Elastin, arterial mechanics, and cardiovascular disease

Austin J. Cocciolone, Jie Z. Hawes, Marius C. Staiculescu, Elizabeth O. Johnson, Monzur Murshed, and Jessica E. Wagenseil

1Department of Biomedical Engineering, Washington University, St. Louis, Missouri; 2Department of Mechanical Engineering and Materials Science, Washington University, St. Louis, Missouri; and 3Faculty of Dentistry, Department of Medicine, and Shriners Hospital for Children, McGill University, Montreal, Quebec, Canada

Submitted 2 February 2018; accepted in final form 6 April 2018

INTRODUCTION

Elastin is an extracellular matrix (ECM) protein with a unique biochemical structure that provides entropic elasticity, allowing the large arteries to reversibly expand and relax with every cardiac cycle. Elastin is the main component of elastic fibers that provide the large artery elasticity necessary for proper cardiovascular function in vertebrate animals. Insufficiency of elastin or disorganization, improper assembly, fragmentation, and biochemical modifications of elastic fibers change the passive mechanical behavior of the large arteries and affect cardiovascular mechanics at multiple length scales. Both genetic and acquired cardiovascular diseases are associated with elastin and elastic fiber defects and the resulting changes in arterial mechanics. Tropoelastin, the soluble precursor to elastin, and the degradation products of fragmented elastic fibers also have important chemical signaling properties.

Elastin is highly hydrophobic, extensively cross-linked, and is assembled into elastic fibers in a dynamic process involving cells, cell surface receptors, and numerous elastic fiber associated proteins. Mutations in the elastin gene cause supravalvular aortic stenosis (SVAS), associated with elastin insufficiency, and autosomal dominant cutis laxa-1 (ADCL1), associated with tissue-specific defects in elastic fiber assembly. SVAS mutations appear throughout the gene, but ADCL1 mutations are clustered near the last few exons, suggesting a role for that region in tissue-specific elastic fiber assembly. Since elastin synthesis and elastic fiber assembly are primarily restricted to development and maturation, it is critical to understand the assembly mechanisms for designing interventions in elastinopathies and to recreate elastic fiber structure and function in tissue-engineered arteries. Acquired diseases are generally associated with elastic fiber degradation or biochemical modification. This degradation initiates a destructive arterial remodeling cascade that is difficult to stop and may be impossible to
reverse. As modern medicine has extended life expectancy beyond elastin’s 70-yr half-life, it is imperative to understand how degradation and modification of elastic fibers within the arterial wall contribute to the pathogenesis of acquired diseases.

In this review, we summarize the biochemistry of elastin and elastic fiber assembly process. We discuss measures of passive arterial mechanics that are affected by elastin amounts, elastic fiber integrity, and cross-linking. When elastin amounts are reduced or elastic fiber integrity is compromised, the vascular wall cells compensate by producing other ECM proteins and remodeling arterial geometry that can further alter the mechanical behavior. Additionally, circulating elastin fragments produced during elastic fiber degradation can activate cytokine and inflammatory signaling pathways that further degrade elastic fibers in a positive feedback cycle. We summarize the results of human and animal studies that have advanced our understanding of genetic elastinopathies and the cardiovascular effects of varying elastin gene dosage. We address acquired cardiovascular diseases that are associated with elastic fiber degradation or modification. We briefly discuss advances in generating elastic fibers within tissue-engineered arteries and conclude with suggestions for future research.

ELASTIN

Elastin Characterization

Elastin is an elastic protein polymer that is highly resilient due to extensive posttranslational cross-linking of the monomeric precursor tropoelastin. Reviews focusing on tropoelastin and/or elastin have been written by Vrhovski and Weiss (191) and Kozel et al. (87). Due to the numerous cross-links at lysine residues and having a composition of nearly 75% hydrophobic residues, elastin is extremely hydrophobic (191). The precursor tropoelastin is a 70-kDa protein transcribed from a single elastin gene in the mammalian genome (51, 128, 158). The human elastin gene is 45 kb, located on chromosome 7, and contains 34 exons of which each exon encodes either a hydrophobic or cross-linking domain in an alternating pattern (Fig. 1) (9, 138, 180, 206). Elastin is only present in vertebrate animals, and there is extensive homology among human, chick, bovine, and rat species at the nucleic and amino acid levels (191). During an organism’s lifespan, elastin gene expression and protein synthesis occur within a narrow timeframe initiating late in embryonic development and terminating in adolescence with essentially no de novo elastin production throughout adult life of the animal (15, 26, 34, 119, 133, 142, 145, 163). The narrow timeframe of elastin expression and synthesis is unusual for an ECM component, as generally ECM production is dynamic throughout the organism’s lifespan. Because of elastin’s resilience and a half-life of nearly 70 yr, the limited time period of expression and synthesis is sufficient for the protein to last a lifetime in most species (151, 165).

Regulation of Tropoelastin Expression

Immunohistochemistry studies in the developing chicken lung (71) and aorta (68) have shown that tropoelastin expression correlates with smooth muscle differentiation markers and suggest that transcription of elastin is developmentally regulated. Tropoelastin mRNA levels are decreased in adult compared with neonatal rat lungs, but tropoelastin pre-mRNA levels are similar, suggesting that posttranscriptional regulation turns off tropoelastin expression in adulthood (176). An element in the open reading frame specifically binds a cytosolic protein that destabilizes tropoelastin mRNA. Binding affinity increases in adult compared with neonatal lung fibroblast cells, mediating rapid tropoelastin mRNA decay and decreasing overall mRNA levels. Binding affinity is decreased by transforming growth factor-β1 (TGF-β1) (212), which stimulates tropoelastin expression in human fetal lung fibroblasts by stabilizing its mRNA (89). Active Smads and de novo tropoelastin expression are required for TGF-β1-mediated tropoelastin mRNA stabilization in cell culture (90). TGF-β1 immunoreactivity is present at the sites of arterial elastin expression as detected by in situ hybridization, supporting a role for TGF-β1 regulation of tropoelastin expression in vivo (159). TGF-β1 may additionally regulate tropoelastin expression through interactions with the promoter region in a tissue-specific manner (109). IGF-1, vitamin D, and IL-1β also modulate tropoelastin expression at either the promoter level or through increased mRNA stability (191). Cytokine regulation of tropoelastin expression has been reviewed by Sproul and Argraves (171).

