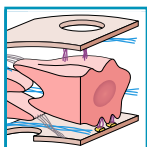# VASCULAR SMOOTH MUSCLE CELLS AND ARTERIAL STIFFENING: RELEVANCE IN DEVELOPMENT, AGING, AND DISEASE

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INSERM, U1116, Vandœuvre-lès-Nancy, France; Université de Lorraine, Nancy, France; IBiTech-bioMMeda, Department of Electronics and Information Systems, Ghent University, Gent, Belgium; Department of Pharmacology, European Georges Pompidou Hospital, Assistance Publique Hôpitaux de Paris, France; PARCC INSERM, UMR 970, Paris, France; and University Paris Descartes, Paris, France

Lacolley P, Regnault V, Segers P, Laurent S. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. *Physiol Rev* 97: 1555–1617, 2017. Published September 27, 2017; doi:10.1152/physrev.00003.2017.— The cushioning function of large arteries encompasses distension during systole and recoil during diastole which transforms pulsatile flow into a steady flow in the microcirculation. Arterial stiffness, the inverse of distensibility, has been implicated in various etiologies of chronic common and monogenic cardiovascular diseases and is a major cause of morbidity and mortality globally. The first components that contribute to arterial stiffening are extracellular matrix (ECM) proteins that support the mechanical load, while the second important components are vascular smooth muscle cells (VSMCs), which not only regulate actomyosin interactions for contraction but mediate also mechanotransduction in cell-ECM homeostasis. Eventually, VSMC plasticity and signaling in both conductance and resistance arteries are highly relevant to the physiology of normal and early vascular aging. This review summarizes current concepts of central pressure and tensile pulsatile circumferential stress as key mechanical determinants of arterial wall remodeling, cell-ECM interactions depending mainly on the architecture of cytoskeletal proteins and focal adhesion, the large/small arteries cross-talk that gives rise to target organ damage, and inflammatory pathways leading to calcification or atherosclerosis. We further speculate on the contribution of cellular stiffness along the arterial tree to vascular wall stiffness. In addition, this review provides the latest advances in the identification of gene variants affecting arterial stiffening. Now that important hemodynamic and molecular mechanisms of arterial stiffness have been elucidated, and the complex interplay between ECM, cells, and sensors identified, further research should study their potential to halt or to reverse the development of arterial stiffness.

I. INTRODUCTION

Hemodynamic homeostasis enables large arteries to transform pulsatile pressure and flow into arteriole continuous pressure and flow with minimal energy dissipation within the vascular wall. The ability of large arteries to distend when they are loaded in a nonlinear behavior defines arterial compliance, which decreases as blood pressure (BP) increases. Arterial compliance depends on the intrinsic material stiffness and the arterial geometry (see **FIGURE 1A**). Arterial stiffness is envi-

