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Killing me un-softly: Causes and mechanisms of arterial stiffness Recent Highlights of ATVB: Early Career Committee Contribution

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Abstract

The aorta is a blood vessel that provides a low resistance path for blood flow directed from the heart to peripheral organs and tissues. However, the aorta has another central hemodynamic function whereby the elastic nature of the aortic wall provides a significant biomechanical buffering capacity complementing the pulsatile cardiac blood flow and this is often referred to as *Windkessel* function. Stiffening of the arterial wall leads to fundamental alterations in central hemodynamics with widespread detrimental implications for organ function. In this review article, we aim to provide a short general overview of some of the most common mechanisms that contribute to increased arterial stiffness, the consequences of arterial stiffening, and the clinical conditions in which arterial stiffness occurs with a focus on recent advancements in the field.

Keywords

arterial stiffness; pathophysiology; wall stress; aging; aortic compliance

Introduction

The aorta, as the major conduit artery of the human body, is primarily thought of as a blood vessel that provides a low resistance path for blood flow directed from the heart to peripheral organs and tissues. However, the aorta has another central hemodynamic function whereby the elastic nature of the aortic wall provides a significant biomechanical buffering capacity complementing the pulsatile cardiac blood flow and this is often referred to as *Windkessel* function. In systole, the compliant aortic wall stretches to accommodate the bolus of blood ejected by the left ventricle, thereby dampening an increase in systolic arterial pressure. In

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diastole, the aortic wall's elastic recoil enables continued aortic blood flow and limits the diastolic drop in arterial pressure.

As a consequence, stiffening of the arterial wall – i.e. the loss of *Windkessel* properties – leads to fundamental alterations in central hemodynamics with widespread detrimental implications for organ function. In this Highlights article we aim provide a short general overview of clinical conditions, mechanisms and effects of arterial stiffening, with a particular focus on recent advancements in the field (Figure 1).

Clinical Significance of Arterial Stiffness

A major functional manifestation of arterial stiffening is a progressive incapacity to dampen the cyclic arterial pressure changes generated by pulsatile cardiac contractions. Arterial stiffness ultimately leads to increased systolic arterial pressure (SAP), as well decreased diastolic arterial pressure (DAP), both of which contribute to an increase in pulse pressure ($PP = SAP - DAP$). Thus, isolated systolic hypertension, the most common form of hypertension among the elderly, is typically due to age-associated increases in aortic stiffness that result in excess morbidity and mortality.¹⁻³

Elevated SAP due to aortic stiffening increases left ventricular afterload^{4, 56} and is associated with left ventricular hypertrophy.⁷ Additionally, aortic stiffness reduces diastolic blood pressure and leads to impaired coronary perfusion.^{8, 9} Hence the coronary perfusion to myocardial demand equilibrium is unbalanced. Clinical studies have demonstrated that aortic stiffness is a strong risk factor and contributor to incident heart failure (HF), including both HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF).^{10, 11} Moreover, increased aortic stiffness may also contribute to severe exercise intolerance in older patients with isolated HFpEF.¹²

Increased, or undamped, pulsatile forces also extend to the vulnerable microcirculation of unprotected organs with low vascular resistance, such as the brain and kidneys.¹³ As such, increased arterial stiffness is associated with cerebral small vessel disease¹⁴ and impaired cognitive function in the elderly,¹⁵⁻¹⁷ as well as in young to middle-aged adults.¹⁸ Just recently, an analysis of the prospective, population-based Age, Gene/Environment Susceptibility (AGES)-Reykjavik study by Din et al. demonstrated that increased carotid arterial stiffness in patients aged >65 years of age is an independent risk factor for incident cerebral micro-bleeds, which frequently occur in older populations and are associated with an increased risk of recurrent stroke, cognitive impairment and dementia, in the deep or infratentorial brain regions.¹⁹ Additionally, arterial stiffness was found to be an independent predictor of stroke, in addition to coronary heart disease, in apparently healthy subjects.²⁰ Unstable atherosclerotic plaques located in the carotid artery are a significant source of cerebrovascular embolism. Interestingly, a recent study by Selwaness and coworkers found that increased aortic stiffness is linked not only to a higher prevalence of carotid atherosclerosis, but also to increased intraplaque hemorrhage as a marker of plaque instability.²¹ With respect to renal function, increased arterial stiffness has been shown to accelerate renal impairment in preexisting chronic kidney disease²² and, in addition, is

associated with incident albuminuria and worsening of glomerular filtration in type 2 diabetes (T2D) patients.²³

Finally, arterial stiffening may not only augment pulsatile hemodynamic stress leading to end organ injury, but may also directly promote arterial pathologies. While it remains controversial as to whether increased arterial stiffness precedes or is a consequence of pathologies such as atherosclerosis,²⁴ a recent study demonstrated that segmental aortic stiffening, and the resultant aortic stiffness gradients, may trigger aortic remodeling leading to abdominal aortic aneurysm (AAA) formation.²⁵ Complementing these findings, research by Zhang et al. conducted in non-human primates indicates that the abdominal segment of aged aortae exhibits the highest regional stiffness²⁶ – thus making the abdominal aorta a predilection site for aneurysm formation. Furthermore, aortic stiffness is elevated in various connective tissue diseases, including Marfan syndrome, that predispose patients to progressive aortic dilatation, potentially resulting in fatal aortic dissection and death.²⁷ In those patients, higher aortic stiffness is associated with higher rates of aortic dilatation and an increased requirement for surgical intervention.²⁷ Mechanistically, recent data published by Crosas-Molist et al. indicate that in Marfan syndrome chronic TGF- β signaling is associated with increased expression of vascular smooth muscle cell (VSMC) contractile markers and actin stress fiber formation, as well as collagen I secretion, ultimately leading to increased VSMC and extracellular matrix (ECM) stiffness.²⁸ On the other hand, active VSMC contraction may serve as a crucial protective mechanism against aortic dissection, as recently suggested by Ferruzzi and colleagues following elegant *ex vivo* experiments in dissection prone murine aortas.²⁹

In the light of these extensive patho-mechanistic implications, it is not surprising that arterial stiffness has emerged as a strong independent predictor of cardiovascular events and all-cause mortality.³⁰ Thus, sufficiently monitoring and effectively targeting arterial stiffening holds great promise to universally address a wide range of cardiovascular complications that lead to increased morbidity and mortality.

Physiological Arterial Compliance and Assessment of Arterial Stiffness

To better understand what influences arterial stiffness, one must understand the physiologic parameters that contribute to arterial compliance. Arteries are conduits comprised of 3 key layers: the intima, media, and adventitia. These layers function together to sense and respond to acute changes in blood pressure via dilation or constriction and respond to chronic changes by undergoing growth and remodeling. The intima is the innermost layer of an artery and is primarily made up of a single layer of endothelial cells on the luminal side of the vessel. The endothelium functions to present an anti-thrombotic surface to flowing blood.³¹ The media consists of concentric layers of elastin, which form the elastic lamellae, fibrillar collagen and VSMCs and it's this layer that provides blood vessels with most of their compliance and the ability to contract and dilate. The outermost layer of the artery is the adventitia and it's primarily comprised of fibroblasts and collagen and functions to give the vessel support and a surface with which to tether onto other tissues. In the case of larger vessels, such as the aorta, the vessel layers also include a network of small blood vessels, called *vasa vasorum*, that help supply oxygen and nutrients to the larger vessel wall.

Between each of the three layers in humans is an elastic lamina, comprised of a fenestrated tube of elastin fibers, that further adds to the vessel's compliance and separates the different layers of the vessel.³² Numerous cell types in the vessel wall, including endothelial cells, vascular smooth muscle cells, and fibroblasts, sense mechanical changes and respond by producing vasoactive molecules, ECM, and ECM-degrading proteases. Several mechanisms that contribute to increases in arterial stiffening and the pathophysiologic conditions linked to increased arterial stiffness are discussed further below.

In vivo, arteries are under constant multiaxial mechanical loading where pulsatile blood pressure distends the vessel and induces a cyclic circumferential stress, whereas blood flow through the vessel lumen induces shear stress along the endothelial cell layer of the vessel wall. Vessels in vivo also exist under an axial load, as demonstrated by their retraction upon excision and removal.³³ Vessels remodel in response to changes in loading conditions. Besides changing geometric parameters such as the inner vessel diameter or vessel wall thickness, vascular remodeling can occur through artery calcification, increased intima-media thickness, and impaired flow-mediated dilation.³⁴ This vascular wall remodeling alters the stiffness or compliance of the vessel and these changes occur to restore mean and local stresses. For example, Matsumoto and Hayashi showed that the different layers of blood vessels thicken to different degrees in response to supra-physiologic loading with the inner layers increasing the most due to the highest stress in that layer.³⁵

In the clinical setting, arterial stiffness can be assessed by numerous non-invasive modalities and there are a number of excellent reviews that focus on this specific topic.³⁶⁻³⁸ Regional quantification of carotid-femoral pulse wave velocity (PWV) is considered the clinical gold standard measurement of arterial stiffness and is used by most studies in the field.³⁹ PWV represents the speed of a pressure wave propagating down a blood vessel and is directly correlated with the vessel's elastic modulus. Increased PWV due to arterial stiffening is the basis for further indices of aortic stiffness, such as augmentation index (AIx).³⁹ Arterial pulse waves traveling from the heart to the periphery are typically reflected at sites of impedance mismatch, such as peripheral branch points or small arterioles. When arteries are compliant, PWV is slow and reflected waves return in diastole, augmenting central DAP. In contrast, when arteries are stiffer and PWV is higher, reflected waves arrive earlier and augment central SAP. This augmentation of central SAP can be visualized through central pulse contour analysis as the pressure difference between the first and the second (augmented) systolic pressure peak. Of note, a recent study by Schultz et al. found that central blood pressure waveform (and therefore AIx) is mainly influenced by aortic compliance (reservoir function) rather than timing of pulse wave reflections⁴⁰.