Coacervation and Cross-Linking

In the arterial wall, tropoelastin is primarily produced from vascular smooth muscle cells (VSMCs) in the media (46, 68), although it can also be produced by endothelial cells (ECs) in the intima (19, 113) and fibroblasts in the adventitia (152, 187). After secretion to the extracellular space, tropoelastin simultaneously undergoes coacervation and cross-linking. Cross-linking is facilitated by enzymes from the lysyl oxidase (LOX) family, in particular LOX and LOX-like-1 (LOXL-1). The role of LOX in elastic fiber assembly has been reviewed by Lucero and Kagan (105). Coacervation is a reversible and thermodynamically driven self-assembly of tropoelastin into globular aggregates that occurs spontaneously at physiological temperatures and is a consequence of the numerous hydrophobic regions in tropoelastin (22, 23, 134, 183, 186, 190). LOX activity appears to depend on coacervation, as LOX demonstrates higher affinity for insoluble, coacervated tropoelastin and is less effective at cross-linking tropoelastin at temperatures below the threshold for spontaneous coacervation (75, 120). While accepted that coacervation is a critical step to cross-linking, it is unclear whether this is due to induced conformational changes in tropoelastin or to tropoelastin sequestering (80, 120, 191).
Through the activity of LOX, an oxidative deamination reaction converts many lysine residues into allysine (143). It is suspected that the presence of an aromatic residue adjacent to and on the COOH-terminal end of lysine sterically inhibits LOX activity, preserving several lysine residues for downstream reactions in the cross-linking process (43). After side chain oxidation, a sequence of spontaneous condensation reactions occurs between the newly formed allysine residues and unreacted lysine residues to form covalent cross-links called desmosine and isodesmosine between tropoelastin units. Desmosine and isodesmosine are unique to elastin and serve as a basis for quantification of elastin amounts in tissues (173).

Elastic Fiber Assembly

Elastin forms the core of elastic fibers and is the most abundant component of the mature structure. The other major component is a scaffold of microfibrils, which are composed mostly of fibrillin-1 and -2 (156, 210). Elastic fiber assembly involves nearly 30 different proteins that are tightly coordinate spatially and temporally (80). The function of only a handful of these associated proteins is understood and elucidation of the function of others is an active area of investigation. Reviews focusing on elastic fiber assembly have been written by Kielty et al. (80), Wagenseil and Mecham (195), and Yanagisawa and Davis (205). Spontaneous tropoelastin coacervation and the cross-linking catalyzed by LOX in the cell surface initiate the assembly process. There is evidence that some of the associated assembly proteins, namely, fibulin (FBLN4 and/or FBLN5, serve to mediate the size and conformation of the developing elastin aggregates (131). These elastin aggregates are then shuttled to the microfibrils that are anchored to the cell surface through integrins. Finally, further cross-linking occurs, again through LOX, to develop the mature elastic fiber that provides elasticity to the large, elastic arteries.

Elastolytic Enzymes

Proteases capable of degrading elastin or elastic fibers are known as elastases. In total, 12 elastases have been identified; however, they are nonspecific to elastin. Neutrophil elastase (Ela-2), cathepsin G (CatG), and proteinase-3 (Pr-3) are serine-class proteases that may have a stronger affinity for tropoelastin than cross-linked elastin. They have been found in atherosclerotic plaques, are secreted from neutrophils, and likely contribute to atherosclerosis progression (111). Ela-2 and CatG are also secreted from VSMCs in the pulmonary artery (83). Four matrix metalloproteinases (MMP-2, MMP-7, MMP-9, and MMP-12) have elastolytic activity. All four MMPs are secreted by macrophages, linking elastic fiber degradation and inflammation. MMP-2 and MMP-9 are also produced by VSMCs and are able to activate latent TGF-β (207). There are three levels of posttranslational regulation of MMP activity. MMPs are initially secreted as inactive pro-MMPs that require proteolytic cleavage of the propeptide, activity is also turned on by the deactivation of a cysteine switch, and MMPs are regulated by specialized inhibitors called tissue inhibitors of metalloproteinase (TIMPs) (189). Another four elastases, also secreted by macrophages, belong to the cysteine class: cathepsin L, S, K, and V (CatL, CatS, CatK, and CatV). CatL, CatS, and CatK are also expressed by VSMCs (111). Pepsin A is the final known elastase, but as a digestive enzyme released in the stomach it is unlikely to play a role in vascular remodeling.

ARTERIAL MECHANICS

Arterial Wall Structure

The structure of the arterial wall is divided into three layers: intima, media, and adventitia. The intima is a single layer of ECs directly adjacent to the arterial lumen that sits on a basement membrane constructed of collagen type IV, laminin, and proteoglycans (208). The middle and largest layer, the media, is further sublayered into lamellar units defined by fenestrated sheets of elastic fibers (Fig. 2), or elastic laminae. These laminae are separated by a region composed of VSMCs, thin elastic fibers, collagen (mostly types I and III), and proteoglycans (27, 32, 64, 123). An internal elastic lamina separates the media from the intima. Elastic fibers and collagen interconnect the elastic laminae forming a continuous network with three-dimensional helical structure in which the fibers are oriented in a near-circumferential direction (123). The number of lamellar units decreases along the arterial tree with larger elastic arteries, such as the descending thoracic aorta, having ~60 units in humans (203) and muscular arteries closer to the periphery having fewer than 3 units. The number of lamellar units scales with physiological pressure and arterial size across mammalian species, providing a constant wall tension/lamellar unit (204).

Cross-sections of a large and small artery from a mouse, demonstrating the number and organization of elastic laminae in the wall, are shown in Fig. 3. An external elastic lamina exists between the media and the outermost layer, the adventitia, in large arteries but is absent in the smallest muscular arteries. The adventitia contains fibroblasts, fibrocytes, and a collagen-rich matrix. Large arteries in large animals contain a vasa vasorum in the adventitia to supply blood to the arterial wall.
outer wall cells that are too far from the arterial lumen for diffusion of oxygen and nutrients across the wall.

The Windkessel Effect

Elastin is only present in vertebrate animals with a closed circulatory system and pulsatile pressure and flow (155). The amount of elastin is highest in the large, elastic arteries closest to the heart and decreases as one moves distally in the cardiovascular system (16). Elastic fibers provide reversible elasticity to the large, elastic arteries. This allows the aorta to deform elastically under an applied hemodynamic load, with no permanent deformation and no energy dissipation when the load is removed. Stephen Hales in 1733 recognized the importance of arterial elasticity in cardiovascular function and Otto Frank in 1895 formalized Hales’ ideas into a mathematical model of the Windkessel effect (132). A Windkessel is an air chamber that was used in fire engines during the 18th century to convert pulsatile pumping into a constant flow of water. In the cardiovascular system, the Windkessel effect dampens the pulsatile flow from the left ventricle (LV), so that the distal vasculature receives almost constant perfusion. Blood ejected from the LV during systole distends the aortic wall and during diastole the aorta reversibly returns to its original configuration. The strain energy stored during systolic deformation is returned as work to further pump blood downstream during diastole. If the Windkessel effect is compromised, for example, by increased arterial stiffness in aged or diseased arteries, the microvasculature of downstream organs, especially in the brain and kidney, can be damaged (125). Furthermore, the Windkessel effect reduces cardiac afterload and perfuses the coronary arteries during diastole; hence, cardiac function can deteriorate when the Windkessel effect is compromised (126).

The Windkessel effect can be compromised through genetic or acquired elastinopathies that alter elasticity of the arterial wall. Elasticity can be altered by increasing wall stiffness or increasing energy dissipation so that less stored strain energy is available to do work on circulating blood. Increased wall stiffness also increases the pulse wave velocity (PWV) of traveling pressure waves in the cardiovascular system. Reflected waves from arterial branch points normally augment diastolic pressure, but increased PWV causes the reflected waves to return earlier in the cardiac cycle and augments systolic blood pressure, leading to systolic hypertension, decreased diastolic perfusion, and increased pulse pressure, which can further damage downstream organs (52).