sioned as a decreased distensibility that represents the relative changes in lumen cross-sectional area for a given change in BP. The distensibility of the arteries contributes to wave propagation and reflection in the arterial tree: the arterial pulse propagates with a certain speed, the pulse wave velocity (PWV), over the arterial tree, whereby it varies continuously in amplitude and shape. The leading clinical concept of arterial stiffness relies on central artery stiffness which has been identified as a major independent risk factor for incident cardiovascular disease and overall mortality (25, 265, 269, 460, 558). Aortic PWV is a reference parameter of central arterial stiffness at the level of large elastic arteries. A complete understanding of arterial stiffness requires integrating peripheral (small artery) stiffness that acts in concert in physiological and pathological settings (459). Specific indexes of arterial stiffness, such as local distensibility or Young's elastic modulus calculated from stress-strain curves, are used in small-sized muscular arteries. Both central and peripheral stiffness encompass the complex interactions between intramural cells and extracellular matrix (ECM)
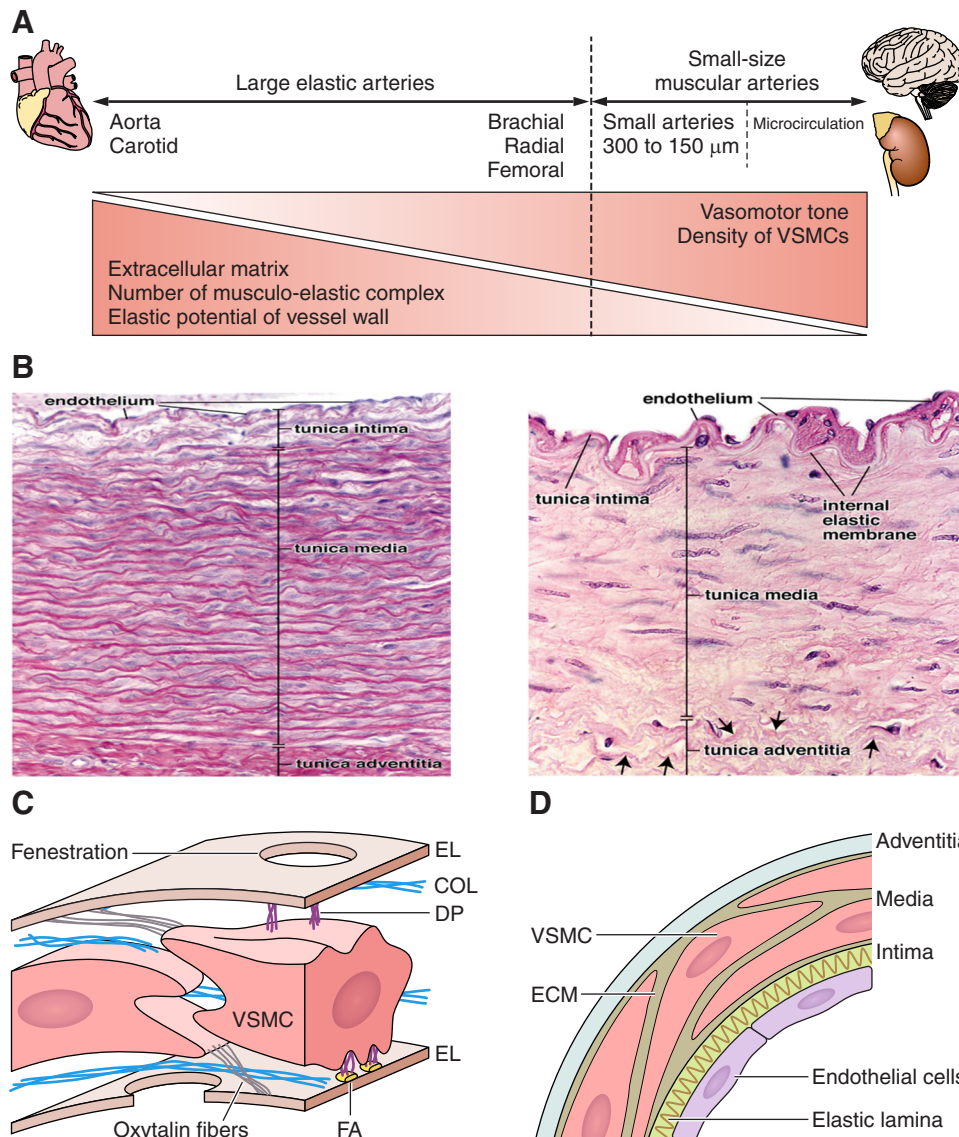
FIGURE 1. Structural and functional heterogeneity of the arterial tree. The structural and functional heterogeneity of the arterial tree allows the large arteries to exert their conduction and compliance function (i.e., to transform pulsatile pressure and flow into a continuous pressure and flow at the site of arterioles to deliver oxygen with a minimal energy dissipation within the vascular wall), and the arterioles to exert distribution of blood flow to target organs. *A*: the red triangle on the left illustrates the main role of arterial compliance (or its inverse: arterial stiffness) of proximal elastic large arteries, ECM, and number of musculo-elastic complexes. The triangular shape thinning toward the right shows that the elastic potential of the arterial wall is reduced because of the progressive reduction in the number of musculo-elastic complexes, from large proximal elastic (aorta, carotid) to medium-sized distal muscular (brachial, radial, femoral) arteries. The red triangle on the right illustrates the main role of vasomotor tone of small arteries and the density in VSMCs. The triangular shape enlarging toward the right shows that the vasomotor function increases as the caliber of small arteries decreases, until the microcirculation. *B*, *left*: histological image of a large elastic artery, clearly displaying the intima-media-adventitia layering. The media consist of concentrically organized musculo-elastic complexes. *Right*: a histological image of a muscular artery is shown. The medial layer is still bounded by the internal and external elastic membrane, but the medial organization in musculo-elastic complexes has entirely disappeared. [*A* and *B* from Resch et al. (443).] *C*: 3-dimensional organization of VSMCs and ECM, within a musculo-elastic complex of a large artery. VSMCs are embedded between two layers of elastic lamellae (EL) and attached to them by dense plaques (DP) corresponding to a focal adhesion complex (FA). Collagen fibers (COL) are running along the elastic lamellae. Elastic lamellae are fenestrated (Fen.). All empty spaces are filled up with other components of ECM. The stiffness of the arterial wall material of large proximal arteries is thus dependent on the stiffness of each component (VSMC, EL, COL), other components of ECM, and their geometrical and functional relationships. Oxytalan fibers (Ox) containing fibrillin attach VSMCs to the elastic lamellae (101). *D*: organization of VSMCs and ECM within a small artery. Only one or two layers of VSMC are present in arterioles. VSMC are loosely dispersed within the ECM. VSMC are separated from endothelial cells by elastic lamina (358).

that regulate mechanical functions and structural integrity of arteries (194) and vary among different-sized vessels.

Initially, arterial stiffening has been attributed mainly to ECM. Research of key molecular/cellular determinants of arterial stiffness has recently expanded this view 1) from the classical components of the ECM (mainly elastin and collagen) to proteins regulating vascular smooth muscle cell (VSMC) tone, cell-ECM interactions, and VSMC stiffness; 2) from shear stress to tensile, pulsatile, circumferential stress, as key mechanical determinants of arterial wall remodeling; and 3) from abnormal macrocirculation to large/small arteries cross-talk, as key determinants of target organ (brain, heart, and kidney) damage in disease (396). The role of ECM proteins, mainly the elastic fiber network, has been extensively reviewed, and mechanical models of cardiovascular development, growth, and adult remodeling vessels have been proposed (561). Initially, vascular remodeling resulting in a smaller external diameter was formally presented in cerebral arterioles in hypertension nearly 30 yr ago (20) and then this concept has since been applied extensively to large arteries in humans. The recent characterization of a general integrin adhesome network and the identification of GTPases and their downstream effectors has revealed new signaling pathways initiated by ECM stiffness and regulating cellular mechanotransduction (186, 524). A detailed discussion of the concept of VSMC plasticity characterized by a phenotypic switching from a normal differentiated contractile state towards a dedifferentiated state with increased proliferative capacities, as well as the redifferentiation process, can be found elsewhere (5, 409). The emerging role of VSMC plasticity in regard to the architecture of cytoskeletal proteins has introduced the notion of VSMC stiffness and cell contraction in the context of arterial stiffness (491).

