy function,

$$J_z^4 = 2b_0 + 2b_{21} I_z \exp(b_{22} I_z^2) + 4b_{31} I_d \exp(b_{32} I_d^2) \cos^2 \alpha_0 + 2b_{21} (1+2E_z) (1+2b_{22} I_z^2) \exp(b_{22} I_z^2) + 4b_{31} (1+2E_z) (1+2b_{32} I_d^2) \exp(b_{32} I_d^2) \cos^2 \alpha_0 \sin^2 \alpha_0, \text{ and} \quad (12)$$

$$J_\theta^4 = 4b_{31} (1+2E_\theta) (1+2b_{32} I_d^2) \exp(b_{32} I_d^2) \cos^2 \alpha_0 \sin^2 \alpha_0. \quad (13)$$

P_{cr}^{theor} of each artery used in biaxial testing was determined using Eqn. 8 with the artery dimensions (diameter and wall thickness) and the fitted material constants as inputs. The average length from the arteries used in the experimental buckling group was used ($l_e = 3.05/2$ mm). The axial stretch range was prescribed from $\lambda_z = 1.0 - 1.4$, and λ_θ was determined iteratively as the value providing the minimum difference between P_{cr}^{theor} (Eqn. 8) and the theoretical lumen pressure (Eqn. 5).

2.6 Statistics

One way ANOVA followed by a Tukey post hoc test was used to compare results between groups using SPSS software. Data are presented as mean $\pm$ sem.

3. Results

3.1 Unloaded dimensions and in vivo stretch ratios

The unloaded dimensions and λ_z^{iv} are shown in Table 1. Similar to previous data (Wagenseil et al. 2005), *Eln*+/- carotid arteries have smaller unloaded outer diameters and reduced λ_z^{iv} compared to *Eln*+/. Also consistent with previous data (Wan et al. 2010), *Fbln5*-/- carotid arteries have similar unloaded outer diameters compared to *Fbln5*+/, but have reduced λ_z^{iv} . The absolute values of λ_z^{iv} are consistent with previous values measured from images of mouse carotid arteries before and after dissection (Wagenseil et al. 2005), but are lower than values obtained by assuming that at λ_z^{iv} , the axial force is constant with increasing pressure during in vitro tests (i.e. (Wan et al. 2010; Dye et al. 2007; Eberth et al. 2009)).

3.2 Experimental buckling pressure

There are no significant differences in P_{cr}^{exp} between the two wild-type groups. At all axial stretch ratios P_{cr}^{exp} is 10 – 20% lower in *Eln*+/- arteries than *Eln*+ (Fig. 2a). Nine out of 10 *Fbln5*-/- arteries did not buckle at 200 mmHg at an axial stretch ratio of 1.4. To demonstrate the increased P_{cr}^{exp} at high stretch in Fig. 2b, we assumed a linear increase in P_{cr}^{exp} between axial stretches of 1.2 – 1.4 for each *Fbln5*-/- artery. The actual P_{cr}^{exp} values may be higher. At lower axial stretch ratios P_{cr}^{exp} is similar in *Fbln5*-/- and *Fbln5*+/- arteries, but at higher axial stretch ratios P_{cr}^{exp} is 15 – 25% higher in *Fbln5*-/- arteries than *Fbln5*+ (Fig. 2b). We fit a linear relationship between P_{cr}^{exp} and axial stretch ratio ($R^2 = .98 - .99$) to estimate in vivo P_{cr}^{exp} using the average in vivo axial stretch ratios (λ_z^{iv}) in Table 1. The estimated in vivo P_{cr}^{exp} (mmHg) is 157 for *Eln*+/, 88 for *Eln*+/-, 165 for *Fbln5*+/- and 121 for *Fbln5*-/- arteries. P_{cr}^{exp} is above systolic blood pressure for *Eln*+ and *Fbln5*+ mice, but below systolic pressure for *Eln*+/- and *Fbln5*-/- mice (Yanagisawa et al. 2002; Faury et al. 2003; Wagenseil et al. 2005), so it is likely that *Eln*+/- and *Fbln5*-/- arteries buckle in vivo.