Arterial Stiffness and Passive Mechanical Behavior

Arterial stiffness is a structural mechanical property that depends on the material properties and geometry of the arterial wall. Common measurements of stiffness include Peterson’s modulus, distensibility, and PWV (50) and almost always refer to deformation and loading in the circumferential direction. We will call these measurements “structural stiffness” values. Peterson’s modulus and distensibility can be measured in vivo or in vitro, while PWV is usually measured in vivo. PWV can be determined simply and reproducibly in the clinic by recording the time it takes for a blood pressure pulse wave to travel a specified distance in the arterial tree (usually from the carotid to the femoral artery) (124). PWV is a measure of structural stiffness because the value is related to Young’s modulus of the arterial wall (a material property), wall thickness, arterial diameter, and density of blood according to the Moens-Korteweg equation (50). The derivation of the Moens-Korteweg equation assumes that the aorta is a perfect cylinder with no tapering or branches, made up of a linear elastic, isotropic material that experiences small deformations during the cardiac cycle, none of which are precisely true. While PWV is a useful measure of structural arterial stiffness, the assumptions accompanying its use must be kept in mind.

Arterial wall material properties are nonlinear and vary with the direction of deformation and loading (i.e., circumferential vs. axial); hence, they cannot be quantified by a single measurement, such as Young’s modulus in the Moens-Korteweg equation. However, estimates of arterial material properties can be obtained by linearizing the mechanical behavior within a given deformation or loading range in a specified direction to provide an incremental Young’s modulus for comparison across samples (50). Material stiffness values can also be obtained by fitting constitutive models to detailed in vitro mechanical testing data and calculating derivatives of the conjugate stress and strain measurements (41). Fitting constitutive models allows comparison of material behavior and stored strain energy for any loading condition in any direction but requires multiple experimental protocols for each sample and is dependent on the suitability of the chosen constitutive equation.

The passive mechanical behavior of large, elastic arteries is determined mostly by the amount and organization of elastic fibers and collagen fibers in the wall. VSMCs contribute to arterial mechanics through active contraction or relaxation and by producing the surrounding ECM proteins, but they do not contribute substantially to the passive mechanical behavior (38). Although there is only one elastin protein, there are multiple collagen proteins. The most abundant arterial collagens are types I and III, with lesser amounts of types IV, V, and VI. The amounts and transmural organization of collagen types depend on location in the cardiovascular tree (64). Elastic arteries show nonlinear behavior with maximum distensibility.
near physiological pressures and reduced distensibility at high pressures (150), presumably to protect the cells and protein components from irreversible damage due to excessive deformation. Selective digestion of elastic fibers or collagen fibers with proteases indicates that the resistance to stretch in elastic arteries at low pressures is due to elastic fibers, while the resistance to stretch at high pressures is due to collagen fibers (33, 42, 146, 164). Experiments on the aorta from mice lacking elastin (Eln\(^{-/-}\)) have demonstrated a reduction in the circumferential material stiffness at low pressure, consistent with the observed role of elastic fibers in protease experiments (82). However, experiments on arteries from elastin haploinsufficient mice (Eln\(^{+/+}\)) with 50–60% of normal elastin levels show similar circumferential stress-stretch behavior to wild-type (WT) mice (Fig. 4), which has been attributed to remodeling of the cardiovascular system during development and maturation (39, 197).

Remodeling of the arterial wall in response to genetic or acquired elastinopathies is important for determining the physiologic mechanical behavior. Production and reorganization of collagens (198) or other ECM components, such as proteoglycans (70), are often observed in response to defects in or degradation of the elastic fibers. Additional collagen deposition or a shift in the ratio of different types of collagens in the wall may increase stiffness of the wall and affect the Windkessel function and strain energy storage capacity (194). Additional proteoglycans may pool in the wall and provide a catalyst for arterial dissections (148).

**Energy Storage and Return**

Because elastin is a highly resilient protein, it allows the wall of the large arteries to deform with little energy loss on loading and unloading. Energy loss is small but finite at 15–20%, and this value appears to be consistent across various species (48). Energy loss is attributed to viscous losses from VSMCs (201) or proteoglycans (200). The relative contributions of different components of the viscoelastic behavior of arteries have been determined using protease digestion in a similar manner to the elastic contributions for different pressure ranges. Recent experiments on newborn mouse aortae lacking elastin (Eln\(^{-/-}\)), lysyl oxidase (Lox\(^{-/-}\)), or fibulin-4 (Fbln4\(^{-/-}\)) have shown that properly assembled and cross-linked elastic fibers are necessary for low energy loss (82). Many investigations do not include energy loss in mechanical studies on large elastic arteries, but this may be

![Fig. 4. Effects of elastin amounts on arterial mechanical behavior. A–D: representative pressure-outer diameter (A and B) and circumferential Cauchy stress-stretch ratio (C and D) curves (197) for the ascending aorta (ASC; A and C) and left common carotid (LCC; B and D) from wild-type (WT; 100% elastin), Eln\(^{+/+}\) (50–60% elastin), and hBAC-mNull (30–40% elastin) mice. Decreased elastin amounts shift the pressure-diameter behavior for both artery locations, but the difference between Eln\(^{+/+}\) and hBAC-mNull pressure-diameter behavior for the LCC is negligible. For both artery locations, the stress-stretch behavior is similar between WT and Eln\(^{+/+}\) mice but becomes more nonlinear at lower stretch ratios for hBAC-mNull mice. The data highlight the importance of examining structural (pressure-diameter) and material (stress-stretch) mechanical behavior as well as differences between artery locations in the structural response to reduced elastin amounts. The results suggest that mouse arteries can remodel to maintain WT material behavior when elastin amounts are 50–60% of normal but cannot fully remodel and adapt the material behavior when elastin amounts are 30–40% of normal.](https://www.ajpheart.org/content/10.1152/ajpheart.00087.2018/Fig4)
an important consideration in diseases with highly fragmented elastic fibers.

ELASTIN AS A SIGNALING MOLECULE

Tropoelastin

While we have focused so far on the mechanical effects of structural changes in elastic fibers, elastin and its degradation products have chemical signaling effects as well. For example, soluble tropoelastin can regulate the cell phenotype. The addition of tropoelastin to the culture media of VSMCs from Eln−/− mice inhibits proliferation (76) and increases stiffness as measured by atomic force microscopy (37). Karmik et al. (77) showed that a specific domain within tropoelastin, VGVAPG, induces actin polymerization in VSMCs through a G protein-coupled pathway. The VGVAPG peptide is a potent chemoattractant for VSMCs (77), fibroblasts, and monocytes (162). Sites have been identified at the COOH terminus and central region of tropoelastin that mediate binding to α5β3- and αvβ3-integrins, which are involved in numerous signaling pathways (96, 149). A nonintegrin-mediated interaction between tropoelastin and the cell membrane has also been identified through the binding of fractionated tropoelastin to heparin sulfated proteoglycans (14). Tropoelastin expression is detectable in the adult human aorta at decreasing levels with aging (44), implying that reduced tropoelastin as well as degradation and modification of existing elastic fibers may modulate age-related changes in the arterial wall.