The purpose of this review is to provide a translational approach of arterial stiffness spanning the understanding of the molecular determinants of mechanical homeostasis focused on VSMCs to the physiology of normal and age-related vascular diseases. We will contrast global large artery stiffness, i.e., that of the vascular wall structure as a whole, in the context of prevailing hemodynamic forces to the prominent role played by VSMC tone in small-sized muscular arteries. Our current understanding of the reciprocal ability of VSMCs to organize the ECM network in response to mechanical signals will be discussed in section II. Key bioengineering concepts to better understand how qualitative and quantitative changes in the components (both stiff and elastic) of the arterial wall translate into an increase in stiffness of large arteries, and the cross-talk between macro- and microcirculation will be highlighted in section III. All these hemodynamic notions based on VSMC phenotype will be described during development, normal and early vascular aging (EVA), with particular focus on

vascular inflammation, stem cells, and calcification in section IV. Section V will focus on the identification of VSMC gene variants involved in arterial stiffness using recent advances in gene analysis. The last part of this review (sects. VI and VII) will focus on physiopathology and clinical aspects in monogenic and polygenic diseases. In three monogenic diseases of the arterial wall, characterized either by arterial rupture and dissection (Marfan and Ehlers-Danlos syndrome), or by stenosis and ischemia (Williams syndrome), VSMCs are the target of intrinsic gene defects that are responsible for changes in ECM structural integrity. We will then try to integrate these mechanical concepts in polygenic diseases to analyze the role of VSMCs in the mechanisms of arterial stiffening in hypertension, diabetes, chronic kidney disease, and atherosclerosis.

II. PHYSIOLOGY OF VASCULAR SMOOTH MUSCLE CELLS AND ARTERIAL STIFFENING

A. Presence and Distribution of VSMCs in the Circulation

Before focusing on the current knowledge on molecular mechanisms/processes that control VSMC contribution to large artery stiffening, we first briefly review some general statements on the origin and distribution of VSMCs. Recent reviews give a historical overview of embryological origins of VSMCs (507).

Several cell lineages have been identified as VSMC progenitors, and their destiny is determined by factors present in their environment (143, 322, 570). As for the aorta, its base will be populated by cells originating from the secondary heart field, while the ascending aorta, arch, and common carotid arteries are populated by primordial VSMCs from the neural crest. The proepicardium gives rise to VSMCs in coronary arteries. The descending thoracic aorta will be populated by somites, and the abdominal aorta by splanchnic mesoderm.

Traditionally, mesenchymal cells are considered to be primordial cells of mesodermal origin with a multipotent differentiation potential giving rise to fibroblasts, osteoblasts, chondroblasts, adipocytes, VSMCs, and endothelial cells (ECs) as well as stromal cells (66). A broad set of markers defines the vascular smooth muscle lineage throughout the vasculature, although no specific markers for VSMC progenitors have been identified so far. There is compelling evidence that embryonic stem cells are capable of differentiating into both ECs and VSMCs and thereby contribute to vascular development (292, 324, 413). The best-studied regulatory events guiding differentiation pathways are mediated by growth factors. Differentiation of progenitor stem cells into VSMCs can be initiated by transforming growth

factor-β (TGF-β) (10) or platelet-derived growth factor (PDGF)-BB, while vascular endothelial growth factor (VEGF) promotes EC differentiation (100, 582). VSMCs can find their origin also from pluripotent circulating cells, EC transition, adventitial myofibroblasts, and pericytes (178).

An increasing number of articles have raised similarities between pericyte and VSMC differentiation (12, 323). Pericytes derived from mesoangioblasts are defined as cells surrounding the basement membrane of microvascular ECs and serve to maintain their progenitor phenotype. It is not trivial to unequivocally differentiate pericytes from VSMCs as there are no single markers such as desmin or PDGF receptor-B, and distinction is based on cell body morphology. Pericytes play a role in small vessel permeability especially in the brain circulation by modulating endothelial cell-cell junctions. In aging for example, the loss of pericytes induces increased endothelial permeability and promotes neurodegeneration (24). After brain ischemia-reperfusion, pericyte contraction leads also to capillary constriction, but mechanisms for blood flow regulation are under investigation. Because of their relative plasticity, it has also been suggested that pericytes may possess stem cell or progenitor cell potential. This capacity may serve pericytes in the adventitia of large arteries to generate VSMCs or myofibroblasts and participate into vessel wall repair in conjunction with inflammation or fibrosis. Indeed, pericytes in fibrosis were shown to constitute a source of myofibroblast precursors expressing α-smooth muscle actin (α-SMA). The loss of pericytes appears to play a key role in the early phase of diabetic retinopathy. It is now generally accepted that defected or absence of pericytes even though ECs are intact, may explain microvascular changes in pathology (404).

The structure of blood vessels organized in lamellar units (an elastic lamella and adjacent VSMCs) varies along the arterial tree (FIGURE 1, B AND C) (204). The aorta and proximal branches contain the greatest number of medial elastic layers (from 5 in mouse to 72 in sow). The seminal works of Wolinsky and Glagov (575) have shown that the total number of elastic lamellar units and the internal diameter are nearly proportional and that the tension per aortic lamellar unit is exerted in a very narrow range after adjustment for the animal size and for a given arterial site. The muscle cell layers increase in amount in distal portions of elastic arteries, i.e., medium-sized musculo-elastic arteries such as radial arteries and in smaller arteries referred to as muscular arteries (diameter from 100 to 400 μm). VSMCs are arranged in a helical pattern around the vessel lumen, with a decreasing pitch in the more peripheral vessels (FIGURE 1D). The elastin-to-collagen ratio and the surrounding ECM/VSMC ratio decrease from the thoracic aorta to distal arteries (106, 149). VSMCs decrease in arterioles (<100 μm), and only ECs and pericytes remain in the capillaries. In

muscular arteries, the luminal diameter is co-determined by the contractile state of VSMCs.