3.3 Experimental mechanical behavior and fitted material parameters

Average outer diameter-pressure relationships at λ_z^{iv} and axial stretch-reduced force relationships at 100 mmHg are shown in Fig. 3. Similar to previous results (Faury et al. 2003; Wagenseil et al. 2005; Wan et al. 2010), *Eln*+/- and *Fbln5*-/- carotid arteries have smaller outer diameters at high pressures compared to their wild-type controls, indicating increased circumferential stiffness of the arterial wall. *Fbln5*-/- arteries have increased axial force at all overlapping axial stretch ratios compared to *Fbln5*+/- arteries, consistent with previous results showing that axial force in *Fbln5*-/- carotid arteries becomes nonlinear at lower axial stretch ratios than *Fbln5*+ (Wan et al. 2010). In contrast, *Eln*+/- arteries have

similar or slightly lower axial forces compared to *Eln*^{+/+} at most axial stretch ratios, consistent with previous data for axial stretch-stress relationships (Wagenseil et al. 2005).

Material parameters in the Fung and four-fiber strain energy functions were fit for each artery using the unloaded dimensions (Table 1) and mechanical test data. Example fits for each group are shown in Fig. 4 (Fung) and Fig. 5 (four-fiber). For the Fung strain energy function (Table 2), there are no significant differences between parameters for the *Eln*^{+/-} and *Eln*^{+/+} groups or the *Eln*^{+/+} and *Fbln5*^{+/+} groups. There are significant differences between 5/7 parameters for the *Fbln5*^{-/-} and *Fbln5*^{+/+} groups. The b_0 constant is five times lower and the exponential constants ($b_1 - b_3$, b_5 , b_6) are 4 – 90 times higher, showing more nonlinear stress-strain behavior in *Fbln5*^{-/-} compared to *Fbln5*^{+/+} arteries. The constants for wild-type mouse carotid arteries are similar to those obtained previously with the Fung strain energy function (Gleason et al. 2008).

For the four-fiber strain energy function (Table 3), there are no significant differences between parameters for the *Eln*^{+/-} and *Eln*^{+/+} groups or the *Eln*^{+/+} and *Fbln5*^{+/+} groups. There are significant differences between 4/8 parameters for the *Fbln5*^{-/-} and *Fbln5*^{+/+} groups. The b_{12} , b_{22} , and b_{32} constants are 2 – 22 times higher in *Fbln5*^{-/-} arteries compared to *Fbln5*^{+/+}, indicating increased nonlinearity of all four fiber families. The angle, α_0 , is about 10° larger in *Fbln5*^{-/-} arteries than *Fbln5*^{+/+}, predicting a more circumferential orientation of the diagonal fibers. Previous results showed increased nonlinearity for the axial and diagonal fiber families in *Fbln5*^{-/-} carotid arteries, but not for the circumferential fibers and no difference in the angle of the diagonal fibers (Wan et al. 2010). For both genotypes, the fitted values for b_0 are lower than Wan et al. (2010), but the values for b_{11} , b_{21} , and b_{31} are higher, demonstrating a trade-off between isotropic and anisotropic stress contributions in the model, which may be related to the loading protocols used for fitting.

There is no significant difference in the average fit error between strain energy functions (.033 ± .003 for Fung and .031 ± .003 for four-fiber). This is in contrast to previous data that showed the four-fiber family model to have about 20% less error in fitting than the Fung model for mouse carotid arteries (Gleason et al. 2008). The parameter values and fitting error are sensitive to the loading range for the experimental data. The applied pressure was higher and the axial stretch range was lower in this study than in Gleason et al. (2008), which may alter the fitting error for different strain energy functions. Additionally, the choice of error function can determine whether the strain energy function fits better at high or low stretch ratios (Ferruzzi et al. 2013) and different error functions were used in this study compared to Gleason et al. (2008).

3.4 Theoretical buckling pressure

The material parameters (Table 2 or 3) and unloaded dimensions (Table 1) for each artery were used to calculate the theoretical buckling pressure (P_{cr}^{theor}) (Fig. 6). For the wild-type groups, P_{cr}^{theor} is within 8 – 43% of P_{cr}^{exp} for both strain energy functions, with underpredictions at low axial stretch, overpredictions at high axial stretch, and the best match between predicted and experimental values at $\lambda_z = 1.3$ (8 – 12% difference). For *Eln*

+/- arteries, P_{cr}^{theor} is within 17 – 54% of P_{cr}^{exp} for both strain energy functions across the axial stretch range, but is always lower than P_{cr}^{exp} . For *Fbln5*-/- arteries, P_{cr}^{theor} is within 0.1 – 603% of P_{cr}^{exp} for the Fung strain energy function and 6 – 135% for the four-fiber strain energy function. The P_{cr}^{theor} values from the Fung strain energy function have a more nonlinear dependence on axial stretch than those calculated with the four-fiber strain energy function, resulting in overprediction of the critical buckling pressure at high stretch for most groups.