Elastin-Derived Peptides

Elastic fibers can be degraded and fragmented by mechanical fatigue, calcification, glycation, lipid peroxidation, and protease digestion (35). The resulting elastin-derived peptides (EDPs) are bioactive and retain the VGVAPG peptide sequence found in tropoelastin, rendering similar signaling capability. EDP levels in the blood are increased in humans with obliterative arteriosclerosis of the legs, type IIb hyperlipidemia, hypertension, diabetes, and ischemic heart disease (45). Whether the increased levels of EDPs are a cause or consequence of the disease is unknown. Recent studies in mice have suggested that while elastic fiber fragmentation may be secondary to the genetic or acquired disease, circulating EDPs contribute to further disease progression. Chronic doses of EDPs, as well as VGVAPG peptides, increase the size of atherosclerotic plaques at the aortic root in two different atherosclerosis susceptible mouse models (47). EDPs are capable of binding the lactose-insensitive receptor found on the surface of macrophages, implicating a role for EDPs in macrophage migration in inflammation and atherosclerosis progression (107). A single dose of EDPs, as well as VGVAPG peptides, causes hyperglycemia in mice (162). Rat aortic VSMCs incubated with EDPs show increased expression of typical bone proteins, which may contribute to arterial calcification (167). An antibody to VGVAPG blocks EDP signaling to inflammatory cells and protects against elastase-induced emphysema in mice (63). VGVAPG antibody also reduces aortic elastic fiber fragmentation, macrophage infiltration, and TGF-β1 activity in a mouse model of aortic aneurysm due to reduced fibrillin-1 levels (54). Finally, VGVAPG sequence-carrying EDPs have been shown to interact with the galecin-3 receptor, which is involved in melanoma chemotaxis and expression of MMP-2 and MMP-3 (141). A review summarizing the effects of elastin degradation and EDPs in cardiovascular aging and disease has been written by Duca et al. (35).

The Elastin Receptor Complex

Many of the chemical signaling effects of tropoelastin and/or EDPs are thought to be mediated by the elastin receptor complex (ERC) (111). The ERC is composed of two membrane-bound subunits, protective protein/cathepsin A (PPCA) and neuraminidase-1 (Neu-1), plus elastin-binding protein (EBP). EBP (also known as S-Gal) is a splice variant of lysosomal β-galactosidase that retains the ability to bind galactosugars but has lost enzymatic activity. In addition, the splice variant has a 32-peptide sequence capable of binding tropoelastin (59). In elastogenesis, EBP is thought to be responsible for shuttling tropoelastin to the cell surface, and antagonistic interaction between EBP and galactose or lactose may release tropoelastin to the extracellular space (58). Neu-1 appears to control signal transduction by ERC through the catalytic conversion of ganglioside to lactosylceramide (153). Downstream signaling of the ERC diverges depending on cell type but converges at the activation of ERK1/2 (35).

TGF-β Sequestration

TGF-β is secreted from cells in the form of a large latent complex (LLC) that includes a latency-associated peptide (LAP) and a latent TGF-β-binding protein (LTBP). LTBP localizes latent TGF-β to the ECM. LTBP1 and LTBP3 bind well to all three TGF-β isoforms (147). LTBP1 has specific interactions with fibrillin-1 microfibrils (129). Although direct interactions of LTBP3 with fibrillin-1 have not been shown, LTBP3 protein is reduced in the aorta of fibrillin-1 knockout (Fbln1−/−) mice (213). In the early stages of elastic fiber assembly, microfibrils are abundant and there is little elastin. Throughout late embryonic development and early maturation, elastin synthesis increases and eventually obscures the microfibrils (196). It is possible that elastin accumulation indirectly regulates TGF-β availability by modulating LLC binding to microfibrils. TGF-β1 immunoreactivity and tropoelastin expression colocalize in the developing rat aorta and decrease with age (159). This has been interpreted as evidence of TGF-β1-regulated expression of tropoelastin in vivo, but it could also be interpreted as tropoelastin-regulated availability of TGF-β1 as elastin accumulates on microfibrils. TGF-β1 signaling is altered when arterial elastic fibers are not assembled properly and through proteolytic cleavage of elastic fiber-associated proteins, indicating bidirectional effects between TGF-β1 activity and elastic fiber integrity (61). Elastinopathies that alter elastin amounts or elastic fiber integrity may then affect TGF-β activity (185).

CARDIOVASCULAR DISEASE

Several genetic and acquired cardiovascular diseases are associated with elastin and elastic fiber defects. The defects include altered amounts of elastin and improper assembly, fragmentation, and modification of elastic fibers. Additional reviews on cardiovascular diseases associated with elastin or elastic fiber defects have been written by Baldwin et al. (7) and Milewicz et al. (115).
**Genetic Elastinopathies**

Supravalvular aortic stenosis. SVAS (OMIM no. 185500) is a rare (~1:20,000 births) congenital obstruction of the LV outflow tract. The defining defect is a lesion in the aorta at the sinotubular junction (STJ) as a result of VSMC hypertrophy, increased collagen, and reduced/disorganized elastic fibers in the medial layer (29, 136). Often there are additional morphologic defects in other elastic arteries, but the arteries affected and severity of the defects are patient specific. There are three forms of SVAS: autosomal dominant familial, sporadic, and Williams-Beuren syndrome (WBS; OMIM no. 194050) associated. The genetic origin defines the form of SVAS. Familial and sporadic SVAS arise from loss-of-function mutations to the elastin gene that are either inherited or de novo, respectively. A variety of mutations have been reported, including translocations, partial deletions, and point mutations (Fig. 1), often resulting in a truncated elastin protein. In WBS-associated SVAS, the entire elastin gene, along with 25–27 other genes, is encompassed in a large deletion on chromosome 7 (140). In all cases, one functional elastin gene remains and sporadic mutations are more common than inherited mutations (5).

SVAS can present as one of two different types. Type I (discrete) is defined by a localized narrowing at the STJ. Type II (diffuse) shows uniform narrowing beginning at the STJ and extending as far as the aortic arch or even the descending aorta (97). The ratio of the type I to type II incidence is ~7:3 (202). Due to the rarity of SVAS, data correlating type I or II with specific elastin mutations are not available (30). In addition to the primary STJ lesion, vascular defects occur in other arteries. Often the aortic valve leaflets are hypertrophic, resulting in partial fusion of the leaflets to the STJ (118). Thirty-nine percent of patients with SVAS have bicuspid aortic valve (30). Pulmonary artery stenosis is present in 83% of patients with SVAS (112, 202). Lesions in the right ventricular outflow tract are also common (30). Coronary arteries can exhibit fibrodysplasia and may have stenotic lesions (160). Because coronaries are muscular arteries, it is unclear whether the lesions are a direct result of the elastinopathy or are a secondary pathology.