Stiffening processes may not uniformly affect the arterial tree⁴¹ and segmental arterial stiffening may be critical for arterial pathologies, such as AAA formation;⁴² therefore, local assessment of arterial stiffness is desirable. This can be achieved by quantification of the fractional change in arterial diameter produced by cyclic systolic-diastolic pressure change. Arterial wall displacement is usually achieved via ultrasound devices. Additionally, aortic PWV may be measured locally using magnetic resonance (MR) imaging, allowing for spatially differentiated evaluation of stiffening.⁴¹

Given the popularity of mouse models to study various pathologies of cardiovascular disease (CVD), including arterial stiffness, reliable methods to quantify arterial biomechanics are essential. As such, the aforementioned *in vivo* metrics of arterial stiffness, such as PWV (either determined through Doppler measurements,^{43, 44} applanation tonometry,⁴⁵ or MRI⁴⁶) or local arterial compliance and distensibility^{25, 47, 48} have been established for use in murine studies. Those methods are indispensable for longitudinal *in vivo* studies measuring arterial stiffness but only allow indirect assessment of biomechanical properties. In contrast, *ex vivo* tests allow for comprehensive biomechanical testing employing a variety of loading protocols. At the macroscale, the mechanical stress – strain relationship may be obtained via tensile testing employing simple uniaxial strip or ring tests or more complex planar biaxial tests. However, planar tensile testing does not employ physiologically relevant mechanical loading conditions for arterial vessels. In this regard, pressure myography systems may be better suited to obtain vascular pressure-diameter or force-length relations as a direct measure of vascular circumferential or axial stiffness.^{47, 49} For dissection of vascular mechanical properties at the cellular or even sub-cellular level, established methods such as atomic force microscopy (AFM),²⁸ nanoindentation, or micropipette aspiration are available. While these have proven to be a powerful tools to assess individual cell stiffness, it remains to be determined if changes in cell stiffness *in vitro* directly correlate with changes in vessel stiffness *in vivo*.

Underlying Mechanisms of Arterial Stiffening

Mechanical factors, such as hemodynamic forces, and humoral factors, including hormones like angiotensin II (Ang II), salt and glucose, all function to influence vessel remodeling. The changes that ultimately result in artery stiffening can occur in ways that are both common and different in common diseases, such as hypertension, diabetes mellitus, and aging. Herein we highlight the most common mechanisms that contribute to changes in arterial stiffness with a focus on mechanistic insights recently identified by studies published in ATVB.

Changes in Extracellular Matrix

The vessel wall is comprised of ECM proteins including collagen, elastin, glycoproteins and proteoglycans. Collagen and elastin function to provide structural integrity and elasticity. ECM stability is assured by the intra- and intermolecular covalent cross-linking of elastin and collagen, initiated by lysyl oxidase (LOX), a copper-dependent amine oxidase. Conversely, matrix metalloproteases (MMPs), through their proteolytic effects, function to degrade the ECM by creating uncoiled collagen and broken/frayed elastin. Therefore, an appropriate balance of LOX and MMP activity is necessary to maintain vascular compliance. Indeed the importance of collagen/elastin ratios and collagen and elastin disarray was recently demonstrated in monkeys. The study by Zhang, et al. published in ATVB clearly demonstrated that aortic stiffness increases with age; however, the most severe increases in aortic stiffness were observed in the abdominal aorta, where values in young monkeys equaled or exceeded values of thoracic aortic stiffness in old monkeys.²⁶ Altogether, these results suggest that regional differences exist between the abdominal and thoracic regions of the aorta that ultimately differentially impact arterial stiffness.

LOX deficiency in mice is associated with aortic aneurysm, tortuosity, and rupture⁵⁰, suggesting that LOX activity is essential to maintaining the elastic features of blood vessels. While obesity and aortic stiffness have been linked, clear mechanistic insight was lacking. Recent studies demonstrated that ob/ob and db/db mice exhibit higher aortic PWVs and lower aortic compliance⁵¹⁻⁵³ and that Zucker fatty rats have greater stiffness.⁵⁴ Mechanistically, a recent ATVB study demonstrated that obesity results in aortic stiffening in both humans and mice and that this is, in part, mediated through LOX downregulation, leading to elastin fragmentation and a significant increase in pulse wave velocity.⁵⁵

Vascular cells, as well as inflammatory cells, produce ECM proteins, as well as the various MMPs capable of degrading collagen and elastin. Further degradation of the basement membrane ECM occurs through the activation of MMP-2 and MMP-9, which have gelatinase activity.⁵⁶ Increases in MMP enzymatic activity is regulated by increases in gene expression, activation by cleavage of pro-MMP protein, through MMP–MMP interactions, and by plasmin, thrombin, and reactive oxygen species (ROS).^{57, 58} Tissue inhibitors of MMPs, or TIMPs, counter this response and are critical for balancing the vessel remodeling process.⁵⁶ Furthermore, the deposition of proteoglycans also contributes to thickening of the vessel wall ECM and, thus, vessel wall stiffness. Using a proteomics approach, a group recently examined the protein extracts of well-defined, homogenous, non-atherosclerotic left mammary artery samples from 10 patients with high PWV and 9 with low PWV by quantitative proteome analysis. Interestingly, the study demonstrated that changes in the amounts of small leucine-rich proteoglycans, known to be involved in collagen fibrillogenesis, and of some non-fibrillar collagens, in addition to alterations in proteins related to human arterial smooth muscle, are associated with increased arterial stiffness.⁵⁹

Data from patients previously demonstrated that mutations of the gene (TGFB2) that codes the TGF- β type II receptor predispose patients to thoracic aortic aneurysms and dissections^{60, 61} and that these mutations also lead to decreased expression of contractile proteins in medial SMCs in response to TGF- β .⁶² Jay Humphrey's group published a study in ATVB earlier this year demonstrating that postnatal disruption of TGF- β type II receptor *Tgfb2* in SMCs compromises both active (contractile) and passive (structural) biaxial biomechanical properties in the murine thoracic aorta.²⁹ Furthermore, they found that daily in vivo treatment with rapamycin largely preserves or restores biaxial contractile properties, but not passive structural properties.

Vascular Calcification and VSMC Senescence

Arterial calcification is associated with CVD events and mortality, independent of vessel type, and early studies noted that older patients typically exhibit both greater arterial stiffness and arterial calcification, suggesting an association. Arterial stiffening is known to involve ECM changes, such as those described in the previous section, and it is thought that these changes may be exacerbated by increases in arterial calcification.⁶³ However, recent evidence suggests that vascular remodeling in the presence of increased stiffness may contribute to medial and intimal calcification.⁶⁴ A recent study in individuals without prevalent CVD showed in a multivariable-adjusted model, both higher carotid-femoral pulse wave velocity and central pulse pressure are associated with greater thoracic aorta

calcification and abdominal aorta calcification, whereas higher augmentation index was associated with abdominal aortic calcification.⁶⁵

Circulating hormones like Ang II influence vessel remodeling and modulate vascular stiffness. Ang II, for example, stimulates collagen formation, triggers matrix remodeling and vascular hypertrophy, depresses nitric oxide (NO)-dependent signaling, increases reactive oxygen species production, and reduces elastin synthesis.⁶⁶ Numerous studies focused on understanding the molecular mechanisms by which Ang II affects aging have shown that Ang II accelerates VSMC senescence in vitro and in vivo,^{67, 68} suggesting a detrimental pro-senescent role of Ang II in vascular aging. Furthermore, it has been shown previously that Sirtuin (SIRT) 1, a nicotinamide adenine dinucleotide dependent deacetylase, inhibits Ang II-induced cell hypertrophy and senescence.^{69, 70} $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) is a subtype of nAChR and is reported to be involved in hypertension end-organ damage. A study by Li, et al. tested the role of $\alpha 7$ nAChR in Ang II-induced senescence of VSMCs and found that activation of $\alpha 7$ nAChR alleviates Ang II-induced VSMC senescence through promoting NAD⁺-SIRT1 pathway, suggesting that $\alpha 7$ nAChR may be a potential therapeutic target for the treatment of Ang II-associated vascular aging disorders.⁷¹ Interestingly, another ATVB publication by Gardner, et al. suggested that senescent VSMCs may be active participants in CVD processes and assume a pro-inflammatory state and secrete factors that promote chemotaxis of mononuclear cells in vitro and in vivo and that release active MMP-9, secrete less collagen, and prime endothelial cells and VSMCs to a pro-inflammatory state.⁷² And while this was established in a model of atherosclerosis, whether this VSMC phenotype plays a direct role in arterial stiffness remains to be investigated.