The differences between groups for the predicted buckling pressure (Fig. 6) are consistent with the experimental data (Fig. 2). For *Fbln5*-/- arteries, both strain energy functions predict a large increase in P_{cr}^{theor} with axial stretch and significantly higher values compared to *Fbln5*+/+ at $\lambda_z = 1.3$ and 1.4. This is supported by experimental observations that 9/10 *Fbln5*-/- arteries did not buckle at the maximum applied pressure of 200 mmHg for $\lambda_z = 1.4$. Additional buckling experiments at higher applied pressures are necessary to better compare P_{cr}^{theor} and P_{cr}^{exp} at high axial stretch for *Fbln5*-/- arteries.

4. Discussion

4.1 Effects of genetic defects in elastic fibers on biaxial mechanical behavior and experimental critical buckling pressure

Eln+/- mice have about 60% of the normal elastin amounts, smaller, thinner arteries, and increased blood pressure (Faury et al. 2003). *Fbln5*-/- mice have disordered elastic fibers, tortuous arteries, and high blood pressure (Nakamura et al. 2002; Yanagisawa et al. 2002). Previous studies show that *Eln*+/- and *Fbln5*-/- arteries have increased circumferential arterial stiffness and reduced in vivo axial stretch ratios compared to their wild-type controls (Wagenseil et al. 2005; Wan et al. 2010). The current study confirms past characterization of the circumferential mechanical behavior and shows that *Fbln5*-/- arteries have more significant stiffening than *Eln*+/- arteries, compared to their respective controls (Fig. 3a). The current study also adds new information about the axial mechanical behavior for arteries with altered elastic fibers, showing that *Fbln5*-/- arteries have significant stiffening in the axial direction compared to *Fbln5*+/+, while *Eln*+/- arteries are slightly less stiff than *Eln*+/+ in the axial direction (Fig. 3b). These data show that reductions in elastin amounts and defects in elastic fiber assembly may not necessarily cause similar changes in mechanical behavior, especially in the axial direction.

Eln+/- arteries display a consistent decrease in buckling pressure at all axial stretch values, while *Fbln5*-/- arteries display an increase in buckling pressure at high axial stretch values, compared to their respective controls. Although in different genetic backgrounds, the two wild-type control groups have similar buckling behavior (Fig. 2). These results show that while some simple measurements (i.e. circumferential stiffness and in vivo axial stretch ratio) may be comparable in *Eln*+/- and *Fbln5*-/- arteries, buckling behavior is very different. The increased axial stiffness of *Fbln5*-/- arteries is most likely responsible for their increased buckling pressure, while the decrease in axial stiffness of *Eln*+/- arteries accounts for their decreased buckling pressure. However, the critical buckling pressure

depends on the three-dimensional mechanical properties, mechanical loading, and geometry of the arteries (Han 2008), and a complete characterization of these properties is necessary to better understand the contribution of genetic modifications in elastic fibers to arterial buckling.

Our data suggest that *Eln*+/- and *Fbln5*-/- arteries are buckled in vivo at systolic pressures. Note that our experiments were done under static pressure in a fluid bath, which is different from the in vivo state of the artery with pulsatile pressure and surrounding tissue support. Hence, our experimental results cannot be directly translated into quantitative in vivo buckling predictions. However, the qualitative trends remain true and if the arteries are buckled in vivo, this can lead to remodeling and arterial tortuosity. While arterial tortuosity has not been described in *Eln*+/- mice, *Eln*+/- carotid arteries form looped or curved shapes upon excision, suggesting residual torsion (Wagenseil et al. 2005). Carotid artery tortuosity has been observed in vivo for newborn mice with no elastin expression (*Eln*-/-) (Wagenseil et al. 2009). Arterial tortuosity in the descending thoracic aorta has been observed in vivo in *Fbln5*-/- mice (Nakamura et al. 2002). Additional in vitro experiments and modeling of different regions of the arterial tree may help predict which sections are prone to buckling and the development of tortuosity.

4.2 Comparison of theoretical and experimental critical buckling results

For most arteries and both strain energy functions, the theoretical buckling pressures are lower than the experimental values at low axial stretch and higher than the experimental values at high axial stretch (Fig. 6). One explanation for the difference at low axial stretch is that we chose a relatively stringent criterion for P_{cr}^{exp} (Fig. 1) to ensure that the artery was buckled. Future experimental studies with less stringent buckling criteria may show better agreement between P_{cr}^{exp} and P_{cr}^{theor} at low axial stretch. At high axial stretch, P_{cr}^{theor} showed the biggest differences from P_{cr}^{exp} in groups with high axial stiffness (i.e. P_{cr}^{theor} at $\lambda_z = 1.4$ for *Fbln5*-/- arteries was greater than both wild-types, which were greater than *Eln*+/-). This implies that the theoretical buckling pressures are overly influenced by the axial stiffness compared to the experimental buckling pressures. This could be due to differences between the experimental and theoretical mechanical behavior at high axial stretch or limiting assumptions in the theoretical analysis of buckling pressure.