During development VSMCs undergo ultrastructural changes (72) and exhibit different phenotypic states related to the expression of an increasing number of cytoskeletal and extracellular molecules, the earliest markers being an actin isoform specific for VSMCs, α-SMA, the fibronectin isoform comprising the spliced extradomain (ED), thrombospondin-1, and elastin (TABLE 1) (100). At the midstage of differentiation, VSMCs express smooth muscle protein 22-α (SM22-α) also called transgelin, SM-actinin, h1-calponin, h-caldesmon, and metavinculin. After birth, the mature and fully differentiated VSMCs express SM-1 myosin heavy chain (MHC), smoothelin (551), and desmin as well as a repertoire of contractile proteins required for regulation of hemodynamic resistance (408). Proteome and secretome mapping of VSMCs have identified hundreds of proteins differentially expressed along the arterial tree or in response to various stimuli or pathological conditions (68, 106, 438). Comparative analysis of proteomes of human aortic, umbilical, and pulmonary artery VSMCs revealed greater differences between human umbilical artery VSMCs and aortic or pulmonary artery VSMCs, in particular in proteins involved in glycolysis and gluconeogenesis and cytoskeleton proteins (filamin and vimentin), than between aortic and pulmonary artery VSMCs (438). Additionally, Akt, NF-κB, c-AMP response element-binding protein (Creb), and tumor protein TP53 were shown to be linked with many of the differentially expressed proteins in a functional network analysis. Comparison of proteome profiles of VSMCs from peripheral musculo-elastic (femoral) and proximal elastic (aorta) arteries has revealed that 25% of the total identified proteins are expressed differentially (106). Proteins involved in cytoskeleton organization are more highly expressed in VSMCs from the aorta while proteins regulating the cell cycle network are more highly expressed in VSMCs from the femoral artery.

While extensive evidence has been accumulated on the involvement of small G proteins in ECs in vascular development, several findings support a crucial role of G protein-coupled receptor (GPCR) signaling in VSMCs for their recruitment to nascent vessels and vessel stabilization. The guanine nucleotide exchange factor C3G has been identified as a key regulator of the recruitment of supporting cells that differentiate into pericytes and VSMCs during blood vessel maturation (560). α-Parvin regulates RhoA and Rho-kinase (RhoK)-mediated signaling to provide persistent and directed migration of VSMCs and thus normal coverage of endothelial tubes (365).

In adult vessels, VSMC progenitor cells are present in a niche environment in the adventitial layer where transcription of VSMC marker genes is silenced to maintain the progenitor phenotype (323). Initially VSMCs express

Table 1. *Vascular smooth muscle cell differentiation markers*

VSMC Marker	*Gene* Model	Other Cell Types Expressing the Marker	Function and/or Comments	Reference Nos.
		Early differentiated VSMCs		
α-SMA (α-smooth muscle actin)	*Acta2* gene α-SMA$^{-/-}$ mouse	Myofibroblasts, pericytes, lymph nodes, activated pancreatic stellate cells	Structural protein that oligomerizes to form thin filaments and thereby regulates vascular motility and contractility.	355
			Impaired vascular contractility and blood pressure homeostasis in α-SMA$^{-/-}$ mice. Overexpression of α-SMA decreases proliferation and migration of VSMCs via Rac1 inhibition.	477
				77
EDA$^+$FN (fibronectin extra domain A)	*Fn1* gene	Fibroblasts, macrophages, platelets, ECs, mesangial cells	Vascular intimal proliferation.	111
	Fn-EDA$^{-/-}$ and Fn-EDA$^{+/+}$ mice		Fn-EDA$^{-/-}$ mice exhibited prolonged times to FeCl$_3$-induced carotid thrombosis.	425
	Fn-EDA$^{-/-}$apoE$^{-/-}$ mouse		Fn-EDA$^{-/-}$apoE$^{-/-}$ mice exhibited less inflammatory response via TLR4 signaling after cerebral ischemia-reperfusion injury.	103
Thrombospondin-1	*Thbs1* gene	ECs, fibroblasts, megakaryocytes, neutrophils, glial cells, tumor cells, pneumocytes, keratinocytes, osteoblasts	ECM cellular glycoprotein, inducer of VSMC chemotaxis and proliferation.	171
	Tsp1$^{-/-}$ mouse		SMC phenotypic changes and neointima formation in a carotid artery ligation model were delayed and impaired in Tsp1$^{-/-}$ mice. Tsp1$^{-/-}$apoE$^{-/-}$ mice exihibited attenuated VSMC migration associated with enhanced fibrotic lesions and plaque necrotic core formation.	368, 369
	Tsp1$^{-/-}$apoE$^{-/-}$ mouse			
Elastin	*Eln* gene	Almost all cell and tissue types, mainly expressed in vessels, lung, and skin	Elasticity.	561
	Eln$^{+/-}$ mouse		Eln$^{+/-}$ mice exhibit increased number of lamellar units.	
		Midstage of differentiation		
SM22-alpha (transgelin)	*Tagln* gene	Skeletal, cardiac, visceral smooth muscle	Formation of stress fiber and vessel contractility.	503
	Sm22$^{-/-}$ mouse		Sm22$^{-/-}$ mice exhibited enhanced arterial inflammation through activation of ROS-mediated NF-κB pathways after carotid denudation.	502
			Sm22$^{-/-}$ mice developed medial chondrogenesis after carotid denudation	
SM-actinin	*Actn1* gene	Almost all cell and tissue types, mainly expressed in megakaryocytes and platelets	Cross linker of actin filament, cell adhesion, and migration.	375
			Actn1 mutations in human caused macrothrombocytopenia.	
Calponin	*Cnn1* gene	Myoepithelial cells, interstitial cells, fibroblasts, tumor cells, visceral smooth muscle	Actin-binding protein involved in smooth muscle contraction.	342, 343