Increased Oxidative Stress

In addition alterations in ECM composition and organization, arterial stiffness is strongly affected by VSMC tone and endothelial cell signaling. Endothelial cell-derived NO is a key modulator of VSMC tone, which is also modified by mechano-stimulation, in part, because of cell stretch and changes in calcium signaling. The bioavailability of NO can be reduced by increased production of ROS caused by increases stress and hormones, such as Ang II. Indeed it has been well described that Ang II stimulation of VSMCs leads to increased ROS (superoxide) production via NADPH oxidase enzymes and that this increased superoxide can, in turn, react with NO to form peroxynitrite and other highly reactive species that promote abnormal vascular tone. For more comprehensive reviews on NADPH oxidase derived ROS and their effects on vascular signaling, see the following references:⁷³⁻⁷⁵. The melanocortin 1 receptor (MC1-R) is expressed by vascular endothelial cells and has been shown to enhance NO bioavailability and vasodilator function upon pharmacological stimulation. Interestingly, a recent study demonstrated that deficiency in MC1-R signaling is associated with increased arterial stiffness and impairment in endothelium-dependent vasodilatation, suggesting a physiological role for MC1-R in the regulation of arterial tone. In addition to increases in ROS in response to humoral factors, mechanotransduction can also lead to increases in ROS production and this has been reviewed previously (see ⁷⁶⁻⁷⁸). Furthermore, changes in blood flow from laminar to more turbulent flow has also been shown to increase ROS production^{79, 80} Interestingly, a recent study published in ATVB

showed in patients with flow reversal during diastole (present in a 1/3 of study participants) that flow reversal in peripheral arteries is accompanied by vascular dysfunction and aortic stiffening;⁸¹ however, if this is due to increased ROS production remains to be investigated.

In addition, recent studies suggest that mitochondrial dysfunction plays an important role in aging and impairing vascular function.^{82, 83} A comprehensive study by Zhou, et al.⁴⁴ presented data to support that prolonged exposure to increased mitochondrial oxidative stress decreases aortic compliance and induces cardiac dysfunction. Indeed they provided evidence that superoxide dismutase (SOD) 2 deficiency over a lifetime is sufficient to induce aortic stiffening, decrease aortic compliance, and cause cardiac dysfunction. Additionally, they showed that aortic stiffening with aging in SOD2^{+/-} mice is associated with structural changes in the aortic wall in vivo, with increased collagen content and ruptures in elastin laminae. Moreover, they find that SOD2 deficiency also increases collagen I expression, decreases elastin expression, and increases MMP-2 expression and activity in aged SMCs. Furthermore, they demonstrated that SOD2 deficiency over a lifetime increases SMC apoptosis in aged mice and sensitizes SMCs to staurosporine-induced increases in cleaved caspase-3 and cleaved PARP levels.⁴⁴ This prolonged SOD2 deficiency impairs cell survival, increases inflammatory signaling responses and increases aortic stiffness with aging.⁴⁴

Conditions Linked to Arterial Stiffness

Aging

Epidemiological studies clearly indicate that age is the dominant risk factor for CVD.⁸⁴ One critical mechanism linking age to increased cardiovascular risk may be age-related stiffening of conduit arteries such as the aorta. In fact, a reduction of elastic properties (stiffening) is the main manifestation of arterial aging in conduit arteries and, vice versa, aging is the main factor leading to arterial stiffening.⁸⁵⁻⁸⁷ Thus, the concept that vascular (biological) age is better related to prognosis than chronological age is rapidly evolving. Therefore, understanding the mechanisms how chronological aging interferes with arterial elasticity offers the exciting opportunity to uncouple chronological from vascular aging. Arterial aging comes along with a wide spectrum of vascular alterations, including ECM remodeling and calcification, VSMC senescence and apoptosis, as well as inflammation and oxidative stress (see above). Those phenomena synergistically result in “age-related medial degeneration and sclerosis”,⁸⁸ the substrate for age-related arterial stiffening.

As conduit arteries age, there is an increase in vascular wall stiffness mainly due to alterations of the ECM structure leading to an imbalance between collagen and elastin.^{86, 89, 90} Due to thinning and fracture of the elastic laminae mechanical load is transferred to collagen fibers, which are 100–1,000 times stiffer than elastic fibers.⁹¹ Elastin fragmentation may result from age-related material fatigue and fracture⁹² as well as increased MMP-mediated proteolysis. Indeed, increased expression of MMP-2 is evident in the medial layer of aged rodents^{93, 94} and is localized to sites of fragmented elastin.⁹⁵ Moreover, high serum MMP levels (MMP-2 and MMP-9) were associated with increased arterial stiffness, as measured by PWV, in healthy individuals and patients with isolated systolic hypertension.⁹⁶ Notably, aortic stiffness and elastase activity are influenced by

MMP-9 gene polymorphisms.⁹⁷ Furthermore, there is an increase in arterial collagen synthesis and deposition with age. Collagen isoforms found in the aorta are mainly (80–90%) of type I and III, with some type IV⁹⁸⁻¹⁰⁰ and their concentration gradually increases after the age of 50.^{99, 101}

The arterial wall may also stiffen due to calcification of the elastic lamellae, termed medial elastocalcinosis (MEC).¹⁰² The presence of calcium deposits in the media of large arteries increases significantly with age.^{103, 104} Further, in animal models of MEC there is a strong correlation between aortic calcium content and arterial stiffness.^{105, 106} Interestingly, medial calcification is associated with local expression of mineralization-regulating proteins that are normally expressed in osteogenesis.¹⁰⁷ This observation gave rise to the now widely accepted concept that vascular calcification is an active cell-driven process characterized by osteogenic differentiation of vascular cells. Indeed, VSMCs may acquire an osteogenic phenotype, expressing bone/mineralization-associated proteins (e.g., Runx2, Sox9, Msx2)^{108, 109} that actively regulate arterial calcification.¹¹⁰⁻¹¹² Additionally, recent data indicate that the osteogenic transcription factor Runx2, independently of its known role as a regulator of vascular calcification, may induce arterial fibrosis and stiffness.⁴⁹

Another age-related alteration to arteries is the development of chronic, low-grade inflammation in both rodents¹¹³⁻¹¹⁵ and humans (i.e., “inflammaging”).^{101, 116, 117} There is ample evidence that vascular and systemic inflammation is associated with (or precedes) conduit arterial stiffening.¹¹⁸⁻¹²³ Mechanisms of inflammatory vascular stiffening include the induction of MMPs, calcification and fibrosis by inflammatory stimuli and cells.^{49, 56, 107, 110, 124, 125} Additionally, aged vessels exhibit chronic oxidative stress due to increased ROS production (e.g., via leakiness of the mitochondrial respiratory chain or increased NADPH oxidase activity) as well as defective anti-oxidant mechanisms (e.g., age-dependent downregulation of SOD2).¹²⁶⁻¹²⁹ Mechanistically, oxidative stress may increase vascular collagen production^{44, 126} and augmented medial elastin fragmentation, partly due to increased expression of MMP-2 and MMP-9.^{44, 130, 131}

In addition to chronological aging, preterm birth also represents a risk factor for cardiovascular disorders and mortality in adult life. In this respect, a recent ATVB study by Odri Komazec et al. demonstrated significantly increased aortic stiffness in five- to seven-year-old children born at a gestational age <32 weeks, possibly due to inadequate elastin synthesis.¹³² Thus, one could argue that those children are already born with significantly progressed arterial biological age.

Metabolic Disease

Accelerated arterial stiffening – or rapid arterial biological aging – is a major complication of diabetes mellitus.^{133, 134} Importantly, arterial stiffness predicts the development of CVD and mortality in the patients with T2D.¹³⁵ Similar to age-related arterial stiffening the process of diabetes-accelerated arterial stiffening includes enhanced levels of oxidative stress¹³⁶ eliciting pro-fibrotic mechanisms as well as MMP-mediated elastin fragmentation.^{49, 137} Additionally, medial arterial calcification is frequently found in diabetic patients¹⁰⁷ and MMP-induced elastin degradation may promote calcification.¹³⁸ Underlining the significance of MMPs for diabetic arterial remodeling, a recent study

published in ATVB by Goncalves et al. found plasmatic levels of MMP-12 (macrophage elastase) to be elevated in T2D patients and positively correlated with arterial stiffness – hence indicating a potential of MMP-12 as a biomarker and possibly as a therapeutic target of diabetic arterial stiffness.¹³⁹

As another increasingly prevalent and clinically relevant metabolic disorder, childhood obesity promotes immediate cardiovascular damage, well in advance of adulthood.¹⁴⁰ Investigating the role of arterial biomechanics as a potential mechanistic link, a recent meta-analysis of 15 studies by Cote et al. revealed that child/adolescent obesity is associated with greater arterial stiffness.¹⁴¹ Moreover, according to a study conducted by Rider et al. in obese children and adults an elevated hepatic fat content (as assessed by ¹H- magnetic resonance spectroscopy) correlated to increased arterial stiffness, partly via increasing serum triglyceride levels (suggesting a liver fat – triglycerides – arterial stiffness pathway).¹⁴² Additionally, a cross-sectional study of 2284 Framingham Heart Study participants revealed that nonalcoholic fatty liver disease without overt CVD exhibit a broad spectrum of vascular dysfunction, including increased arterial stiffness, as recently reported by Long et al.¹⁴³ Pointing towards therapeutic opportunities, a meta-analysis of 3 randomized controlled trials by Petersen et al. (involving 1259 participants) recently indicated that modest weight loss (mean 8% of total body weight), achieved with diet and lifestyle changes seems to improve arterial stiffness.¹⁴⁴

Vitamin D deficiency and hyperparathyroidism are associated with increased cardiovascular risk. Through interference with the renin-angiotensin-system, modulation of VSMC proliferation, calcification as well as vascular wall inflammation a deregulated Vitamin D/ parathormone (PTH) system may affect arterial stiffness as a mechanism to increase cardiovascular risk.¹⁴⁵ However, a longitudinal analysis of 2580 Multi-Ethnic Study of Atherosclerosis (MESA) participants by Gepner et al. revealed that neither baseline PTH nor vitamin D concentrations were associated with changes in arterial stiffening during nearly a decade of follow-up. Yet, the study demonstrated a cross-sectional association between arterial stiffness and high PTH.¹⁴⁵