We examined the material parameters of each group to better understand the buckling pressure predictions, especially the large P_{cr}^{theor} for *Fbln5*-/- arteries at high axial stretch. For the Fung strain energy function, the constants b_2 , b_4 , and b_5 indicate the contribution of the Greens strain in the axial direction. These parameters are 2 – 12 times higher in the *Fbln5*-/- group compared to the other groups. However, the axial parameters do not completely determine the buckling pressure, because b_2 , b_4 , and b_5 for *Eln*+/- arteries in the Fung strain energy function are not reduced compared to *Eln*+/-, despite lower predicted buckling pressures. For the four-fiber strain energy function, the diagonal and axial fibers are important for determining the response to axial stretch. For the diagonal fibers, b_{31} and b_{32} are about two times greater in *Fbln5*-/- arteries than other groups. For the axial fibers, b_{21} is comparable to other groups, but b_{22} is 3 – 12 times greater in *Fbln5*-/- arteries than other groups. As for the Fung strain energy function, axial parameters do not completely

determine the critical buckling pressure, because *Eln*+/- arteries show reductions in b_{31} and b_{21} compared to *Eln*+/, but comparable b_{32} values and increases in b_{22} , despite lower predicted buckling pressures. Our observations are consistent with previous analyses (Liu et al. 2014) and confirm that axial mechanical behavior of the artery is important, but not alone, in determining the critical buckling pressure.

We used two strain energy functions that have been shown to describe the mechanical behavior of mouse carotid arteries (Gleason et al. 2008). We found similar fitting errors for both strain energy functions and similar differences between P_{cr}^{theor} and P_{cr}^{exp} for most points. However, P_{cr}^{theor} for the Fung strain energy function has a more nonlinear dependence on axial stretch than the four-fiber strain energy function, leading to significant overpredictions at $\lambda_z = 1.4$, especially in *Fbln5*-/- arteries with high axial stiffness. Overall, the predictions with the four-fiber strain energy function best match the experimental relationship between buckling pressure and axial stretch.

The theoretical buckling pressures in this study were calculated based on steady-state equilibrium of a buckled artery (Lee et al. 2012; Liu et al. 2014). Goriely and Vandiver (2010) develop a theoretical analysis of buckling instability in growing arteries. They find that the axial stretch-critical buckling pressure relationship depends on the values of differential axial growth and residual axial stretch. For similar residual stretch values, higher growth values are associated with increasing nonlinearity of the axial stretch-critical buckling pressure relationship. The high critical buckling pressure in *Fbln5*-/- arteries at large axial stretch suggests that axial growth parameters may be higher in *Fbln5*-/- arteries than all other groups. Rachev (2009) develops a theoretical analysis of buckling stability in arteries subject to periodic pressure, which is a better representation of the in vivo state than a static pressure load. Buckling under periodic pressure occurs as a parametric resonance that depends on a combination of load parameters, including mean arterial pressure, pressure amplitude, pressure frequency, axial stretch, and axial length. However, experimental results have shown that the effect of periodic pressure is small at normal heart rates (Liu and Han 2012). Even though our theoretical calculations under static pressure cannot be directly translated into in vivo predictions of arterial buckling under more complicated loading conditions, the static theoretical model can be used for comparison to our static experimental results and the model helps us to better understand the contributions of elastic fibers to arterial buckling behavior, and provide guidance for further in vivo studies.