Hypertension is common in patients with WBS even at early ages (139) and may be linked to increased arterial structural stiffness due to reduced elastin amounts. Kozel et al. (85) measured PWV in 77 patients with WBS and found an increase versus healthy control subjects. However, the increased PWV was independent of whether or not the patient was hypertensive (85). While hypertension and increases in arterial structural stiffness generally develop coincidently in acquired cardiovascular disease, their dissociation in WBS may indicate that reduced elastin amounts are a common causal factor. Patients with SVAS have an increased risk of sudden cardiac death, with patients with WBS having the highest risk (202). The most common cause of sudden cardiac death is myocardial ischemia, likely resulting from coronary obstruction and LV hypertrophy (11). Ischemia could also be caused by reduced coronary perfusion during diastole due to a compromised Windkessel effect. Compromised arterial Windkessel function would also increase cardiac afterload and could contribute to LV hypertrophy.

**Autosomal dominant cutis laxa-1.** ADCL1 (OMIM no. 123700) is a rare connective tissue disorder in which the skin is loose, is hyperextensible, and has redundant folds. Patients may have cardiovascular pathologies including bicuspid aortic valve, dilation of the aorta or pulmonary artery, and pulmonary emphysema (55). The majority of reported ADCL1 cases have a frame shift mutation within the last five exons of the ELN gene (Fig. 1). The result of the frame shift is usually an elongation of tropoelastin protein that is believed to form larger aggregates during coacervation and impair the ability of elastin to bind to the microfibrillar scaffold during elastic fiber assembly. Callewaert et al. (17) demonstrated impaired tropoelastin deposition onto microfibrils, increased TGF-β signaling, and indications of endoplasmic reticulum stress in cultured fibroblasts from patients with ADCL1. In nearly 50% of ADCL1 cases, aneurysms are present in the aortic root, aortic arch, or pulmonary artery. Aneurysm development is likely a combined result of reduced elastic fiber synthesis and increased susceptibility to elastic fiber degradation (36).

**Mouse Models of Genetic Elastinopathies**

Targeted deletion of the Eln gene in mice was first reported by Li et al. in 1998 (98). Eln−/− mice represent an extreme elastinopathy and provide insights into the role of elastin in arterial mechanics, VSMC phenotype modulation, and cardiovascular disease. The same year, Li et al. showed that Eln+/− mice demonstrate some of the cardiovascular phenotypes observed in patients with SVAS, such as increased arterial structural stiffness and an increased number of arterial lamellar units but do not have the local aortic lesions observed in SVAS (99). A mouse model of WBS with a large deletion that includes the Eln gene (100) has a similar cardiovascular phenotype to Eln−/− mice (49). Eln+/− mice have been used to study cardiovascular effects of reduced elastin amounts and the associated increases in arterial structural stiffness.

Due to the insertion of a short exon after exon 4 in the mouse and the loss of two exons (34, 35) in the human during primate evolution, the mouse Eln gene has 37 exons, whereas the human Eln gene has 34 exons (178). The human Eln gene also shows unique alternative splicing patterns (40). To create a mouse model to study Eln mutations in human disease, Hirano et al. expressed Eln gene in a bacterial artificial chromosome (BAC) in Eln−/− mice (60). These hBAC-mNull mice only express human elastin and have total elastin amounts at ~30–40% of normal (60). hBAC-mNull mice also show VSMC hypertrophy and aortic luminal narrowing representative of SVAS lesions (72). Sugitani et al. engineered a single base deletion associated with ADCL1 within the Eln gene in a BAC and expressed it in mice (175). The mutant elastin was incorporated into elastic fibers in the mouse skin and lung and compromised tissue function. The mutant elastin was incorporated at low levels into the mouse aorta and did not affect tissue function. The results show tissue specific differences in elastic fiber assembly and highlight the utility of “humanized” mice for studying human disease (175).

**Effects of graded elastin amounts in mice.** Eln−/−, Eln+/−, and hBAC-mNull mice allow investigation of how graded amounts of elastin affect cardiovascular function. They are relevant for determining threshold amounts of elastin necessary for restoring cardiovascular function in genetic and acquired elastinopathies, as well as in tissue-engineered arteries. Each mouse model is discussed in additional detail below. Compar-
isons of arterial mechanics, wall structure, and morphology for WT mice with 100% of normal elastin amounts, Eln<sup>+/−</sup> mice with 50–60% of normal elastin amounts, and hBAC-mNull mice with 30–40% of normal elastin amounts are shown in Figs. 4, 5, and 6, respectively.

0% ELASTIN. Eln<sup>+/−</sup> mice die within a few days of birth due to VSMC overproliferation in the aortic wall leading to luminal occlusion that is similar to the aortic root lesion observed in SVAS (98). Eln<sup>+/−</sup> mice have normal blood pressure and aortic structural stiffness at embryonic day 18 (192) but high blood pressure and increased aortic structural stiffness at birth (193). Aortas from Eln<sup>+/−</sup> mice have decreased aortic material stiffness compared with WT mice in the low pressure range but similar aortic material stiffness to WT mice in the high pressure range (82). VSMCs from Eln<sup>+/−</sup> mice have higher proliferation rates (76) and lower material stiffness values (37), and these phenotypes can be partially reversed by the addition of tropoelastin to the cell culture medium. Treatment with the mechanistic target of rapamycin (mTOR) inhibitor rapamycin decreases VSMC overproliferation in Eln<sup>+/−</sup> mice but does not extend their lifespan (101). 3-Integrin expression is upregulated in the Eln<sup>+/−</sup> aorta, and genetically reducing 3-integrin dosage extends the lifespan of Eln<sup>+/−</sup> mice by ~2 days (116). Pharmacological inhibition of 3-integrin with cilengitide reduces VSMC overproliferation in the Eln<sup>+/−</sup> aorta and may be a novel treatment to prevent SVAS (116).

Fig. 5. Effects of elastin amounts on arterial wall structure and composition. A–I: Histological sections (93) of the left common carotid artery from wild-type (WT) mice with 100% elastin (A, D, and G), Eln<sup>+/−</sup> mice with 50–60% elastin (B, E, and H), and hBAC-mNull mice with 30–40% elastin (C, F, and I). A–C: Verhoeff-Van Gieson (VVG)-stained sections show black elastic laminae, brown muscle tissue, and pink collagen. The elastic laminae are thinner, more numerous, and have more diffuse staining as elastin amounts decrease. D–F: picrosirius red (PSR)-stained sections show collagen fibers in red and other material in yellow. In WT arteries, red collagen clearly outlines the yellow elastic laminae. In Eln<sup>+/−</sup> and hBAC-mNull arteries, the collagen staining overlaps with the elastic laminae. G–I: hematoxylin and eosin (H&E)-stained sections show cell nuclei in purple and other material in pink. Scale bars = 20 μm.