Pseudoxanthoma elasticum is an inherited metabolic disorder resulting from mutations in the ATP binding cassette subfamily C member 6 (*ABCC6*) gene. The vascular phenotype is characterized by progressive calcification and fragmentation of elastic fibers. However, until recently the consequences for arterial function remained obscure. In a model of *Abcc6*^{-/-} mice, Kauffenstein and coworkers demonstrated increased expression of osteogenic and chondrogenic differentiation markers accompany increased arterial stiffness, as well as enhanced myogenic tone in resistance arteries.⁴⁷

Sleep Disturbances

While adequate sleep is critical for cardiovascular health, epidemiological studies have demonstrated an increased risk for CVD for both short and long sleep duration.¹⁴⁶ Moreover, aberrations to circadian rhythm meet with pathological consequences. For example, shift work significantly elevates the incidence of CVD.¹⁴⁷ In these conditions, arterial stiffness again may, in part, confer the deleterious cardiovascular effects. Indeed it was shown by Kim et al. that extreme (short and long) sleep duration, as well as poor sleep

quality, is associated with increased arterial stiffness in young and apparently healthy participants.¹⁴⁸ Mechanistically, in a study by Anea et al., arterial stiffening in cardiac clock-mutant mice was linked to increased vascular expression of MMP-2 and MMP-9.⁴³

Smoking

Smoking, a major reversible cardiovascular risk factor, has been linked to increased arterial stiffness¹⁴⁹. However, while demonstrating an association between smoking status and inflammatory biomarkers as well as subclinical atherosclerosis, a recent analysis of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort (that enrolled 6814 participants without evident CVD) by McEvoy et al. did not find a consistent association between smoking and local carotid and aortic distensibility¹⁵⁰. This finding is somewhat surprising and interpretations are rather speculative. For instance, smoking may differentially affect specific arterial segments. As such, studies using carotid-femoral PWV as the clinical gold standard to assess arterial stiffness on a regional scale might be helpful to further evaluate this discrepancy.

Poor Cardiovascular Health

The assessment of cardiovascular health (CVH) as proposed by the American Heart Association CVH score enables an integrated approach to evaluate the combinatorial effects of smoking, physical activity, body mass index, diet, blood glucose, total cholesterol and blood pressure on cardiovascular endpoints. As such, epidemiological data suggest that the CVH score is inversely associated with the incidence of CVD. A recent study conducted by Gaye and colleagues now sheds light on potential mechanisms that may promote the protective or adverse effects of ideal or poor CVH on CVD. Analyzing data from 9155 participants, an observational community-based study ideal CVH was associated with substantially less arterial stiffness and thickness. Strikingly, the difference in carotid arterial stiffness between ideal and poor CVH corresponds on average to 15 years difference in chronological age – thus identifying poor CVH a dramatic accelerator for arterial biological aging.¹⁵¹

Conclusions

Arterial stiffening is a feature of physiological vascular aging that is accelerated in variety of pathological conditions with increased cardiovascular risk, such as T2D. Of note, arterial stiffness by itself is a strong risk factor for a broad spectrum of cardiovascular diseases, including arterial hypertension, heart failure, myocardial infarction and stroke. Moreover, arterial stiffening appears to precede the onset of overt end-organ disease. Thus, increased arterial stiffness may represent a critical causal link between cardiovascular risk and eventual disease and might therefore qualify as a universal target for therapeutic intervention.

In order to test this intriguing hypothesis it is essential to demonstrate that reducing arterial stiffness is effective in reducing cardiovascular morbidity and mortality. As a first step, a variety of prognostically relevant cardiovascular drugs, such as angiotensin-converting enzyme inhibitors, Ang II receptor blockers, β -receptor blockers, aldosterone antagonists or

statins were shown to reduce arterial stiffness.¹⁵² However, using those agents it is difficult to discern whether modulation of arterial stiffness is of any additional benefit unrelated to the drugs' primary effects (e.g., anti-hypertensive or cholesterol lowering). Moreover, many antihypertensive interventions will lower the arterial distending pressure and unload the arterial wall mechanically - therefore functionally reducing arterial stiffness to some extent. However, to achieve a more effective impact on arterial stiffness beyond mere blood pressure control it may be important to therapeutically address the intrinsic biomechanical properties of the arterial wall. To this end, the increasing recognition of epigenetic regulators (such as microRNAs) in the vascular system may yield a variety of novel therapeutic targets to counteract structural arterial remodeling that underlies arterial stiffening.¹⁵³

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References

1. Safar M, Levy B, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation*. 2003; 107:2864–9. [PubMed: 12796414]
2. Safar M, Nilsson P. Pulsatile hemodynamics and cardiovascular risk factors in very old patients: background, sex aspects and implications. *J Hypertens*. 2013; 31:848–57. [PubMed: 23449020]
3. Franklin SS, Gustin Wt, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation*. 1997; 96:308–15. [PubMed: 9236450]
4. Nichols WW, O'Rourke MF, Avolio AP, Yaginuma T, Murgu JP, Pepine CJ, Conti CR. Effects of age on ventricular-vascular coupling. *Am J Cardiol*. 1985; 55:1179–84. [PubMed: 3984897]
5. O'Rourke MF. Diastolic heart failure, diastolic left ventricular dysfunction and exercise intolerance. *J Am Coll Cardiol*. 2001; 38:803–5. [PubMed: 11527637]
6. Strait JB, Lakatta EG. Aging-associated cardiovascular changes and their relationship to heart failure. *Heart Fail Clin*. 2012; 8:143–64. [PubMed: 22108734]
7. Toprak A, Reddy J, Chen W, Srinivasan S, Berenson G. Relation of pulse pressure and arterial stiffness to concentric left ventricular hypertrophy in young men (from the Bogalusa Heart Study). *Am J Cardiol*. 2009; 103:978–84. [PubMed: 19327426]
8. Watanabe H, Ohtsuka S, Kakihana M, Sugishita Y. Coronary circulation in dogs with an experimental decrease in aortic compliance. *J Am Coll Cardiol*. 1993; 21:1497–506. [PubMed: 8473662]
9. Ikonomidis I, Lekakis J, Papadopoulos C, Triantafyllidi H, Paraskevaidis I, Georgoula G, Tzortzis S, Revela I, Kremastinos DT. Incremental value of pulse wave velocity in the determination of coronary microcirculatory dysfunction in never-treated patients with essential hypertension. *Am J Hypertens*. 2008; 21:806–13. [PubMed: 18497732]
10. Chirinos JA, Kips JG, Jacobs DR Jr, Brumback L, Duprez DA, Kronmal R, Bluemke DA, Townsend RR, Vermeersch S, Segers P. Arterial wave reflections and incident cardiovascular events and heart failure: MESA (Multiethnic Study of Atherosclerosis). *J Am Coll Cardiol*. 2012; 60:2170–7. [PubMed: 23103044]
11. Tsao CW, Lyass A, Larson MG, Levy D, Hamburg NM, Vita JA, Benjamin EJ, Mitchell GF, Vasan RS. Relation of Central Arterial Stiffness to Incident Heart Failure in the Community. *J Am Heart Assoc*. 2015:4.