4.3 Clinical relevance

Arterial buckling is an instantaneous lateral deflection caused by increasing the internal pressure past the critical point for stable equilibrium (Han 2008) or by a parametric resonance that occurs during a critical combination of load parameters for unstable equilibrium (Rachev 2009). In both steady and pulsatile pressure analyses, high mean pressure is the major destabilizing factor and buckling is reversible if the mean pressure is subsequently decreased. However, sustained buckling and the loss of mechanical stability, could lead to the development of tortuous arteries in vivo. The mechanical factors that lead to buckling are risk factors for arterial tortuosity, including high blood pressure, weakened wall mechanical properties, and decreased axial stretch (Han 2012). Surgically reducing

axial stretch in animal models causes arterial buckling (Zhang et al. 2014) and leads to arterial tortuosity (Jackson et al. 2005). Similar buckling occurs in veins, as well (Badel et al. 2013; Martinez et al. 2010). Alterations in elastic fibers are associated with genetic diseases characterized by tortuous arteries including arterial tortuosity syndrome (Wessels et al. 2004), cutis laxa (Urban and Davis 2014), Loeys-Dietz syndrome (Nakajima et al. 2014), and Williams syndrome (Winter et al. 1996). Cutis laxa is associated with mutations in fibulin-5 that lead to profound defects in elastic fiber formation (Urban and Davis 2014), similar to *Fbln5*^{-/-} mice. Williams syndrome is caused by deletions within chromosome seven which includes the elastin gene and leads to elastin heterozygosity (Ewart et al. 1993), similar to *Eln*^{+/-} mice. Mouse models of elastic fiber diseases allow us to further investigate the relationship between genetic defects in elastic fibers and arterial buckling and test theoretical models that may predict the development of tortuosity in vivo.

4.4 Limitations

We chose two commonly used strain energy functions to evaluate in this study, but other strain energy functions could be investigated, (i.e. (Zulliger et al. 2004)). Our best-fit parameter values may be influenced by the range of starting values, the loading protocols for the experimental data, and our choice of error function (Eqn. 7). Our average theoretical and experimental buckling pressures were compared from different sets of arteries. Our experimental applied pressures were limited to 200 mmHg and not all arteries buckled at this value. Our theoretical calculations assume a steady-state pressure load.

4.5 Conclusions

We measured arterial buckling pressure in arteries from genetically-modified mice with elastic fiber defects. Reduced elastin in *Eln*^{+/-} arteries reduces buckling pressure, while disrupted elastic fibers in *Fbln5*^{-/-} arteries increases buckling pressure at high axial stretches, compared to their respective controls. Theoretical buckling pressure calculated with a four-fiber strain energy function fit to biaxial mechanical test data better matched the dependence of experimental buckling pressure on axial stretch than values calculated with a Fung strain energy function. The experimental and theoretical results can help us better understand the contribution of genetic defects in elastic fibers to arterial mechanics, arterial buckling and the development of arterial tortuosity.

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Highlights

- Buckling pressure may be related to the development of arterial tortuosity
- Mouse arteries with reduced elastin amounts have decreased buckling pressure
- Arteries without fibulin-5 have increased buckling pressure at high axial stretch
- Theoretical models can predict experimental buckling pressure
- A four-fiber strain energy function best predicts buckling behavior

Figure 1. Definition of experimental critical buckling pressure (P_{cr}^{exp})

Eln^{+/+} artery mounted in the myograph at an axial stretch (λ_z) of 1.0 at 0 mmHg and inflated to 35 and 60 mmHg. The outer edges (solid lines) and centerlines (dotted lines) of the unloaded (black lines) and inflated (grey lines) arteries were defined and overlaid using Matlab. P_{cr}^{exp} was defined as the pressure at which the centerline of the loaded artery had a maximum displacement greater than the radius of the unloaded artery. This can be seen when the grey dotted line moves to the right of the right hand black solid line. Scale bar = 100 μ m.

Figure 2. Experimental critical buckling pressure (P_{cr}^{exp}) in mouse arteries with altered elastic fibers

Carotid arteries from mice with reduced elastin amounts ($Eln^{+/-}$) have decreased buckling pressures compared to wild-type ($Eln^{+/+}$) (a). Carotid arteries from mice with disrupted elastic fibers due to lack of fibulin-5 expression ($Fbln5^{-/-}$) have similar buckling pressures at low axial stretch (λ_z), but increased buckling pressures at high λ_z compared to wild-type ($Fbln5^{+/+}$) (b). * = 9/10 $Fbln5^{-/-}$ arteries did not buckle at $\lambda_z = 1.4$ at the maximum applied pressure of 200 mmHg. To show the high P_{cr}^{exp} for $Fbln5^{-/-}$ arteries that did not buckle, we assumed P_{cr}^{exp} increased linearly between axial stretches of 1.2 – 1.4. P_{cr}^{exp} may be even higher. \$ = $p < .05$ between genotypes, $N = 10/\text{group}$. Values are mean $\pm$ sem.