Continued

VSMC Marker	Gene Model	Other Cell Types Expressing the Marker	Function and/or Comments	Reference Nos.
	Cnn1$^{-/-}$ mouse		Cnn1$^{-/-}$ mice displayed increased spontaneous arterial baroreflex and a blunted α-adrenergic response to phenylephrine.	307
			Overexpressing human CNN1 suppressed neointimal formation following carotid ligation injury.	
Caldesmon	Cald1 gene hCaD$^{-/-}$ mouse	h-CaD isoform in smooth muscle and l-CaD in nonmuscle cells	Inhibitor of ATPase activity.	154, 155
			70% mortality at birth of hCaD$^{-/-}$ mouse which exhibited ventral hernia and slower relaxation of smooth muscle.	
Metavinculin	Vcl gene	Muscle tissue, platelets	Major constituent of focal adhesion and/or signaling via integrins and cadherins.	581
	Vinculin$^{-/-}$ mouse		No live vinculin$^{-/-}$ mice, reduced population of cardiomyocytes.	
		Fully differentiated VSMCs		
Desmin	Des gene	Pericyte, skeletal, cardiac, visceral smooth muscle	Constituent of intermediate filaments involved in smooth muscle dilation and contraction.	314
	Des$^{-/-}$ mouse		Des$^{-/-}$ mice showed decreased dilatory and contractile functions in resistance arteries.	
Smooth muscle myosin heavy chain	Myh11 gene	Smooth muscle lineages	Structural protein that oligomerizes to form thick filaments and thereby regulates vascular motility and contractility.	82, 366
	SM2$^{-/-}$ mouse		Aortic rings from SM2$^{-/-}$ null mouse exhibited increased nonmuscle myosin heavy chain-dependent contraction to potassium.	
Smoothelin-B	Smtn gene	Visceral SMCs	Contractile phenotype marker and thin filament regulatory protein, highly expressed in muscular arteries and modestly expressed in elastic arteries.	442, 551
	Smtn-B$^{-/-}$mouse		Smtn-B$^{-/-}$ mice displayed decreased arterial contractility associated with elevated mean arterial pressure and cardiac hypertrophy.	

VSMC, vascular smooth muscle cell; EC, endothelial cell; TLR, Toll-like receptor; ROS, reactive oxygen species.

a proliferative and migratory phenotype and synthesize ECM proteins. A quiescent and contractile phenotype is characteristic of mature VSMCs. All these phenotypes are present in the media of all arteries along the arterial tree with a majority being contractile cells. VSMCs can be differentiated on the basis of two main morphological phenotypes, spindle-shaped and epithelioid cells, to which can be added the thin elongated and the senescent cells. These morphologically different subtypes most likely mirror the functional classification of contractile (spindle-shaped) and synthetic (epithelioid) VSMCs (323, 324).

Thus VSMC lineage diversity is an important determinant of specific properties of artery wall cells in different segments of vascular tree and of heterogeneous patterns of vascular diseases.