12. Hundley WG, Kitzman DW, Morgan TM, Hamilton CA, Darty SN, Stewart KP, Herrington DM, Link KM, Little WC. Cardiac cycle-dependent changes in aortic area and distensibility are reduced in older patients with isolated diastolic heart failure and correlate with exercise intolerance. *J Am Coll Cardiol*. 2001; 38:796–802. [PubMed: 11527636]
13. O'Rourke MF, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension*. 2005; 46:200–4. [PubMed: 15911742]
14. Poels MM, Zaccai K, Verwoert GC, Vernooij MW, Hofman A, van der Lugt A, Witteman JC, Breteler MM, Mattace-Raso FU, Ikram MA. Arterial stiffness and cerebral small vessel disease: the Rotterdam Scan Study. *Stroke*. 2012; 43:2637–42. [PubMed: 22879099]
15. Scuteri A, Brancati AM, Gianni W, Assisi A, Volpe M. Arterial stiffness is an independent risk factor for cognitive impairment in the elderly: a pilot study. *J Hypertens*. 2005; 23:1211–6. [PubMed: 15894897]
16. Waldstein SR, Rice SC, Thayer JF, Najjar SS, Scuteri A, Zonderman AB. Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. *Hypertension*. 2008; 51:99–104. [PubMed: 18025297]
17. Fukuhara M, Matsumura K, Ansai T, Takata Y, Sonoki K, Akifusa S, Wakisaka M, Hamasaki T, Fujisawa K, Yoshida A, Fujii K, Iida M, Takehara T. Prediction of cognitive function by arterial stiffness in the very elderly. *Circ J*. 2006; 70:756–61. [PubMed: 16723799]
18. Pase MP, Himali JJ, Mitchell GF, Beiser A, Maillard P, Tsao C, Larson MG, DeCarli C, Vasan RS, Seshadri S. Association of Aortic Stiffness With Cognition and Brain Aging in Young and Middle-Aged Adults: The Framingham Third Generation Cohort Study. *Hypertension*. 2016; 67:513–9. [PubMed: 26754644]
19. Ding J, Mitchell GF, Bots ML, Sigurdsson S, Harris TB, Garcia M, Eiriksdottir G, van Buchem MA, Gudnason V, Launer LJ. Carotid arterial stiffness and risk of incident cerebral microbleeds in older people: the Age, Gene/Environment Susceptibility (AGES)-Reykjavik study. *Arterioscler Thromb Vasc Biol*. 2015; 35:1889–95. [PubMed: 26112009]
20. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation*. 2006; 113:657–63. [PubMed: 16461838]
21. Selwaness M, van den Bouwhuijsen Q, Mattace-Raso FU, Verwoert GC, Hofman A, Franco OH, Witteman JC, van der Lugt A, Vernooij MW, Wentzel JJ. Arterial stiffness is associated with carotid intraplaque hemorrhage in the general population: the Rotterdam study. *Arterioscler Thromb Vasc Biol*. 2014; 34:927–32. [PubMed: 24482373]
22. Ford ML, Tomlinson LA, Chapman TP, Rajkumar C, Holt SG. Aortic stiffness is independently associated with rate of renal function decline in chronic kidney disease stages 3 and 4. *Hypertension*. 2010; 55:1110–5. [PubMed: 20212269]
23. Bouchi R, Babazono T, Mugishima M, Yoshida N, Nyumura I, Toya K, Hanai K, Tanaka N, Ishii A, Uchigata Y, Iwamoto Y. Arterial stiffness is associated with incident albuminuria and decreased glomerular filtration rate in type 2 diabetic patients. *Diabetes Care*. 2011; 34:2570–5. [PubMed: 21972413]
24. Hansen L, Taylor WR. Is increased arterial stiffness a cause or consequence of atherosclerosis? *Atherosclerosis*. 2016; 249:226–7. [PubMed: 27133480]
25. Raaz U, Zollner AM, Schellinger IN, Toh R, Nakagami F, Brandt M, Emrich FC, Kayama Y, Eken S, Adam M, Maegdefessel L, Hertel T, Deng A, Jagger A, Buerke M, Dalman RL, Spin JM, Kuhl E, Tsao PS. Segmental aortic stiffening contributes to experimental abdominal aortic aneurysm development. *Circulation*. 2015; 131:1783–95. [PubMed: 25904646]
26. Zhang J, Zhao X, Vatner DE, McNulty T, Bishop S, Sun Z, Shen YT, Chen L, Meining GA, Vatner SF. Extracellular Matrix Disarray as a Mechanism for Greater Abdominal Versus Thoracic Aortic Stiffness With Aging in Primates. *Arterioscler Thromb Vasc Biol*. 2016; 36:700–6. [PubMed: 26891739]
27. Prakash A, Adlakha H, Rabideau N, Hass CJ, Morris SA, Geva T, Gauvreau K, Singh MN, Lacro RV. Segmental Aortic Stiffness in Children and Young Adults With Connective Tissue Disorders: Relationships With Age, Aortic Size, Rate of Dilation, and Surgical Root Replacement. *Circulation*. 2015; 132:595–602. [PubMed: 26115544]

28. Crosas-Molist E, Meirelles T, Lopez-Luque J, Serra-Peinado C, Selva J, Caja L, Gorbenko Del Blanco D, Uriarte JJ, Bertran E, Mendizabal Y, Hernandez V, Garcia-Calero C, Busnadiago O, Condom E, Toral D, Castilla M, Forteza A, Navajas D, Sarri E, Rodriguez-Pascual F, Dietz HC, Fabregat I, Egea G. Vascular smooth muscle cell phenotypic changes in patients with Marfan syndrome. *Arterioscler Thromb Vasc Biol.* 2015; 35:960–72. [PubMed: 25593132]
29. Ferruzzi J, Murtada SI, Li G, Jiao Y, Uman S, Ting MY, Tellides G, Humphrey JD. Pharmacologically Improved Contractility Protects Against Aortic Dissection in Mice With Disrupted Transforming Growth Factor-beta Signaling Despite Compromised Extracellular Matrix Properties. *Arterioscler Thromb Vasc Biol.* 2016; 36:919–27. [PubMed: 26988590]
30. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol.* 2010; 55:1318–27. [PubMed: 20338492]
31. Schwartz SM, deBlois D, O'Brien ER. The intima. Soil for atherosclerosis and restenosis. *Circ Res.* 1995; 77:445–65. [PubMed: 7641318]
32. Holzapfel GA, Weizsacker HW. Biomechanical behavior of the arterial wall and its numerical characterization. *Comput Biol Med.* 1998; 28:377–92. [PubMed: 9805198]
33. Boron WF, BE. International. 2nd. Saunders/Elsevier; Philadelphia, PA: 2009. Medical physiology : a cellular and molecular approach; p. 2009
34. J. H. Cardiovascular solid mechanics : cells, tissues, and organs. Springer; New York: 2002.
35. Matsumoto T, Hayashi K. Stress and strain distribution in hypertensive and normotensive rat aorta considering residual strain. *J Biomech Eng.* 1996; 118:62–73. [PubMed: 8833076]
36. Oliver JJ, Webb DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol.* 2003; 23:554–66. [PubMed: 12615661]
37. O'Rourke M, Staessen J, Vlachopoulos C, Duprez D, Plante G. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens.* 2002; 15:426–44. [PubMed: 12022246]
38. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J.* 2006; 27:2588–605. [PubMed: 17000623]
39. Cecelja M, Chowienzyk P. Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis.* 2012;1.
40. Schultz MG, Davies JE, Hardikar A, Pitt S, Moraldo M, Dhutia N, Hughes AD, Sharman JE. Aortic reservoir pressure corresponds to cyclic changes in aortic volume: physiological validation in humans. *Arterioscler Thromb Vasc Biol.* 2014; 34:1597–603. [PubMed: 24812322]
41. Hickson S, Butlin M, Graves M, Taviani V, Avolio A, McEniery C, Wilkinson I. The relationship of age with regional aortic stiffness and diameter. *JACC Cardiovasc Imaging.* 2010; 3:1247–55. [PubMed: 21163453]
42. Chowienzyk PJ. Aortic Stiffness and Disease: Location is Key. *Circulation.* 2015; 131:1745–7. [PubMed: 25904647]
43. Anea CB, Ali MI, Osmond JM, Sullivan JC, Stepp DW, Merloiu AM, Rudic RD. Matrix metalloproteinase 2 and 9 dysfunction underlie vascular stiffness in circadian clock mutant mice. *Arterioscler Thromb Vasc Biol.* 2010; 30:2535–43. [PubMed: 20829506]
44. Zhou RH, Vendrov AE, Tchivilev I, Niu XL, Molnar KC, Rojas M, Carter JD, Tong H, Stouffer GA, Madamanchi NR, Runge MS. Mitochondrial oxidative stress in aortic stiffening with age: the role of smooth muscle cell function. *Arterioscler Thromb Vasc Biol.* 2012; 32:745–55. [PubMed: 22199367]
45. Leloup AJ, Fransen P, Van Hove CE, Demolder M, De Keulenaer GW, Schrijvers DM. Applanation tonometry in mice: a novel noninvasive technique to assess pulse wave velocity and arterial stiffness. *Hypertension.* 2014; 64:195–200. [PubMed: 24752435]
46. Parczyk M, Herold V, Klug G, Bauer WR, Rommel E, Jakob PM. Regional in vivo transit time measurements of aortic pulse wave velocity in mice with high-field CMR at 17.6 Tesla. *J Cardiovasc Magn Reson.* 2010; 12:72. [PubMed: 21134260]