Figure 3. Circumferential and axial mechanical behavior

Average outer diameter-pressure relationships at λ_z^{iv} (a) and average axial stretch-reduced force relationships at 100 mmHg (b) from the biaxial mechanical test protocols. *Eln*^{+/-} and *Fbln5*^{-/-} arteries show increased circumferential stiffness compared to their respective controls. *Eln*^{+/-} arteries show similar or slightly reduced axial stiffness compared to *Eln*^{+/+}, while *Fbln5*^{-/-} arteries show increased axial stiffness compared to *Fbln5*^{+/+}. N = 6 – 8/group for outer diameter-pressure curves. N = 3 – 7/group for axial stretch-reduced force curves. The numbers of axial samples are lower because axial stretch ratios were determined individually for each artery, so not all samples were stretched in the same range. Axial stretches of 1.35 and 1.40 are the only values that have enough samples in all groups for statistical comparison. * = P < .05 between *Eln*^{+/+} and *Eln*^{+/-} and \$ = P < .05 between *Fbln5*^{+/+} and *Fbln5*^{-/-}.

Figure 4. Example fits for the Fung strain energy function

Mechanical test data from *Eln*^{+/+} (a,b), *Eln*^{+/-} (c,d), *Fbln5*^{+/+} (e,f), and *Fbln5*^{-/-} (g,h) carotid arteries were fit with a Fung strain energy function by minimizing the differences between the experimental and theoretical pressures and reduced axial forces (Eqn. 7) to determine the material constants shown in Table 2.

Figure 5. Example fits for the four-fiber strain energy function

Mechanical test data from *Eln*^{+/+} (a,b), *Eln*^{+/-} (c,d), *Fbln5*^{+/+} (e,f), and *Fbln5*^{-/-} (g,h) carotid arteries were fit with a four-fiber strain energy function by minimizing the differences between the experimental and theoretical pressures and reduced axial forces (Eqn. 7) to determine the material constants shown in Table 3.

Figure 6. Comparison of experimental (P_{cr}^{exp}) and theoretical (P_{cr}^{theor}) buckling pressures
 Material constants from the Fung or four-fiber strain energy function, along with the unloaded dimensions, were used to calculate P_{cr}^{theor} according to Eqn. 8 for each artery. In general, P_{cr}^{theor} is lower than P_{cr}^{exp} at low axial stretch and higher than P_{cr}^{exp} at high axial stretch. Similar to experimental results, both models predict increased buckling pressure in *Fbln5*^{-/-} arteries at high axial stretch. Note the increased vertical scale for *Fbln5*^{-/-} arteries. Values are mean $\pm$ sem.

Table 1

Unloaded outer diameters (OD) and inner diameters (ID) measured from cut rings of the carotid arteries after mechanical testing. In vivo axial stretch ratios (λ_z^{iv}) measured from images before and after dissection of the artery are also shown. Significant P values are shown in italics.

<i>Eln</i> ^{+/+}				<i>Eln</i> ^{+/-}			
Sample	OD (μm)	ID (μm)	λ_z^{iv}	Sample	OD (μm)	ID (μm)	λ_z^{iv}
1	376	259	1.44	1	341	229	1.04
2	353	252	1.20	2	347	236	1.19
3	440	316	1.38	3	370	246	1.13
4	427	316	1.23	4	368	264	1.11
5	402	282	1.13	5	352	241	1.31
6	409	284	1.49	6	347	246	1.10
7	407	289	1.50				
mean	402	285	1.34	mean	354	244	1.15
sem	11	9	0.06	sem	5	5	0.04
				P (<i>Eln</i> ^{+/+} to <i>Eln</i> ^{+/-})			
					0.013	0.009	.012
<i>Fbln5</i> ^{+/+}				<i>Fbln5</i> ^{-/-}			
Sample	OD (μm)	ID (μm)	λ_z^{iv}	Sample	OD (μm)	ID (μm)	λ_z^{iv}
1	373	241	1.53	1	401	290	1.20
2	383	266	1.31	2	384	277	1.18
3	407	298	1.36	3	359	253	1.19
4	339	230	1.35	4	444	306	1.22
5	365	257	1.33	5	389	274	1.12
6	351	232	1.45	6	370	258	1.14
7	414	287	1.33	7	383	273	1.26
8	403	278	1.25				
mean	379	261	1.37	mean	390	276	1.19
sem	10	9	0.03	sem	10	7	0.02
P (<i>Eln</i> ^{+/+} to <i>Fbln5</i> ^{+/+})				P (<i>Fbln5</i> ^{+/+} to <i>Fbln5</i> ^{-/-})			
	0.339	0.153	.963		0.852	0.552	.013

Table 2
Material parameters and fit errors for the Fung strain energy function. Significant P values are shown in italics.

