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Loss of elastic fiber integrity compromises common carotid artery function: Implications for vascular aging

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Abstract Competent elastic fibers endow central arteries with the compliance and resilience that are fundamental to their primary mechanical function in vertebrates. That is, by enabling elastic energy to be stored in the arterial wall during systole and then to be used to work on the blood during diastole, elastic fibers decrease ventricular workload and augment blood flow in pulsatile systems. Indeed, because elastic fibers are formed during development and stretched during somatic growth, their continual tendency to recoil contributes to the undulation of the stiffer collagen fibers, which facilitates further the overall compliance of the wall under physiologic pressures while allowing the collagen to limit over-distension during acute increases in blood pressure. In this paper, we use consistent methods of measurement and quantification to compare the biaxial material stiffness, structural stiffness, and energy storage capacity of murine common carotid arteries having graded degrees of elastic fiber integrity – normal, elastin-deficient, fibrillin-1 deficient, fibulin-5 null, and elastase-treated. The finding that the intrinsic material stiffness tends to be maintained nearly constant suggests that intramural cells seek to maintain a favorable micromechanical environment in which to function. Nevertheless, a loss of elastic energy storage capability due to the loss of elastic fiber integrity severely compromises the primary function of these central arteries.

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Introduction

The mechanical functionality, structural integrity, and adaptive capacity of central arteries result in large part from cellular regulation of the myriad structural constituents that constitute the wall, and their interactions. Notwithstanding the many contributors to the mechanics of the arterial wall, four are of primary importance: elastic fibers, fibrillar collagens, contractile smooth muscle, and proteoglycans/glycosaminoglycans.[1,2] Of these four, elastic fibers are unique - they alone do not turnover during maturity and they are not effectively repaired or replaced during disease or injury. That is, functional elastic fibers are produced prior to adulthood and they have a half-life on the order of 25–70 years in both mice and humans under normal conditions.[3–6] These fibers are thus uniquely susceptible to damage by mechanical fatigue in addition to normal degradation by proteolysis.[7–9] Because elastic fibers cannot be effectively repaired or replaced, congenital and acquired losses of elastic fiber integrity have irreversible consequences on arterial mechanics and mechanobiology, and thus the physiology and pathophysiology. Genetically induced changes in, proteolytic degradation of, or mechanical damage to elastic fibers contribute, therefore, to central artery stiffening in aging and hypertension, which in turn promote diverse cardiovascular, renovascular, and neurovascular diseases.[10–12] Indeed, loss of elastic fiber integrity also plays direct mechanical roles in atherosclerosis, aneurysms, and dissections.[13–15]

Elastic fibers in central arteries are organized into concentric fenestrated elastic sheets, or laminae, that form a three-dimensional network in the media. These fibers consist primarily of a core of elastin (90%) plus multiple elastin-associated glycoproteins.[2,16] The glycoproteins fibulin-5 and fibrillin-1 are fundamental to the elastogenesis and long term mechanical and biological stability, respectively, of the composite fibers.[17,18] Elastic fibers also associate with proteoglycans and minor collagens (e.g., collagen VIII), though specific contributions of these constituents to mechanical functionality is less clear.[16] Nevertheless, loss of elastic fiber integrity has five primary consequences to the arterial wall: loss of resilience and thus a decrease in elastic recoil,[19] decrease in collagen fiber undulation and thus diminished distensibility and extensibility,[20] loss of cell–matrix interactions and thus altered smooth muscle cell phenotypic activity,[17,21] increased intramural inflammation due to the chemotactic character of elastin degradation products,[9] and possible premature entrance into the cell death cycle, or anoikis.[22]

Among the many important consequences of reduced elastic fiber integrity, we focused on the mechanical functionality of the common carotid artery (CCA) in the mouse. Specifically, we contrasted passive biaxial mechanical behaviors of carotids from control, elastin haploinsufficient ($Eln^{+/-}$), fibrillin-1 deficient ($Fbn1^{mgR/mgR}$), and fibulin-5 null ($Fbln5^{-/-}$) mice with elastase-treated carotids from control mice. In this way, we assessed graded effects on wall structure and function due to elastic fibers ranging from normal to fully compromised. New biaxial data ($Eln^{+/-}$) are presented and compared with previously published data (controls from,[23] elastase-treated and $Fbn1^{mgR/mgR}$ from,[20] and $Fbln5^{-/-}$ from [24]) using new approaches for interpreting the data. By treating control and elastase-treated data as upper and lower bounds of elastic fiber functionality, respectively, we elucidated graded changes in carotid artery mechanics across the three genetic models. Importantly, total elastic energy displays a strong qualitative correlation with the severity of elastic fiber disarray.

Methods

Experimental methods

All animal protocols were approved by the Institutional Animal Care and Use Committee of Yale University. Briefly, mice were euthanized and CCAs were gently excised, cleaned of excess perivascular tissue, cannulated on custom drawn glass pipettes, and placed within a custom biaxial testing system in a Hanks buffered saline at 37 °C[25]; we have shown that this protocol yields a passive behavior.[20] Following methods described previously,[23] we performed cyclic pressure–diameter ($P–d$) tests at three different fixed axial stretches (the *in vivo* value and 5% above and below this value) and cyclic axial force–length ($f–l$) tests at four different fixed pressures (10, 60, 100, 140 mmHg). The latter tests are particularly important for estimating the preferred, or optimal, *in vivo* axial stretch[26] and ensuring robust estimations of material parameters that define biaxial wall properties.[23] All data (luminal pressure, outer diameter, axial force, and axial length, noting that axial stretch is defined as the ratio of the *in vivo* to the unloaded *in vitro* length) were collected using a custom LabView program and used for on-line feedback control of the tests and off-line analysis.

Material characterization

Each of the seven biaxial protocols (three $P–d$ plus four $f–l$) consisted of two cycles of data. We extracted and combined the unloading portion of the final cycle for the seven data sets, each of which consisted of hundreds of data points. The unloading portion of a testing cycle provides information on the energy stored in the tissue that is yet available to work on the blood; that is, it does not include the small amount (typically 3–5%) of energy that dissipates during full pressure–diameter and axial force–length testing. The resulting combined data sets were analyzed via nonlinear regression to identify best-fit values of model parameters in a validated constitutive relation. Details on the constitutive formulation and regression method are given elsewhere.[23,27] Briefly, we use a stored-energy function W containing 8 parameters $(c, c_1^1, c_2^1, c_1^2, c_2^2, c_1^{3,4}, c_2^{3,4}, \alpha_o)$ to model contributions to overall load bearing within the wall by an elastin-dominated isotropic matrix and four families of locally-parallel fibers; these fiber families are set in axial, circumferential, and symmetric diagonal directions to capture phenomenologically the net effects of distributed collagen fibers and their lateral cross-links as well as, in the circumferential direction, passive smooth muscle cells (see Appendix). This constitutive relation describes and predicts well the passive