47. Kauffenstein G, Pizard A, Le Corre Y, Vessieres E, Grimaud L, Toutain B, Labat C, Mauras Y, Gorgels TG, Bergen AA, Le Saux O, Lacolley P, Leftheriotis G, Henrion D, Martin L. Disseminated arterial calcification and enhanced myogenic response are associated with *abcc6* deficiency in a mouse model of pseudoxanthoma elasticum. *Arterioscler Thromb Vasc Biol.* 2014; 34:1045–56. [PubMed: 24675664]
48. Kuo MM, Barodka V, Abraham TP, Steppan J, Shoukas AA, Butlin M, Avolio A, Berkowitz DE, Santhanam L. Measuring Ascending Aortic Stiffness In Vivo in Mice Using Ultrasound. *Journal of Visualized Experiments : JoVE.* 2014:52200.
49. Raaz U, Schellinger IN, Chernogubova E, Warnecke C, Kayama Y, Penov K, Hennigs JK, Salomons F, Eken S, Emrich FC, Zheng WH, Adam M, Jagger A, Nakagami F, Toh R, Toyama K, Deng A, Buerke M, Maegdefessel L, Hasenfuss G, Spin JM, Tsao PS. Transcription Factor *Runx2* Promotes Aortic Fibrosis and Stiffness in Type 2 Diabetes Mellitus. *Circ Res.* 2015; 117:513–24. [PubMed: 26208651]
50. Maki JM, Rasanen J, Tikkanen H, Sormunen R, Makikallio K, Kivirikko KI, Soininen R. Inactivation of the lysyl oxidase gene *Lox* leads to aortic aneurysms, cardiovascular dysfunction, and perinatal death in mice. *Circulation.* 2002; 106:2503–9. [PubMed: 12417550]
51. Katz PS, Trask AJ, Souza-Smith FM, Hutchinson KR, Galantowicz ML, Lord KC, Stewart JA Jr, Cismowski MJ, Varner KJ, Lucchesi PA. Coronary arterioles in type 2 diabetic (db/db) mice undergo a distinct pattern of remodeling associated with decreased vessel stiffness. *Basic Res Cardiol.* 2011; 106:1123–34. [PubMed: 21744279]
52. Sikka G, Yang R, Reid S, Benjo A, Koitabashi N, Camara A, Baraban E, O'Donnell CP, Berkowitz DE, Barouch LA. Leptin is essential in maintaining normal vascular compliance independent of body weight. *Int J Obes (Lond).* 2010; 34:203–6. [PubMed: 19806156]
53. Yang R, Sikka G, Larson J, Watts VL, Niu X, Ellis CL, Miller KL, Camara A, Reinke C, Savransky V, Polotsky VY, O'Donnell CP, Berkowitz DE, Barouch LA. Restoring leptin signaling reduces hyperlipidemia and improves vascular stiffness induced by chronic intermittent hypoxia. *Am J Physiol Heart Circ Physiol.* 2011; 300:H1467–76. [PubMed: 21278136]
54. Sista AK, O'Connell MK, Hinohara T, Oommen SS, Fenster BE, Glassford AJ, Schwartz EA, Taylor CA, Reaven GM, Tsao PS. Increased aortic stiffness in the insulin-resistant Zucker fa/fa rat. *Am J Physiol Heart Circ Physiol.* 2005; 289:H845–51. [PubMed: 15833807]
55. Chen JY, Tsai PJ, Tai HC, Tsai RL, Chang YT, Wang MC, Chiou YW, Yeh ML, Tang MJ, Lam CF, Shiesh SC, Li YH, Tsai WC, Chou CH, Lin LJ, Wu HL, Tsai YS. Increased aortic stiffness and attenuated lysyl oxidase activity in obesity. *Arterioscler Thromb Vasc Biol.* 2013; 33:839–46. [PubMed: 23413430]
56. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res.* 2002; 90:251–62. [PubMed: 11861412]
57. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003; 92:827–39. [PubMed: 12730128]
58. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. *J Clin Invest.* 1996; 98:2572–9. [PubMed: 8958220]
59. Lyck Hansen M, Beck HC, Irmukhamedov A, Jensen PS, Olsen MH, Rasmussen LM. Proteome analysis of human arterial tissue discloses associations between the vascular content of small leucine-rich repeat proteoglycans and pulse wave velocity. *Arterioscler Thromb Vasc Biol.* 2015; 35:1896–903. [PubMed: 26069235]
60. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, Meyers J, Leitch CC, Katsanis N, Sharifi N, Xu FL, Myers LA, Spevak PJ, Cameron DE, De Backer J, Hellemans J, Chen Y, Davis EC, Webb CL, Kress W, Coucke P, Rifkin DB, De Paepe AM, Dietz HC. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in *TGFBR1* or *TGFBR2*. *Nat Genet.* 2005; 37:275–81. [PubMed: 15731757]
61. Pannu H, Fadulu VT, Chang J, Lafont A, Hasham SN, Sparks E, Giampietro PF, Zaleski C, Estrera AL, Safi HJ, Shete S, Willing MC, Raman CS, Milewicz DM. Mutations in transforming growth factor-beta receptor type II cause familial thoracic aortic aneurysms and dissections. *Circulation.* 2005; 112:513–20. [PubMed: 16027248]

62. Inamoto S, Kwartler CS, Lafont AL, Liang YY, Fadulu VT, Duraisamy S, Willing M, Estrera A, Safi H, Hannibal MC, Carey J, Wiktorowicz J, Tan FK, Feng XH, Pannu H, Milewicz DM. TGFBR2 mutations alter smooth muscle cell phenotype and predispose to thoracic aortic aneurysms and dissections. *Cardiovasc Res.* 2010; 88:520–9. [PubMed: 20628007]
63. Dao HH, Essalihi R, Bouvet C, Moreau P. Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension. *Cardiovasc Res.* 2005; 66:307–17. [PubMed: 15820199]
64. Rattazzi M, Bertacco E, Puato M, Faggin E, Pauletto P. Hypertension and vascular calcification: a vicious cycle? *J Hypertens.* 2012; 30:1885–93. [PubMed: 22871891]
65. Tsao CW, Pencina KM, Massaro JM, Benjamin EJ, Levy D, Vasani RS, Hoffmann U, O'Donnell CJ, Mitchell GF. Cross-sectional relations of arterial stiffness, pressure pulsatility, wave reflection, and arterial calcification. *Arterioscler Thromb Vasc Biol.* 2014; 34:2495–500. [PubMed: 25169933]
66. Dzau VJ. Significance of the vascular renin-angiotensin pathway. *Hypertension.* 1986; 8:553–9. [PubMed: 3013773]
67. Herbert KE, Mistry Y, Hastings R, Poolman T, Niklason L, Williams B. Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. *Circ Res.* 2008; 102:201–8. [PubMed: 17991883]
68. Kunieda T, Minamoto T, Nishi J, Tateno K, Oyama T, Katsuno T, Miyauchi H, Orimo M, Okada S, Takamura M, Nagai T, Kaneko S, Komuro I. Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. *Circulation.* 2006; 114:953–60. [PubMed: 16908765]
69. Kim MY, Kang ES, Ham SA, Hwang JS, Yoo TS, Lee H, Paek KS, Park C, Lee HT, Kim JH, Han CW, Seo HG. The PPARdelta-mediated inhibition of angiotensin II-induced premature senescence in human endothelial cells is SIRT1-dependent. *Biochem Pharmacol.* 2012; 84:1627–34. [PubMed: 23000914]
70. Xiong S, Salazar G, Patrushev N, Ma M, Forouzanfar F, Hilenski L, Alexander RW. Peroxisome proliferator-activated receptor gamma coactivator-1alpha is a central negative regulator of vascular senescence. *Arterioscler Thromb Vasc Biol.* 2013; 33:988–98. [PubMed: 23430617]
71. Li DJ, Huang F, Ni M, Fu H, Zhang LS, Shen FM. alpha7 Nicotinic Acetylcholine Receptor Relieves Angiotensin II-Induced Senescence in Vascular Smooth Muscle Cells by Raising Nicotinamide Adenine Dinucleotide-Dependent SIRT1 Activity. *Arterioscler Thromb Vasc Biol.* 2016; 36:1566–76. [PubMed: 27339462]
72. Gardner SE, Humphry M, Bennett MR, Clarke MC. Senescent Vascular Smooth Muscle Cells Drive Inflammation Through an Interleukin-1alpha-Dependent Senescence-Associated Secretory Phenotype. *Arterioscler Thromb Vasc Biol.* 2015; 35:1963–74. [PubMed: 26139463]
73. Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. *Circ Res.* 2015; 116:531–49. [PubMed: 25634975]
74. Lyle AN, Griendling KK. Modulation of vascular smooth muscle signaling by reactive oxygen species. *Physiology (Bethesda).* 2006; 21:269–80. [PubMed: 16868316]
75. Lassegue B, Griendling KK. Reactive oxygen species in hypertension; An update. *Am J Hypertens.* 2004; 17:852–60. [PubMed: 15363831]
76. Chatterjee S, Fisher AB. Mechanotransduction in the endothelium: role of membrane proteins and reactive oxygen species in sensing, transduction, and transmission of the signal with altered blood flow. *Antioxid Redox Signal.* 2014; 20:899–913. [PubMed: 24328670]
77. Hsieh HJ, Liu CA, Huang B, Tseng AH, Wang DL. Shear-induced endothelial mechanotransduction: the interplay between reactive oxygen species (ROS) and nitric oxide (NO) and the pathophysiological implications. *J Biomed Sci.* 2014; 21:3. [PubMed: 24410814]
78. Raaz U, Toh R, Maegdefessel L, Adam M, Nakagami F, Emrich FC, Spin JM, Tsao PS. Hemodynamic regulation of reactive oxygen species: implications for vascular diseases. *Antioxid Redox Signal.* 2014; 20:914–28. [PubMed: 23879326]

79. Willett NJ, Kundu K, Knight SF, Dikalov S, Murthy N, Taylor WR. Redox signaling in an in vivo murine model of low magnitude oscillatory wall shear stress. *Antioxid Redox Signal*. 2011; 15:1369–78. [PubMed: 20712414]
80. Willett NJ, Long RC Jr, Maiellaro-Rafferty K, Sutliff RL, Shafer R, Oshinski JN, Giddens DP, Guldberg RE, Taylor WR. An in vivo murine model of low-magnitude oscillatory wall shear stress to address the molecular mechanisms of mechanotransduction--brief report. *Arterioscler Thromb Vasc Biol*. 2010; 30:2099–102. [PubMed: 20705917]
81. Breton-Romero R, Wang N, Palmisano J, Larson MG, Vasani RS, Mitchell GF, Benjamin EJ, Vita JA, Hamburg NM. Cross-Sectional Associations of Flow Reversal, Vascular Function, and Arterial Stiffness in the Framingham Heart Study. *Arterioscler Thromb Vasc Biol*. 2016; 36:2452–2459. [PubMed: 27789476]
82. Edgar D, Shabalina I, Camara Y, Wredenberg A, Calvaruso MA, Nijtmans L, Nedergaard J, Cannon B, Larsson NG, Trifunovic A. Random point mutations with major effects on protein-coding genes are the driving force behind premature aging in mtDNA mutator mice. *Cell Metab*. 2009; 10:131–8. [PubMed: 19656491]
83. Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. *Circ Res*. 2008; 102:488–96. [PubMed: 18096818]
84. Nichols M, TN., Luengo-Fernandez, R., Leal, J., Gray, A., Scarborough, P., Rayner, M., European Cardiovascular Disease Statistics 2012. European Heart Network. European Society of Cardiology, Sophia Antipolis; Brussels: 2012.
85. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. 2006; 27:2588–605. [PubMed: 17000623]
86. Greenwald S. Ageing of the conduit arteries. *J Pathol*. 2007; 211:157–72. [PubMed: 17200940]
87. O'Rourke M. Arterial aging: pathophysiological principles. *Vasc Med*. 2007; 12:329–41. [PubMed: 18048471]
88. Sawabe M. Vascular aging: from molecular mechanism to clinical significance. *Geriatrics & gerontology international*. 2010; 10(Suppl 1):S213–20. [PubMed: 20590836]
89. Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation*. 1983; 68:50–8. [PubMed: 6851054]
90. Asmar R, Benetos A, London G, Hugue C, Weiss Y, Topouchian J, Laloux B, Safar M. Aortic distensibility in normotensive, untreated and treated hypertensive patients. *Blood Press*. 1995; 4:48–54. [PubMed: 7735497]
91. Wagenseil J, Mecham R. Elastin in large artery stiffness and hypertension. *J Cardiovasc Transl Res*. 2012; 5:264–73. [PubMed: 22290157]
92. O'Rourke M, Hashimoto J. Mechanical factors in arterial aging: a clinical perspective. *Journal of the American College of Cardiology*. 2007; 50:1–13. [PubMed: 17601538]
93. Fleenor BS, Marshall KD, Durrant JR, Lesniewski LA, Seals DR. Arterial stiffening with ageing is associated with transforming growth factor-beta1-related changes in adventitial collagen: reversal by aerobic exercise. *The Journal of physiology*. 2010; 588:3971–82. [PubMed: 20807791]
94. Wang M, Zhang J, Spinetti G, Jiang LQ, Monticone R, Zhao D, Cheng L, Krawczyk M, Talan M, Pintus G, Lakatta EG. Angiotensin II activates matrix metalloproteinase type II and mimics age-associated carotid arterial remodeling in young rats. *The American journal of pathology*. 2005; 167:1429–42. [PubMed: 16251426]
95. Li Z, Froehlich J, Galis ZS, Lakatta EG. Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats. *Hypertension*. 1999; 33:116–23. [PubMed: 9931091]
96. Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR, Wilkinson IB. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol*. 2005; 25:372. [PubMed: 15556929]