Fig. 6. Effects of elastin amounts on arterial morphology. Yellow latex (172) was injected for contrast into the ascending aorta, major branches, and descending thoracic aorta in wild-type (WT; 100% elastin), Eln<sup>+/−</sup> (50–60% elastin), and hBAC-mNull (30–40% elastin) mice. With decreasing elastin amounts, the aortic diameter decreases and length increases. There are also changes in branching morphology with reduced elastin amounts. Scale bars = 1 mm.
increased arterial stiffness but do not have the in-heterozygous deletion observed in WBS (100) have hyperten-
reduced elastin due to a large genetic deletion that mimics the
ceptibility to vascular calcification (Fig. 7) (79). Mice with
deposition (black) along the elastic laminae (Fig. 5) (39, 99). Treatment with the mTOR inhibitor rapamycin
an increased number of lamellar units in the arterial wall (Fig.
in the ascending aorta, have extreme hypertension, and have
These hBAC-mNull mice have 35% of normal elastin amounts
mice (101).
increased arterial structural and material stiffness (Fig. 4) (60). Treatment of
mice (101). Eln^+/− mice show reduced
stenosis in ECM composition and arterial geometry lead to in-
compromised elastic fiber integrity and the subsequent altera-
sions in ECM composition and arterial geometry lead to in-
compromised elastic fiber integrity and the subsequent altera-
sions (194), and Mitchell (117), among others. Targeting ECM
hypertension. The role of large artery stiffness in hypertension
in hypertension (209). TGF-
are not required for SVAS-associated hypertension (85, 139) and that elastin insufficiency likely plays a key role. Modulation of TGF-β availability by emilin-1, an elastic fiber-
associated protein, determines systolic blood pressure in mice, supporting a role for elastic fiber integrity and TGF-β signaling in hypertension (209). TGF-β is an important regulator of ECM remodeling in the arterial wall (57). Inflammatory sig-
naling stimulated by EDPs from elastic fiber degradation may also play a role in hypertension. Reduced amounts of elastin or compromised elastic fiber integrity and the subsequent alterations in ECM composition and arterial geometry lead to in-
creased material and/or structural arterial stiffness. While hyp-
ertension has long been associated with changes in resistance of the small, muscular arteries, it is now appreciated that increased stiffness of the large arteries can contribute to hyper-
The role of large artery stiffness in hypertension
has been reviewed by Greenwald (52), Wagenseil and Mecham
discussed in this review, with a focus on elastic fibers and arterial
mechanics, include hypertension and arterial stiffening, diabetes,
obesity, atherosclerosis, calcification, and aneurysms and dissec-
ions.
Acquired Cardiovascular Diseases
Due to the limited timeframe of elastin synthesis, elastic
fibers that are damaged or degraded with aging or disease are
generally not repaired. Elastic fibers are then replaced by
collagens and proteoglycans that stiffen the arterial wall and
lead to hypertension and damage to downstream organs. The
ratio of elastin to collagen in the human aorta decreases 30–40%
with age from the second to the ninth decade of life (62). In aging
primates, it was recently observed that the elastin-to-collagen ratio
changes with age, but disarray of the remaining elastic and
collagen fibers may be an even more important contributor to
increased arterial stiffness with aging (211). Many acquired dis-
ases represent accelerated or exacerbated versions of the arterial
remodeling observed with aging. The cardiovascular diseases
discussed in this review, with a focus on elastic fibers and arterial
mechanics, include hypertension and arterial stiffening, diabetes,
obesity, atherosclerosis, calcification, and aneurysms and dissec-
ions.
Hypertension and arterial stiffening. Hypertension is fre-
nently observed in conjunction with diseases associated with
defects in elastin or elastic fibers (4). While hypertension is
typically associated with aging, the coincidental occurrence of
hypertension in infants with SVAS indicates that age-related
factors are not required for SVAS-associated hypertension (85,
30–40% ELASTIN. Eln^+/− mice live a normal lifespan but have
hypertension, increased arterial structural stiffness (Fig. 4), and
an increased number of lamellar units in the arterial wall (Fig.
(39, 99). Treatment with the mTOR inhibitor rapamycin
prevents the formation of increased lamellar units in Eln^+/−
mice (101). Eln^+/− arteries show reduced in vivo axial stretch,
residual torsion (197), and increased length (Fig. 6). Increased
arterial structural stiffness precedes increases in blood pressure
compared with WT mice during maturation, indicating that it
may be a causative factor in the development of hypertension
in Eln^+/− mice (94). Eln^+/− mice have indications of diastolic
dysfunction, which may be linked to compromised arterial
Windkessel function and decreased cardiac perfusion during
diastole (95). Eln^+/− mice have impaired glucose metabolism
(28) and altered susceptibility to atherosclerotic plaque accumu-
lation (108, 174). Genetic modifiers of the Eln^+/− cardio-
vascular phenotype have been identified by outcrossing Eln^+/−
mice. Several genes, including Ren1, Ncf1, and Nos1, were
identified that may synergize with elastin insufficiency to
predispose an individual to hypertension and increased arterial
structural stiffness (86). Treatment of Eln^+/− mice with anti-
hypertensive medications improves pulse pressure and sub-
sequently physiologic structural stiffness due to the shift in
operating pressures but does not change material stiffness of
the arteries (56). Treatment of Eln^+/− mice with minoxidil, a
K_{ATP} channel opener and vasodilator, also improves physi-
ologic structural stiffness (84). Eln^+/− mice have reduced sus-
ceptibility to vascular calcification (Fig. 7) (79). Mice with
reduced elastin due to a large genetic deletion that mimics the
heterozygous deletion observed in WBS (100) have hyperten-
sion and increased arterial stiffness but do not have the in-
creased number of lamellar units observed in Eln^+/− mice (49).

30–40% ELASTIN. Eln^-/- mice can be rescued by genetic
insertion of a BAC expressing the human elastin gene (60).
These hBAC-mNull mice have 35% of normal elastin amounts
in the ascending aorta, have extreme hypertension, and have
increased arterial structural and material stiffness (Fig. 4) (60).
Increased arterial structural stiffness has been confirmed in
vivo using ultrasound imaging and may be caused by additional
collagen amounts in the aortic media (Fig. 5) and
increased wall thickness (72). While lesion development in
SVAS is attributed to VSMC overproliferation in the aortic
media, Jiao et al. (72) showed that deficient circumferential
growth that limits outward expansion of the aorta, combined
with normal VSMC proliferation, is a mechanism for luminal
narrowing in hBAC-mNull mice. Inhibition of mTOR with
rapalogs does not prevent luminal narrowing in hBAC-mNull
mice but does prevent the observed collagen fibrosis and
arterial structural stiffening (73). hBAC-mNull mice have a
longer aorta than WT mice (Fig. 6), and the axial growth may
be a compensation mechanism for the deficient circumferential
growth (67).

Fig. 7. Effects of elastin amounts on medial calcification caused by matrix Gla protein (MGP) deficiency. Mgp^-/- mouse arteries show extensive mineral deposition (black) along the elastic laminae (A), which is markedly reduced
(arrows) in Mgp^-/-/Eln^+/− mice (B). Thin plastic sections of the thoracic aortae from 2-wk-old mice were stained with von Kossa and van Gieson (79).
Scale bars = 20 μm.
increased stiffness of the large arteries does not precede hypertension in SHRs. SHRs have reduced elastin amounts, smaller arterial diameter, and increased structural stiffness of the mesenteric resistance arteries compared with WKY rats, suggesting that increased resistance of the distal arteries contributes to hypertension development in SHRs (13). With aging, aortic material stiffness dramatically increases in SHRs compared with WKY rats (110). The increased stiffness has been attributed to endothelial dysfunction (154), increases in advanced glycation end products (6), and decreases in the elastin-to-collagen ratio (20) in the aged SHR compared with WKY aorta. These results imply that the SHR is not an appropriate model to investigate large artery stiffness in the development of hypertension but is an appropriate model to investigate the mechanisms of ECM remodeling and increased arterial stiffness in aging coupled with hypertension.