B. VSMC-ECM Interactions

1. Mechanobiology

A process of mechanical homeostasis between ECM and VSMCs is a fundamental concept in arterial stiffness (194). These interactions coexist with both homocellular (VSMC-
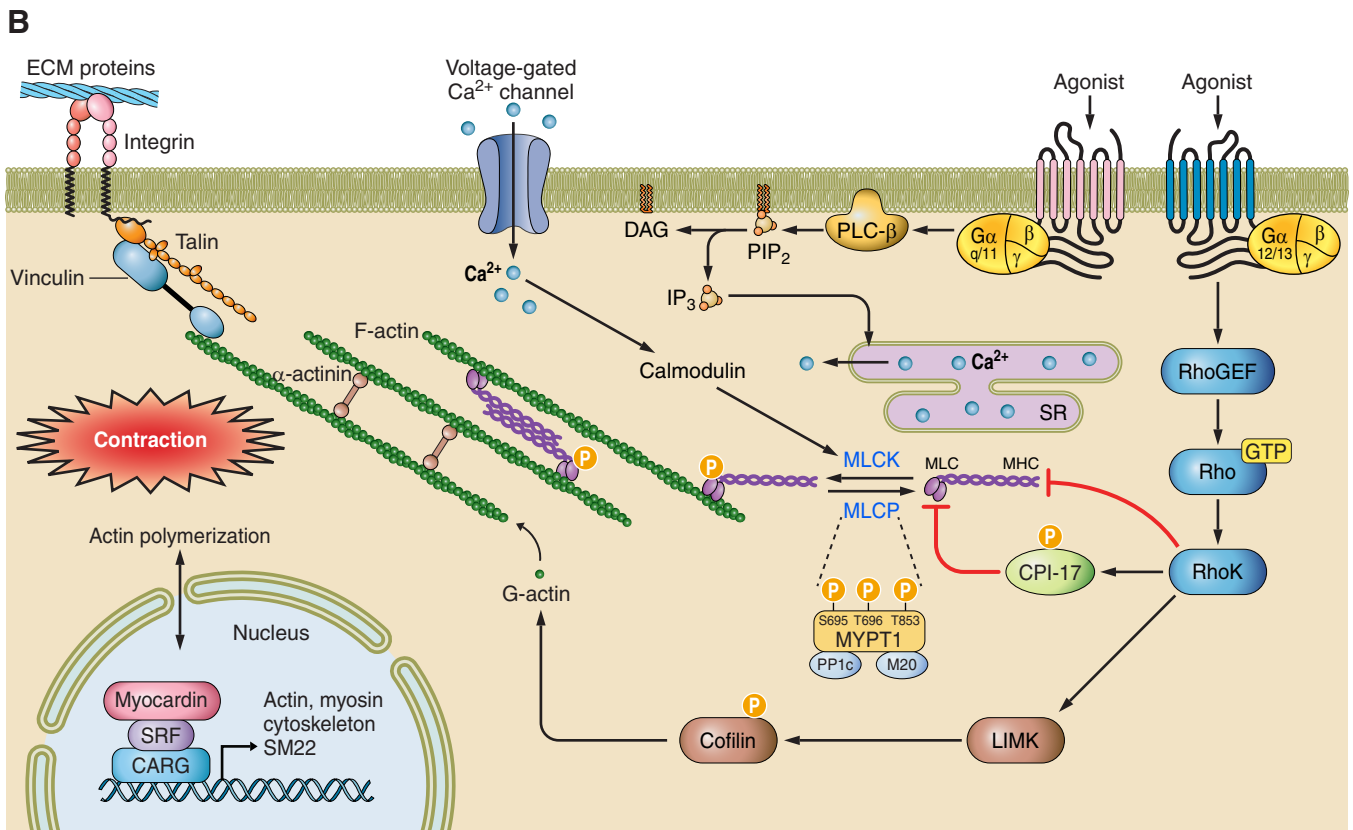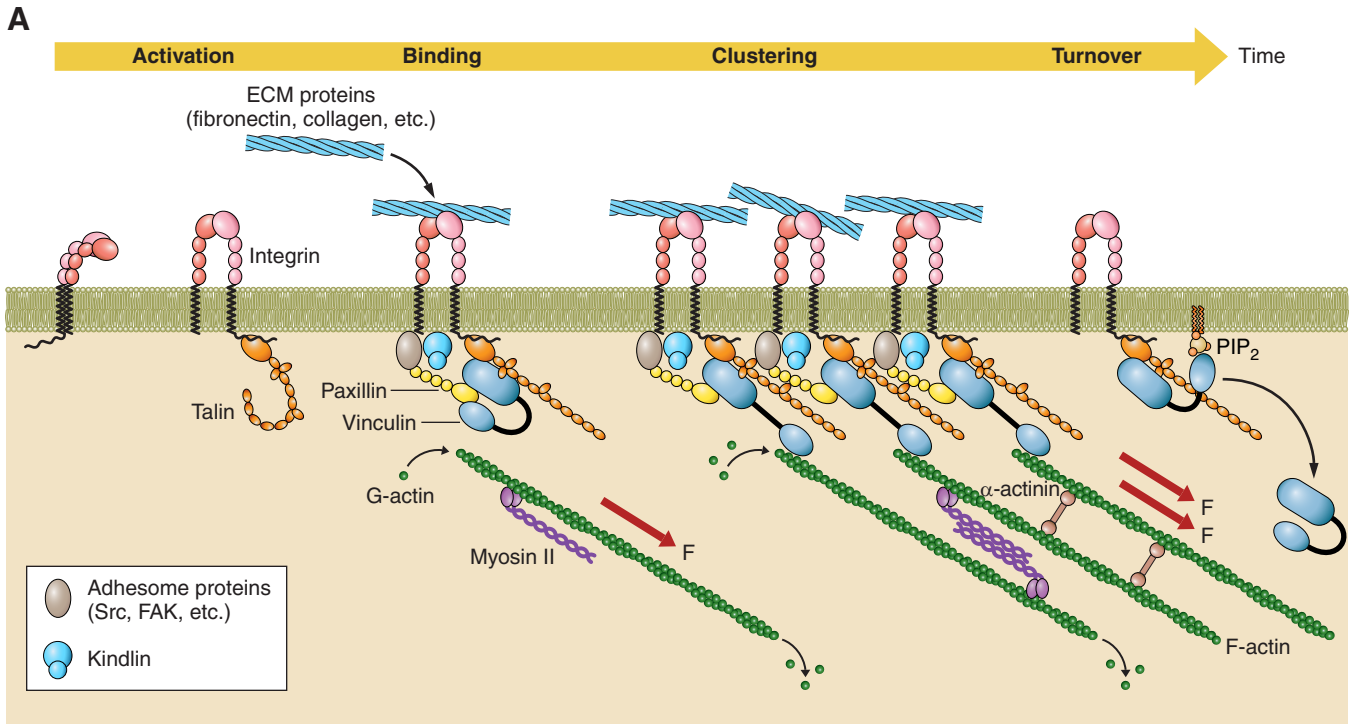
VSMC) and heterocellular (VSMC-EC) interactions mediated by gap junctions (connexins) and adherent junctions (cadherins). The major constituents of the ECM are, on the one hand, elastic and collagen fibers and on the other hand glycosaminoglycans and related proteoglycans. The role of VSMCs in the synthesis of soluble and cross-linked elastin as well as in the formation of collagen fibers has been consistently demonstrated during the 1970s using ultrastructural analyses by Ross's group (379, 457, 458). The heterogeneity of the adventitial elastin network in small arteries from different vascular beds serves for accommodating longitudinal changes in arterial length and prestressed conditions (179). Internal elastic lamellae are characterized by fenestrations **(FIGURE 1C)** whose number and size participate in the mechanical adaptation of the arterial wall during hypertension (41). In addition to the organization of internal elastic lamellae and the adventitial network, fine elastic fibers present in the media may act to connect VSMCs to ECM through elastin receptors that become disorganized with aging (113). Cell-ECM interactions involve collagen and elastin proteins, adhesion proteins, and transmembrane receptors, mainly integrins, which link at focal adhesion (FA) sites the associated integrin linker proteins (such as talin, kindlin, and vinculin) to the actomyosin cytoskeleton and GPCRs.

Integrins are crucial for ECM deposition and vascular phenotype. Integrins are $\alpha\beta$-heterodimeric receptors present on the surface of nonactivated cells in a low-affinity state **(FIGURE 2A)**. They shift to a high-affinity state through inside-out signaling, thus increasing their avidity for ligands. Ligand-occupied integrins in turn transduce outside-in signals that orchestrate many cellular responses. Dynamic inside-out and outside-in signaling events more than likely operate in concert in a self-reinforcing feedback loop. In VSMCs in vivo, the β_1 subunit pairs with α_1, α_3, α_4, α_5, α_6, α_7, and α_8 subunits that play different roles in attachment and migration by acting as laminin-binding, Arg-Gly-Asp (RGD) motif or collagen receptors (361). The α_v subunit pairs with the β_3 subunit and the α_6 subunit with the β_4 subunit to form additional subgroups of RGD receptors and laminin-binding integrins, respectively. There is no integrin gradient along the arterial tree (as, for instance, for the ECM composition between large and small arteries). Complexity and redundancy of the integrin repertoire are a signature of VSMC mechanotransduction. Elucidation of the functional roles of integrins has benefited from tissue-specific transgenic mice **(TABLE 2)**.

