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Research Paper

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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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 and 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 CO_2 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 to 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 (0–175 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 to 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 s/step), while the axial stretch cycles were performed by manual rotation of the micrometer attached to one end of the carotid artery (~10 μm/s). 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 μm 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)],