97. Yasmin, McEniery CM, O'Shaughnessy KM, Harnett P, Arshad A, Wallace S, Maki-Petaja K, McDonnell B, Ashby MJ, Brown J, Cockcroft JR, Wilkinson IB. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. *Arterioscler Thromb Vasc Biol.* 2006; 26:1799–805. [PubMed: 16709939]
98. Jacob MP. Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie.* 2003; 57:195–202. [PubMed: 12888254]
99. Cattell MA, Anderson JC, Hasleton PS. Age-related changes in amounts and concentrations of collagen and elastin in normotensive human thoracic aorta. *Clinica chimica acta; international journal of clinical chemistry.* 1996; 245:73–84. [PubMed: 8646817]
100. Wagenseil J, Mecham R. Vascular extracellular matrix and arterial mechanics. *Physiol Rev.* 2009; 89:957–89. [PubMed: 19584318]
101. Wang M, Zhang J, Jiang LQ, Spinetti G, Pintus G, Monticone R, Kolodgie FD, Virmani R, Lakatta EG. Proinflammatory profile within the grossly normal aged human aortic wall. *Hypertension.* 2007; 50:219–27. [PubMed: 17452499]
102. Dao H, Essalihi R, Bouvet C, Moreau P. Evolution and modulation of age-related medial elastocalcinosi: impact on large artery stiffness and isolated systolic hypertension. *Cardiovasc Res.* 2005; 66:307–17. [PubMed: 15820199]
103. Elliott RJ, McGrath LT. Calcification of the human thoracic aorta during aging. *Calcified tissue international.* 1994; 54:268–73. [PubMed: 8062142]
104. Blumenthal HT, Lansing AI, Wheeler PA. Calcification of the Media of the Human Aorta and Its Relation to Intimal Arteriosclerosis, Ageing and Disease. *The American journal of pathology.* 1944; 20:665–87. [PubMed: 19970771]
105. Essalihi R, Dao HH, Yamaguchi N, Moreau P. A new model of isolated systolic hypertension induced by chronic warfarin and vitamin K1 treatment. *American journal of hypertension.* 2003; 16:103–10. [PubMed: 12559675]
106. Niederhoffer N, Lartaud-Idjouadiene I, Giummelly P, Duvivier C, Peslin R, Atkinson J. Calcification of medial elastic fibers and aortic elasticity. *Hypertension.* 1997; 29:999–1006. [PubMed: 9095090]
107. Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, Zeller T, Hilaire C, Shanahan C. Medial vascular calcification revisited: review and perspectives. *Eur Heart J.* 2014; 35:1515–25. [PubMed: 24740885]
108. Tyson KL, Reynolds JL, McNair R, Zhang Q, Weissberg PL, Shanahan CM. Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. *Arterioscler Thromb Vasc Biol.* 2003; 23:489–94. [PubMed: 12615658]
109. Speer MY, Yang HY, Brabb T, Leaf E, Look A, Lin WL, Frutkin A, Dichek D, Giachelli CM. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. *Circ Res.* 2009; 104:733–41. [PubMed: 19197075]
110. Sun Y, Byon C, Yuan K, Chen J, Mao X, Heath J, Javed A, Zhang K, Anderson P, Chen Y. Smooth muscle cell-specific runx2 deficiency inhibits vascular calcification. *Circ Res.* 2012; 111:543–52. [PubMed: 22773442]
111. Shao J, Sierra O, Cohen R, Mecham R, Kovacs A, Wang J, Distelhorst K, Behrmann A, Halstead L, Towler D. Vascular calcification and aortic fibrosis: a bifunctional role for osteopontin in diabetic arteriosclerosis. *Arterioscler Thromb Vasc Biol.* 2011; 31:1821–33. [PubMed: 21597007]
112. Bostrom K, Jumabay M, Matveyenko A, Nicholas S, Yao Y. Activation of vascular bone morphogenetic protein signaling in diabetes mellitus. *Circ Res.* 2011; 108:446–57. [PubMed: 21193740]
113. Zou Y, Yoon S, Jung KJ, Kim CH, Son TG, Kim MS, Kim YJ, Lee J, Yu BP, Chung HY. Upregulation of aortic adhesion molecules during aging. *The journals of gerontology Series A, Biological sciences and medical sciences.* 2006; 61:232–44.
114. Csizsar A, Labinskyy N, Smith K, Rivera A, Orosz Z, Ungvari Z. Vasculoprotective effects of anti-tumor necrosis factor-alpha treatment in aging. *The American journal of pathology.* 2007; 170:388–98. [PubMed: 17200210]

115. Lesniewski LA, Durrant JR, Connell ML, Henson GD, Black AD, Donato AJ, Seals DR. Aerobic exercise reverses arterial inflammation with aging in mice. *American journal of physiology Heart and circulatory physiology*. 2011; 301:H1025–32. [PubMed: 21622824]
116. Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE, Seals DR. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. *Circ Res*. 2007; 100:1659–66. [PubMed: 17478731]
117. Donato AJ, Black AD, Jablonski KL, Gano LB, Seals DR. Aging is associated with greater nuclear NF kappa B, reduced I kappa B alpha, and increased expression of proinflammatory cytokines in vascular endothelial cells of healthy humans. *Aging cell*. 2008; 7:805–12. [PubMed: 18782346]
118. Booth AD, Wallace S, McEniery CM, Yasmin, Brown J, Jayne DR, Wilkinson IB. Inflammation and arterial stiffness in systemic vasculitis: a model of vascular inflammation. *Arthritis and rheumatism*. 2004; 50:581–8. [PubMed: 14872502]
119. Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, Toutouza M, Stefanadis C. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation*. 2005; 112:2193–200. [PubMed: 16186422]
120. Roman MJ, Devereux RB, Schwartz JE, Lockshin MD, Paget SA, Davis A, Crow MK, Sammaritano L, Levine DM, Shankar BA, Moeller E, Salmon JE. Arterial stiffness in chronic inflammatory diseases. *Hypertension*. 2005; 46:194–9. [PubMed: 15911740]
121. Amar J, Ruidavets JB, Peyrieux JC, Mallion JM, Ferrieres J, Safar ME, Chamontin B. C-reactive protein elevation predicts pulse pressure reduction in hypertensive subjects. *Hypertension*. 2005; 46:151–5. [PubMed: 15956115]
122. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension*. 2005; 46:1118–22. [PubMed: 16216991]
123. Maki-Petaja KM, Hall FC, Booth AD, Wallace SM, Yasmin, Bearcroft PW, Harish S, Furlong A, McEniery CM, Brown J, Wilkinson IB. Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. *Circulation*. 2006; 114:1185–92. [PubMed: 16952987]
124. Quiding-Jarbrink M, Smith DA, Bancroft GJ. Production of matrix metalloproteinases in response to mycobacterial infection. *Infection and immunity*. 2001; 69:5661–70. [PubMed: 11500442]
125. McEniery CM, Wilkinson IB. Large artery stiffness and inflammation. *Journal of human hypertension*. 2005; 19:507–9. [PubMed: 15647777]
126. Fleenor BS, Seals DR, Zigler ML, Sindler AL. Superoxide-lowering therapy with TEMPOL reverses arterial dysfunction with aging in mice. *Aging cell*. 2012; 11:269–76. [PubMed: 22168264]
127. Sindler AL, Fleenor BS, Calvert JW, Marshall KD, Zigler ML, Lefer DJ, Seals DR. Nitrite supplementation reverses vascular endothelial dysfunction and large elastic artery stiffness with aging. *Aging cell*. 2011; 10:429–37. [PubMed: 21276184]
128. Li M, Chiu JF, Mossman BT, Fukagawa NK. Down-regulation of manganese-superoxide dismutase through phosphorylation of FOXO3a by Akt in explanted vascular smooth muscle cells from old rats. *The Journal of biological chemistry*. 2006; 281:40429–39. [PubMed: 17079231]
129. El Assar M, Angulo J, Rodriguez-Manas L. Oxidative stress and vascular inflammation in aging. *Free radical biology & medicine*. 2013; 65:380–401. [PubMed: 23851032]
130. Rajagopalan S, Meng X, Ramasamy S, Harrison D, Galis Z. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. *J Clin Invest*. 1996; 98:2572–9. [PubMed: 8958220]
131. Castier Y, Brandes R, Leseche G, Tedgui A, Lehoux S. p47phox-dependent NADPH oxidase regulates flow-induced vascular remodeling. *Circ Res*. 2005; 97:533–40. [PubMed: 16109921]
132. Odri Komazec I, Posod A, Schwienbacher M, Resch M, Pupp Peglow U, Kiechl S, Baumgartner D, Kiechl-Kohlendorfer U. Aortic Elastic Properties in Preschool Children Born Preterm. *Arterioscler Thromb Vasc Biol*. 2016