Because the composition of FAs are cell- and ligand-specific and highly regulated in a dynamic fashion, comparative proteomics have identified a consensus adhesome of 60 proteins that need to be combined with phosphoproteome to identify signaling pathways regulating FA dynamics (332). FAs transmit external mechanical forces or internal cell contractile force in the outside-in or inside-out direction through the integrin receptors. In response to mechanical load applied through the integrins, talin rod undergoes unfolding and subsequently more domains bind to the vinculin head domain which increases the strength of the actin-integrin attachment **(FIGURE 2A)**. Clustering of integrins at FAs produces changes in protein conformation or the association and dissociation rate of protein complex assembly. In parallel, VSMCs actively reorganize ECM and crosslinking through activation of FA. The accumulation of these proteins may recruit more integrins leading to an enlargement of FAs. Integrin clustering is highly sensitive to ECM stiffness. On soft ECM, the level of talin extension is not sufficient to induce the recruitment of vinculin that connects to F-actin. The slip bond behavior of these interactions limits mechanotransmission and may accelerate FA turnover. A stiff ECM induces a complete talin rod unfolding, and the binding of vinculin reinforces integrin clustering to form a catch bond. The mechanical linkage between ECM, integrins, and actomyosin defines the molecular clutch (524). Binding of phosphoinositide to vinculin displaces F-actin and causes FA turnover. Later on, integrative models recapitulating mechanotransduction processes have been conceptualized on FA recruitment and strengthening, defining the lifetime of the whole adhesion process varying from seconds to minutes. FA protein recycling is regulated by endocytic pathways both in proliferative and migratory cells as well as in some but not all mature differentiated cells (424). Such turnover of FAs plays a role in arterial stiffness (468). All data

FIGURE 2. Major mechanisms regulating focal adhesion and vascular smooth muscle cell contraction. A: dynamics of focal adhesion formation. In resting state, integrins are present on VSMCs in an inactive "bent" conformation. Recruitment of talin and binding to the β-cytoplasmic tail induces integrin to adopt an extended form that enables strong ligation with specific ECM proteins. Binding of talin to actin filaments via activation of vinculin promotes nascent focal complexes. Final maturation of focal adhesions depends on ECM stiffness and involves clustering of integrins and recruitment of additional adhesome proteins such as kindlin, paxillin, and α-actinin, which in turn increases actin polymerization and contractile capacity. Disassembly of the actin cytoskeleton and interaction of vinculin with PIP_2 regulates focal adhesion turnover. B: schematic representation of signaling pathways of smooth muscle acto-myosin activity. Intracellular calcium is increased either via opening of voltage-gated Ca^{2+} channels or release from sarcoplasmic reticulum through activation of G protein-coupled receptors coupled to $G\alpha_{q/11}$ proteins and subsequent inositol trisphosphate (IP_3) production. Myosin light chain (MLC20) is phosphorylated by the Ca^{2+}/calmodulin-activated MLC kinase (MLCK), which in turn increases acto-myosin interaction and contraction. Activation of Rho-family small GPTases and their downstream effectors (Rho-associated protein kinase, RhoK) decreases the activity of MLC phosphatase (MLCP) with its regulatory subunit, myosin phosphatase target subunit 1 (MYPT1), directly or also through phosphorylation of C-kinase-activated protein phosphatase-1 inhibitor (CPI-17). Another target of RhoK is LIM kinase, which phosphorylates cofilin, leading to actin polymerization and serum response factor (SRF) activation. Incorporation of G-actin into polymerizing the actin network through proteins of integrin-based adhesion structures participates in vasoconstriction.

Table 2. *Integrin expression and role in vascular smooth muscle cell functions*

Integrin	ECM Ligands	Expression in Macro- or Microcirculation	Involvement in VSMC Functions	Insights From Animal Models
$\alpha_1\beta_1$	COL I, COL II, COL III, COL IV, COL VIII, LN 1	Highly expressed on aortic VSMCs (316)	ANG II-induced proliferation of VSMC (57)	Genetic deficiency in α_1 integrin in mice inhibited FMD without affecting receptor-mediated endothelium-dependent or endothelium-independent dilation, and myogenic tone (315)
		Low expression in mesenteric artery (165)		
$\alpha_3\beta_1$	LNs, FN, COL I	The α3A variant is highly expressed on VSMCs (97)	Binding with maspin inhibits VSMC migration (19)	$\alpha_3\beta_1$ Integrin-null mice die during the neonatal period (241)
$\alpha_4\beta_1$	FN, VCAM-1, OPN	Expressed on aortic VSMCs during development (114)	Promote VSMC transition to myofibroblasts and proliferation (441)	α_4 Integrin-deficient mouse embryos exhibited failure of pericyte-VSMC interaction during blood vessel development (148)
		Expressed in cremaster arterioles (564)	Involvement in arteriole vasoconstriction (564)	
$\alpha_5\beta_1$	FN, OPN	Expressed on aortic VSMCs	ANG II and PDGF increases $\alpha_5\beta_1$-mediated adhesion of VSMCs to FN (221)	α_5 Integrin-null mice are embryonically lethal (449)
		Expressed in microvascular SMCs (525)	Binding with maspin inhibits VSMC migration (19)	
		Expressed in cerebral muscular arteries (88)	Homocysteine promotes VSMC migration via the $\alpha_5\beta_1$/FAK/paxillin/Rac1 pathway (214)	
			Neointimal formation in response to TGF-β involves overexpression of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ (290)	
			Involvement of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ in micromyogenic tone (525)	
			Involvement in myogenic tone in cremaster arterioles, cerebral and renal arteries (15, 88, 338)	
			Enhancement of L-type Ca^{2+} channel current in cremaster arterioles (577)	
$\alpha_6\beta_1$	CCN1, LNs	Expressed on carotid VSMCs (344)	CCN1 stimulates adhesion of VSMCs via $\alpha_6\beta_1$ and neointimal hyperplasia (344)	α_6 Integrin-null mice die at birth (96)
$\alpha_6\beta_4$	LNs	Expressed on SMCs of small vessels (93)	Involved in hemidesmosomes (359) but its role in the vasculature has not been identified	
$\alpha_7\beta_1$	LNs	Highly expressed in aortic VSMCs (584)	Role in the maintenance of the VSMC differentiated phenotype and in their interaction with laminins (584). Negatively regulates proliferation through ERK activation to promote VSMC contractile phenotype (573)	α_7 Integrin-null mice displayed pronounced neointimal formation after carotid artery ligation (573)
$\alpha_8\beta_1$	FN, tenascin, vitronectin	Highly expressed in aortic VSMCs (479)	Marker of differentiation, involvement in assembly of FAs and negative regulator of VSMC migration (590)	Most mice lacking the α_8 gene die soon after birth due to kidney defects (371)