Genetic factors associated with selective breeding of inbred rat strains are linked to changes in amount and organization of the aortic ECM. Behmoaras et al. (10) found that aortic, blood pressure, elastin, collagen and cytokine amounts, and elastic laminae structure are different among seven inbred rat strains. Of the seven strains, the Brown-Norway rat (BNR) has the lowest aortic elastin amount and lowest elastin-to-collagen ratio, leading to increased structural stiffness of the aorta compared with young LOU control rats (130). BNRs are normotensive, suggesting that they are a good model for investigating interactions between elastin and arterial stiffness that are independent of changes in blood pressure. Treatment of cultured BNR VSMCs with K+ channel openers (minoxidil or diazoxide) increases tropoelastin transcription and mRNA stability. Treatment of BNRs with minoxidil or diazoxide increases aortic elastic fiber content (169). In contrast, treatment of Eln+/− mice with minoxidil increases arterial size and stimulates differential expression of 127 matrix or matrix-associated genes but does not show an elastin specific effect (84). Therapeutic options for increasing elastic fiber content may depend on the primary cause of the elastinopathy.

Diabetes. In diabetes, the body does not produce or respond to insulin appropriately, leading to increased blood glucose levels. In vitro studies have examined the effects of increased glucose on VSMCs, isolated arterial elastin, and arterial mechanical behavior. High-glucose media, with the addition of EDPs and TGF-β1, increase expression of osteogenic genes in cultured VSMCs, and may lead to arterial calcification associated with diabetes (168). Incubation of isolated arterial elastin in high-glucose solution increases the storage and loss modulus, indicating an increase in material stiffness and viscous energy loss (102). After 48 h of incubation in a high-glucose solution, Zou and Zhang (214) found an increase in the material stiffness of isolated arterial elastin in the longitudinal direction but not in the circumferential direction. With incubation times up to 28 days, Wang et al. (199) found an increased material stiffness in both longitudinal and circumferential directions as well as increased hysteresis (or energy loss). The mechanical changes in isolated arterial elastin have been attributed to the dehydrating effects of glycation (102), but it is unclear how these results translate in vivo for an intact arterial wall with ECs acting as an impermeable barrier. Assuming that the glucose can reach the medial layers, increased glucose may lead to glycation of elastic fibers that alters stiffness and protease susceptibility.

In a rat diabetic model, glycation increases over time in arterial samples as diabetes develops but is unchanged in control rats (182). Diabetic rats also have reduced elastin protein levels in aortic extracts as well as an increase in elastolytic activity (91). A decreased elastin-to-collagen mRNA ratio and increased structural stiffness of the aorta are observed in the leptin receptor-deficient mouse model for type 2 diabetes (db/db). Interestingly, the same study (78) found opposite trends in the elastin-to-collagen mRNA ratio and structural stiffness for the db/db coronary artery. Human patients with diabetes show a similar decrease in elastin-to-collagen protein ratios in aortic tissues (179) and an increase in circulating CatS levels (103). There are increased EDP levels in children with diabetes (122), indicating that degradation of elastic fibers may occur in diabetes that contributes to arterial stiffening and inflammatory signaling. EDPs may also contribute to insulin resistance and the onset of diabetes with aging (12). These results suggest that high glucose associated with diabetes may physically stiffen elastic fibers as well as make them more susceptible to degradation, altering passive arterial mechanics and initiating a chemical signaling cascade through EDPs.

Obesity. Obesity is a risk factor for increased cardiovascular-related mortality, but it is typically associated with additional cardiovascular risk factors including hypertension and diabetes. Rider et al. (144) observed an increase in aortic PWV in obese patients absent other cardiovascular risk factors, providing evidence for a direct correlation between obesity and aortic stiffness. Elastin is decreased in adipose tissue of obese patients (170); however, investigations on elastin and elastic fiber changes in large arteries from obese patients have yet to be performed. TGF-β1 levels positively correlate with body mass index in humans (184) and are upregulated in adipose tissue from obese humans (2) and mice (157). Cathepsin elastases, CatK, CatL, and CatS have increased activity with increased body mass index in humans and in the leptin-deficient (ob/ob) mouse model of obesity (92). In ob/ob mice, aortic stiffening was observed by an increase in PWV, an increase in abdominal aorta structural stiffness in vitro, and an increase in thoracic aorta material stiffness as measured by atomic force microscopy. Elastic fiber remodeling in the ob/ob aorta was evidenced by a decrease in LOX protein levels and activity, a decrease in desmosine cross-links, an increase in elastase activity, and a higher degree of fragmentation in the elastic lamellae (21). The observed increase in aortic stiffness with obesity may arise from elastic fiber remodeling through LOX downregulation and an upregulation of elastolytic activity.

Atherosclerosis. Both the synthesis of tropoelastin and degradation of elastic fibers are increased in human atherosclerotic lesions (1). This may be due to the production of tropoelastin (88), metalloproteinases (121), and cathepsins (92) by macrophages involved in atherosclerosis. Elastic fiber degradation allows infiltration of lipids and immune cells into the aortic wall for plaque formation and rupture in mice (188). EDPs resulting from elastic fiber fragmentation further enhance atherogenesis (47). TGF-β1 activity, which may be modulated by elastic fiber degradation, has a wide range of effects in atherosclerosis (181). Elastin insufficiency and the associated increase in arterial structural stiffness do not increase atherosclerotic plaque deposition in mice (108, 174). Together, these
results suggest that elastic fiber degradation and the resulting circulating peptides are key mechanisms linking elastin to atherosclerosis progression.

Calcification. Elastic fiber degradation has been linked to ectopic calcium phosphate mineral deposition (calcification) in the arterial walls, particularly in the medial layer. As demonstrated by Basalyga et al. (8), periadventitial treatment of surgically exposed rat aortas with calcium chloride leads to ectopic mineral deposition along the elastic laminae. Calcified aortas of treated rats show the presence of degraded ECM proteins, including elastin. Later, Khavandgar et al. (79) showed that medial calcification in mice lacking matrix Gla protein (MGP), a potent inhibitor of soft tissue calcification, is elastin dependent. The amount of deposited minerals in Mgp−/− arteries scales with elastin amounts in the cardiovascular tree, with mineral deposition and elastin amounts decreasing from the thoracic to the abdominal aorta. Khavandgar et al. (79) further showed that Mgp−/−Eln+/− mice have markedly reduced arterial mineral deposition compared with Mgp−/− mice (Fig. 7). This genetic experiment provides evidence that elastin content, and not just elastic fiber degradation, is a critical determinant of arterial medial calcification.