133. Stehouwer C, Henry R, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia*. 2008; 51:527–39. [PubMed: 18239908]
134. Schram M, Henry R, van Dijk R, Kostense P, Dekker J, Nijpels G, Heine R, Bouter L, Westerhof N, Stehouwer C. Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. *Hypertension*. 2004; 43:176–81. [PubMed: 14698999]
135. Cruickshank K. Aortic Pulse-Wave Velocity and Its Relationship to Mortality in Diabetes and Glucose Intolerance: An Integrated Index of Vascular Function? *Circulation*. 2002; 106:2085–2090. [PubMed: 12379578]
136. Kayama Y, Raaz U, Jagger A, Adam M, Schellinger IN, Sakamoto M, Suzuki H, Toyama K, Spin JM, Tsao PS. Diabetic Cardiovascular Disease Induced by Oxidative Stress. *International journal of molecular sciences*. 2015; 16:25234–63. [PubMed: 26512646]
137. Chung A, Yang H, Sigrist M, Brin G, Chum E, Gourlay W, Levin A. Matrix metalloproteinase-2 and -9 exacerbate arterial stiffening and angiogenesis in diabetes and chronic kidney disease. *Cardiovasc Res*. 2009; 84:494–504. [PubMed: 19617223]
138. Qin X, Corriere M, Matrisian L, Guzman R. Matrix metalloproteinase inhibition attenuates aortic calcification. *Arterioscler Thromb Vasc Biol*. 2006; 26:1510–6. [PubMed: 16690876]
139. Goncalves I, Bengtsson E, Colhoun HM, Shore AC, Palombo C, Natali A, Edsfeldt A, Duner P, Fredrikson GN, Bjorkbacka H, Ostling G, Aizawa K, Casanova F, Persson M, Gooding K, Strain D, Khan F, Looker HC, Adams F, Belch J, Pinnoli S, Venturi E, Kozakova M, Gan LM, Schneck V, Nilsson J, Consortium S. Elevated Plasma Levels of MMP-12 Are Associated With Atherosclerotic Burden and Symptomatic Cardiovascular Disease in Subjects With Type 2 Diabetes. *Arterioscler Thromb Vasc Biol*. 2015; 35:1723–31. [PubMed: 25953645]
140. Cote AT, Harris KC, Panagiotopoulos C, Sandor GG, Devlin AM. Childhood obesity and cardiovascular dysfunction. *Journal of the American College of Cardiology*. 2013; 62:1309–19. [PubMed: 23954339]
141. Cote AT, Phillips AA, Harris KC, Sandor GG, Panagiotopoulos C, Devlin AM. Obesity and arterial stiffness in children: systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol*. 2015; 35:1038–44. [PubMed: 25633314]
142. Rider OJ, Banerjee R, Rayner JJ, Shah R, Murthy VL, Robson MD, Neubauer S. Investigating a Liver Fat: Arterial Stiffening Pathway in Adult and Childhood Obesity. *Arterioscler Thromb Vasc Biol*. 2016; 36:198–203. [PubMed: 26564820]
143. Long MT, Wang N, Larson MG, Mitchell GF, Palmisano J, Vasan RS, Hoffmann U, Speliotes EK, Vita JA, Benjamin EJ, Fox CS, Hamburg NM. Nonalcoholic fatty liver disease and vascular function: cross-sectional analysis in the Framingham heart study. *Arterioscler Thromb Vasc Biol*. 2015; 35:1284–91. [PubMed: 25745056]
144. Petersen KS, Blanch N, Keogh JB, Clifton PM. Effect of weight loss on pulse wave velocity: systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol*. 2015; 35:243–52. [PubMed: 25414255]
145. Gepner AD, Colangelo LA, Blondon M, Korcarz CE, de Boer IH, Kestenbaum B, Siscovick DS, Kaufman JD, Liu K, Stein JH. 25-hydroxyvitamin D and parathyroid hormone levels do not predict changes in carotid arterial stiffness: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2014; 34:1102–9. [PubMed: 24700125]
146. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur Heart J*. 2011; 32:1484–92. [PubMed: 21300732]
147. Boggild H, Knutsson A. Shift work, risk factors and cardiovascular disease. *Scandinavian journal of work, environment & health*. 1999; 25:85–99.
148. Kim CW, Chang Y, Zhao D, Cainzos-Achirica M, Ryu S, Jung HS, Yun KE, Choi Y, Ahn J, Zhang Y, Rampal S, Baek Y, Lima JA, Shin H, Guallar E, Cho J, Sung E. Sleep Duration, Sleep Quality, and Markers of Subclinical Arterial Disease in Healthy Men and Women. *Arterioscler Thromb Vasc Biol*. 2015; 35:2238–45. [PubMed: 26359509]
149. Yu-Jie W, Hui-Liang L, Bing L, Lu Z, Zhi-Geng J. Impact of smoking and smoking cessation on arterial stiffness in healthy participants. *Angiology*. 2013; 64:273–80. [PubMed: 22649109]

150. McEvoy JW, Nasir K, DeFilippis AP, Lima JA, Bluemke DA, Hundley WG, Barr RG, Budoff MJ, Szklo M, Navas-Acien A, Polak JF, Blumenthal RS, Post WS, Blaha MJ. Relationship of cigarette smoking with inflammation and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2015; 35:1002–10. [PubMed: 25745060]
151. Gaye B, Mustafic H, Laurent S, Perier MC, Thomas F, Guibout C, Tafflet M, Pannier B, Boutouyrie P, Jouven X, Empana JP. Ideal Cardiovascular Health and Subclinical Markers of Carotid Structure and Function: The Paris Prospective Study III. *Arterioscler Thromb Vasc Biol.* 2016; 36:2115–24. [PubMed: 27585698]
152. Nilsson PM, Boutouyrie P, Laurent S. Vascular Aging: A Tale of EVA and ADAM in Cardiovascular Risk Assessment and Prevention. *Hypertension.* 2009; 54:3–10. [PubMed: 19487587]
153. Maegdefessel L, Rayner KJ, Leeper NJ. MicroRNA regulation of vascular smooth muscle function and phenotype: early career committee contribution. *Arterioscler Thromb Vasc Biol.* 2015; 35:2–6. [PubMed: 25520518]

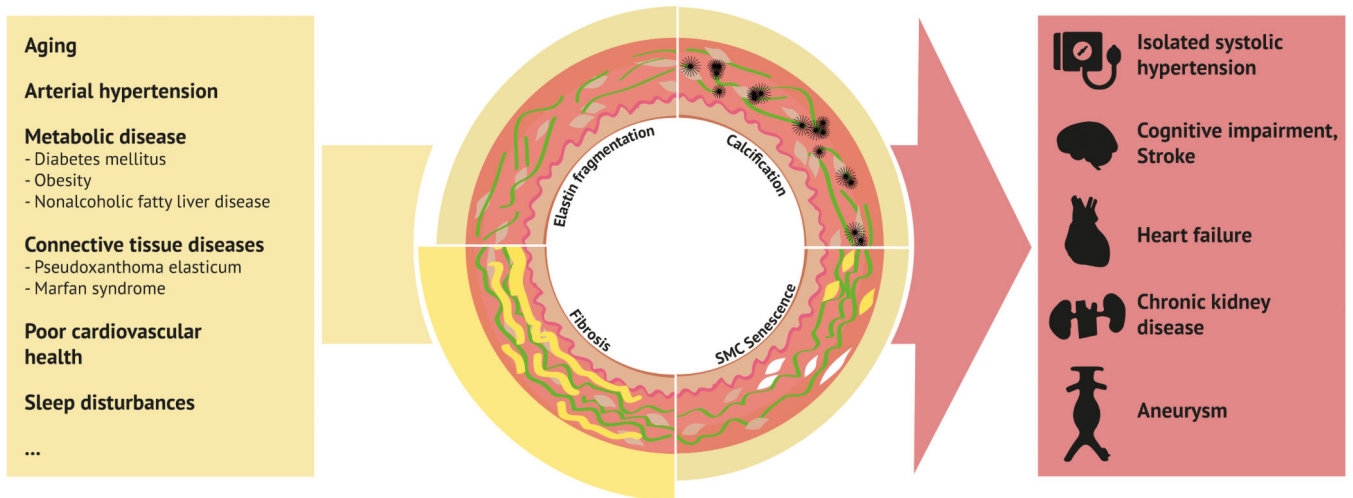


Figure 1. Arterial stiffness: contributing factors

Clinical conditions, mechanisms and effects associated with increased arterial stiffness.

Green lines represent elastic lamellae, yellow areas indicate fibrosis, black spots, black spots mark medial calcium depositions, fusiform structures indicate VSMCs.