<i>Eln+/+</i>									
Sample	b_0 (kPa)	b_1	b_2	b_3	b_4	b_5	b_6	fit error	
1	323	0.090	0.148	0.338	0.056	0.000	0.030	0.044	
2	125	0.197	0.387	0.249	0.109	0.027	0.000	0.031	
3	59	0.467	0.557	1.269	0.277	0.523	0.000	0.045	
4	26	0.421	1.052	4.908	0.272	1.171	0.000	0.068	
5	328	0.170	0.118	0.168	0.068	0.000	0.111	0.025	
6	38	0.636	0.579	2.522	0.130	0.140	0.000	0.032	
7	252	0.135	0.383	0.376	0.091	0.179	0.000	0.053	
mean	165	0.302	0.461	1.404	0.143	0.291	0.020	0.043	
sem	51	0.078	0.119	0.665	0.035	0.162	0.016	0.006	
<i>Eln+/-</i>									
Sample	b_0 (kPa)	b_1	b_2	b_3	b_4	b_5	b_6	fit error	
1	180	0.107	0.306	0.515	0.074	0.213	0.000	0.047	
2	31	0.330	0.990	3.490	0.126	0.793	0.000	0.020	
3	125	0.156	0.190	0.439	0.085	0.000	0.041	0.048	
4	32	0.380	1.142	4.436	0.231	1.299	0.000	0.045	
5	48	0.353	1.117	3.359	0.083	0.990	0.000	0.063	
6	41	0.442	0.862	1.937	0.177	0.620	0.000	0.034	
mean	76	0.295	0.768	2.363	0.129	0.653	0.007	0.043	
sem	25	0.054	0.170	0.680	0.026	0.198	0.007	0.006	
P (<i>Eln++</i> to <i>Eln+/-</i>)	0.753	1.000	0.642	0.976	0.757	0.998	0.980	0.999	
<i>Fbln5+/+</i>									
Sample	b_0 (kPa)	b_1	b_2	b_3	b_4	b_5	b_6	fit error	
1	88	0.213	0.559	1.076	0.132	0.396	0.000	0.038	
2	39	0.360	1.367	4.455	0.160	1.488	0.000	0.038	
3	490	0.093	0.164	0.148	0.061	0.055	0.000	0.019	
4	737	0.031	0.137	0.045	0.035	0.052	0.000	0.027	
5	27	0.218	1.186	4.639	0.000	0.203	0.023	0.025	

6	171	0.086	0.632	1.061	0.136	0.719	0.000	0.054
7	117	0.181	0.819	2.547	0.196	1.036	0.000	0.009
8	50	0.185	1.955	5.052	0.402	2.624	0.000	0.039
mean	215	0.171	0.852	2.378	0.140	0.822	0.003	0.031
sem	92	0.036	0.220	0.737	0.044	0.313	0.003	0.005
P (<i>Fbln</i>^{+/+} to <i>Fbln</i>^{5+/+})	0.928	0.871	0.912	0.970	1.000	0.927	0.996	0.351
<i>Fbln</i>^{5-/-}								
Sample	b₀ (kPa)	b₁	b₂	b₃	b₄	b₅	b₆	fit error
1	25	0.971	3.877	7.860	0.505	3.796	0.000	0.015
2	34	1.192	2.724	3.964	0.555	2.627	0.692	0.023
3	11	0.446	7.595	21.410	0.491	9.765	0.000	0.028
4	9	2.258	4.247	17.325	0.000	3.797	0.612	0.017
5	161	0.604	0.830	1.338	0.285	0.552	0.353	0.011
6	45	0.598	1.912	1.605	0.029	0.239	0.000	0.010
7	19	0.956	3.366	8.528	0.265	3.095	0.146	0.006
mean	43	1.003	3.507	8.861	0.304	3.410	0.258	0.016
sem	20	0.231	0.813	2.942	0.086	1.191	0.113	0.003
P (<i>Fbln</i>^{5+/+} to <i>Fbln</i>^{5-/-})	0.191	<.001	0.001	0.031	0.147	0.031	0.016	0.134

Table 3

Material parameters and fit errors for the four-fiber strain energy function. Significant P values are shown in italics.