Currently, the mechanism of mineral accumulation by elastin and how this process is inhibited by MGP are not well understood. Also, the role of elastin in intimal calcification associated with atherosclerotic plaques is still unknown. Future mechanistic work focusing on these aspects of elastin biology may lead to new therapeutic approaches to treat vascular calcification, for which no treatment is currently available.

Aneurysms and dissections. Degraded and fragmented elastic fibers in the arterial wall are a common histopathology in thoracic and abdominal aortic aneurysms (18, 114). It appears that fragmented elastic fibers or improper elastic fiber deposition is critical to aneurysm formation rather than reduced elastin amounts. ADC1L, characterized by improper elastic fiber deposition, is generally associated with aneurysms, whereas SVAS, characterized by elastin insufficiency, is generally not associated with aneurysms. Additionally, genetic mutations that affect proteins necessary for elastic fiber assembly, such as FBLN4 (66) and LOX (53), cause aneurysms. Loss-of-function mutations in genes that affect TGF-β1 sequestration in the ECM, such as fibrillin-1, are associated with aberrant TGF-β1 activity and aortic aneurysms (31). Fragmented, degraded, or improperly assembled elastic fibers may be more susceptible to protease digestion, allowing aneurysmal dilation over time and stimulating cytokine and inflammatory signaling. Homogenates of abdominal aortic aneurysms show high elastinolytic activity (18). Degraded or improperly assembled elastic fibers allow inflammatory cell infiltration and affect TGF-β1 sequestration, and the released EDPs can enhance macrophage activation (24) and TGF-β1 activity (54).

Researchers have capitalized on the relationship among elastic fiber degradation, inflammatory cell activation, and chemokine signaling to generate a variety of chemically stimulated models of abdominal aortic aneurysms and dissections in animals (3, 25, 106). The models include intraluminal or periadventitial application of elastase, periadventitial incubation with calcium chloride, and subcutaneous infusion of angiotensin II. Several of the models have been extended to create thoracic aortic aneurysms and dissections (69, 74). It is important to note that many of the chemically induced animal models demonstrate aortic dilation and aneurysm formation but do not progress to aortic rupture or dissection (161). Models that progress to rupture and dissection will be critical for translating the results of animal studies to treatment of human disease.

Tissue engineering

Increasing elastin amounts in genetic elastopathies or repairing fragmented elastic fibers in acquired cardiovascular diseases are challenging problems. Tissue engineering, however, encounters the most challenging problem, recreating functional elastic fibers de novo. Elastogenesis has been called the “holy grail” of arterial tissue engineering, as the lack of mature elastic fibers in tissue-engineered constructs results in inferior mechanical properties compared with native arteries (135). Long and Tranquillo (104) showed that the type of scaffold and cells chosen affects elastogenesis in tissue-engineered arteries, with increased elastin amounts secreted from neonatal compared with adult cells and on fibrin compared with collagen scaffolds. They also used chemokines known to increase steady-state elastin mRNA expression, but the resulting elastic fiber structure in their constructs does not resemble arterial elastic laminae. Trying to replicate in vivo loading conditions, Kim et al. (81) showed that long-term application of circumferential cyclic strain increases tropoelastin mRNA and protein expression along with increasing the material stiffness and ultimate tensile strength of tissue-engineered arteries, although the mechanical properties are still inferior to native aorta. Huang et al. (65) recently showed that application of biaxial cyclic strain, more representative of the in vivo loading environment, produces tissue-engineered arteries with evidence of mature elastic fibers and improved mechanical properties compared with circumferentially strained constructs or constructs grown without cyclic strain. A tissue-engineered graft implanted as a pulmonary artery replacement in 8-wk-old lambs exposed to cytokines and mechanical loading present in early maturation and growth showed abundant elastic fiber deposition and ~50% of the insoluble elastin levels of the native pulmonary artery when evaluated 42 wk after implantation (177). Overall, these results demonstrate the importance of reproducing the biochemical and biomechanical environment of developing arteries to encourage elastogenesis in tissue engineering.

Summary and future directions

We have summarized genetic and acquired cardiovascular diseases linked to elastin and elastic fiber defects with a focus on large artery mechanics. Elastin and elastic fibers play a critical mechanical role at multiple length scales from pulse wave reflections in the cardiovascular system, to local material properties of the arterial wall, to interactions of tropoelastin with VSMCs. Elastin is a unique protein due to its limited time period of synthesis, long half-life, multistep assembly process, and high degree of cross-linking that provides mechanical elasticity to the large arteries. Acquired damage to the elastic fibers is especially problematic due to the inability of the organism to generate de novo elastic fibers postadolescence. Despite the increasing therapeutic options for controlling diseases such as hypertension, obesity, and diabetes, elastic fiber damage is currently irreparable, meaning that the cardiovascu-
lar risks associated with arterial stiffness may persist even when other disease aspects are controlled. The process of elastic fiber assembly, especially inducing assembly in vitro or in vivo, is an active and important area of investigation for treating elastinopathies and the associated changes in cardiovascular mechanics, as well as advancing tissue engineering. We would like to highlight the differential effects of reduced elastin amounts compared with improper assembly or degradation of elastic fibers. Reduced elastin amounts lead to altered arterial wall structure, narrowed arterial lumen, increased arterial structural stiffness, and hypertension in humans and mice. In mice, the structural, mechanical, and functional phenotypes scale with elastin amounts. For the most part, mice appear to adapt reasonably well to 50–60% elastin, with more severe phenotypes appearing when elastin amounts drop to 30–40% of normal. The results of the mouse studies suggest that a threshold amount of elastin may provide near-normal outcomes. Hence, therapies that moderately increase elastin amounts, rather than completely restoring them to normal levels, may be sufficient for improved cardiovascular outcomes.

Improperly assembled or degraded elastic fibers alter the passive mechanical properties of the arterial wall. The consequences extend far beyond physical changes to the elastic fibers to the initiation of several arterial remodeling events including VSMC phenotype modulation, ECM deposition, inflammatory cell infiltration, and the release of EDPs. EDPs stimulate cytokine signaling and activate inflammatory cells. The inflammatory cells secrete proteases that contribute to additional elastic fiber degradation in a positive feedback cycle. It is unclear whether the arterial remodeling signaling cascade is similar for genetic diseases with improperly assembled elastic fibers, such as ADCL1, and acquired diseases with elastic fiber degradation, such as diabetes or abdominal aortic aneurysms. However, elastic fiber fragmentation, inflammatory signaling, and arterial stiffening are common factors in many of the cardiovascular diseases discussed in this review. Better understanding of the biochemical and biomechanical signaling associated with improperly assembled or degraded elastic fibers may allow therapeutic interventions that halt or slow down the arterial remodeling cascade and prevent disease progression.

Grants
This work was partially supported by National Heart, Lung, and Blood Institute Grants HL-115560 and HL-105314.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00087.2018 • www.ajpheart.org

Downloaded from www.physiology.org/journal/ajpheart by [individualUser.givenNames] [individualUser.surname] (128.252.020.193) on September 19, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.