<i>Eln+/+</i>									
Sample	b_0 (kPa)	b_{11} (kPa)	b_{12}	b_{21} (kPa)	b_{22}	b_{31} (kPa)	b_{32}	α_0 (deg)	fit error
1	1.74	4.34	0.000	19.07	0.000	24.53	0.050	44.5	0.032
2	9.24	12.07	0.000	18.81	0.001	9.59	0.335	39.5	0.025
3	2.92	10.54	0.168	0.93	0.355	12.69	0.403	44.7	0.047
4	4.49	13.31	0.075	0.81	0.408	9.41	0.454	38.6	0.068
5	7.53	9.19	0.073	14.15	0.000	10.68	0.231	46.1	0.019
6	4.58	16.02	0.163	3.86	0.000	15.88	0.320	39.5	0.024
7	8.15	13.87	0.067	10.19	0.000	19.90	0.250	30.5	0.051
mean	5.52	11.33	0.078	9.69	0.109	14.67	0.292	40.5	0.038
sem	1.07	3.81	0.068	7.97	0.187	5.77	0.133	5.3	0.018
<i>Eln+/-</i>									
Sample	b_0 (kPa)	b_{11} (kPa)	b_{12}	b_{21} (kPa)	b_{22}	b_{31} (kPa)	b_{32}	α_0 (deg)	fit error
1	3.71	9.38	0.001	7.57	0.170	10.02	0.178	39.1	0.043
2	3.13	8.06	0.080	1.65	0.496	8.74	0.338	36.0	0.018
3	3.09	0.82	0.000	10.59	0.000	13.32	0.120	48.2	0.032
4	6.27	9.82	0.108	0.95	0.633	7.42	0.460	39.1	0.044
5	1.75	8.93	0.134	2.84	0.530	12.17	0.288	36.6	0.063
6	3.56	7.80	0.183	3.33	0.288	9.37	0.401	38.7	0.033
mean	3.59	7.47	0.084	4.49	0.353	10.17	0.298	39.6	0.039
sem	0.61	3.35	0.073	3.78	0.242	2.20	0.130	4.4	0.015
P (<i>Eln+/+</i> to <i>Eln+/-</i>)	0.314	0.199	1.000	0.442	0.799	0.749	1.000	0.990	0.999
<i>Fbln5+/+</i>									
Sample	b_0 (kPa)	b_{11} (kPa)	b_{12}	b_{21} (kPa)	b_{22}	b_{31} (kPa)	b_{32}	α_0 (deg)	fit error
1	5.85	8.16	0.060	4.81	0.290	9.02	0.322	38.1	0.038
2	7.13	12.47	0.117	0.12	0.685	8.66	0.459	29.2	0.038
3	5.92	15.20	0.007	15.75	0.000	25.04	0.140	40.2	0.018
4	3.48	9.49	0.000	13.71	0.000	24.68	0.084	31.0	0.026
5	3.84	9.54	0.021	10.50	0.134	18.53	0.275	27.0	0.015

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6	2.35	9.59	0.000	3.23	0.612	9.46	0.299	35.3	0.053
7	6.69	10.10	0.000	3.58	0.597	18.60	0.197	44.2	0.018
8	3.60	16.50	0.000	2.22	1.121	3.03	0.915	35.2	0.034
mean	4.86	11.38	0.026	6.74	0.430	14.63	0.336	35.1	0.030
sem	0.62	1.07	0.015	2.04	0.139	2.89	0.09	2.1	0.005
P (Fbln5+/+ to Fbln5+/+)	0.915	1.000	0.082	0.791	0.578	1.000	0.967	0.203	0.678
Fbln5-/-									
Sample	b₀ (kPa)	b₁₁ (kPa)	b₁₂	b₂₁ (kPa)	b₂₂	b₃₁ (kPa)	b₃₂	α₀ (deg)	fit error
1	4.46	8.19	0.648	3.07	1.192	18.64	0.697	47.4	0.021
2	2.27	4.50	0.625	5.52	0.922	9.64	0.887	44.3	0.029
3	3.26	14.04	0.021	0.55	2.951	19.50	0.428	46.5	0.025
4	6.03	11.19	0.962	6.84	1.677	45.93	0.572	50.5	0.017
5	6.25	8.32	0.755	15.90	0.451	31.26	0.452	44.2	0.018
6	4.56	4.70	0.443	16.13	0.890	21.33	0.547	35.5	0.010
7	3.46	9.58	0.413	5.66	1.080	15.44	0.744	42.1	0.008
mean	4.33	8.65	0.552	7.67	1.309	23.11	0.678	44.4	0.018
sem	0.55	1.29	0.113	2.29	0.307	4.53	0.063	1.8	0.003
P (Fbln5+/+ to Fbln5-/-)	0.953	0.421	<.001	0.991	0.009	0.203	0.035	0.010	0.389