Continued

Table 2.—*Continued*

Integrin	ECM Ligands	Expression in Macro- or Microcirculation	Involvement in VSMC Functions	Insights From Animal Models
$\alpha_v\beta_3$	VN, FN, OPN, LN, TSP, COL I, COL IV, tenascin-C, fibrinogen, prothrombin	Expressed on aortic VSMCs	Mediate adhesion, migration, apoptosis, and proteinase expression of VSMCs (513)	α_v Integrin-null mice die during embryonic development (504)
		Expressed in cremaster arterioles (577)	Antiapoptotic action via activation of NF-κB in VSMC exposed to type I collagen fragment in atherosclerotic lesion (559)	
			Regulate vascular healing (461)	
			Mediate the increase in thrombin generation on VSMC in response to mechanical stretch (333)	
			Reduction of L-type Ca^{2+} channel current in cremaster arterioles (577)	

Reference numbers are given in parentheses. VSMC, vascular smooth muscle cell; COL, collagen; LN, laminin; OPN, osteopontin; CCN1, cysteine-rich angiogenic protein 61; VN, vitronectin; VWF, von Willebrand factor; TSP, thrombospondin; FA, focal adhesion; FAK, focal adhesion kinase; FMD, flow-mediated dilation.

suggest that changes in integrin activity in VSMCs are both a cause and a consequence of ECM changes. A positive feedback loop between intramural FA-mediated mechanotransduction and local hemodynamics endows arterial stiffening which is specific of each different-sized vessel. Arterial stiffness can also induce global hemodynamic changes that promote ECM composition and cell phenotypic changes.

Main regulators of the FA-mediated mechanotransduction are the intracellular signaling molecules [FA kinase (FAK) and Src] and the Rho-family small GTPases which activate the myosin light chain kinase (MLCK) **(FIGURE 2*B*)**. It has been reported in vitro and in vivo that Src- and FAK-mediated tyrosine phosphorylation of FA proteins increased aortic stiffness and contractility (468). Other FA proteins such as cofilin, which mediates the disassembly of actin filaments, and the adaptor protein p130Cas, a substrate for p60 Src kinase involved in the activation of p38 MAPK, also regulate the mechanosensing processes at FAs (166).

The degree of ECM stiffness together with the frequency and amplitude of applied forces govern FA dynamics. It has been previously reported in whole vessel organ culture that steady and cyclic stretch may induce different pathways of mechanotransduction related to FAK-induced ERK1/2 activation. Only static stretch was able to increase FAK phosphorylation via Src and integrin engagement, whereas the downstream signaling ERK1/2 cascade was activated independently of these molecules in cyclic stretch conditions (278). A stiff substrate leads to more spreading and migration of cells. At the opposite, less stiff substrates, by reducing cell attachments and integrin signaling, produce apoptosis called anoikis. The migration of VSMCs towards gradients of substrate stiffness called durotaxis occurs on

fibronectin-coated surfaces but not on lamimin, indicating a key role for ECM composition in this process (164). Many cardiovascular complications such as thoracic aortic aneurysm and dissection can be envisioned as mechanotransduction disorders affecting first VSMC selectively exposed to static or cyclic stretch (182, 194, 196).

2. VSMC plasticity

VSMC plasticity, initially referred to as phenotypic modulation (72), has been conceptualized as the ability of VSMCs to switch from a quiescent contractile phenotype to a more migratory, secretory, proliferating phenotype with remodeling of the ECM (leading to arterial stiffening). The relevant markers of VSMC differentiation are SM-MHC, smoothelin, and intermediate filaments, desmin, and vimentin. Vimentin is prominent in elastic arteries, whereas desmin is present mainly in muscular arteries (572). The loss of these markers and the parallel increase of non-muscle MHC are the most reliable indices of VSMC dedifferentiation (409). The identification of key elements and pathways responsible for VSMC plasticity remains a field of intense and complex research (249), so only oversimplified mechanistic explanations are attempted here.

The states of differentiation of VSMCs are controlled by hemodynamic parameters, growth factors, vasodilation and vasoconstriction pathways, ligand-receptor interactions, and reactive oxygen species (ROS). Hemodynamic parameters are mainly represented by blood flow and BP considering their respective steady and pulsatile components. The time course of phenotypic changes varies depending on the exact location of the vessel along the arterial tree as a function of shear stress and pulse pressure (PP) and

also within the vessel according to the structural modifications and distribution of mechanical forces into the wall (106). VSMCs have the ability to reprogram their expression patterns to organize the ECM network in response to mechanical signals. PDGF-BB and TGF-β1 as well as ANG II, endothelin, thrombin, and norepinephrine act on specific receptors to control proliferation, fibrosis, and ROS production. Recently, the decreased expression of the integrin ligand mindin upon exposure of VSMCs to PDGF-BB was shown to blunt VSMC dedifferentiation through downregulation of Akt/glycogen synthase kinase 3β (GSK-3β)/mammalian target of rapamycin (mTOR)/forkhead box O (FOXO3A-FOXO1) signaling (598). Nitric oxide (NO) synthesized by ECs induces VSMC relaxation and maintains a low level of proliferation. In large elastic arteries, ROS production has an opposite effect favoring major structural mechanisms of arterial stiffness that are collagen synthesis, intimal hyperplasia, and apoptosis (205).

VSMC plasticity is under the control of many regulatory transcriptional pathways, in particular serum response factor (SRF). Binding of SRF to *cis*-regulatory elements called CArG [CC(A/T-rich)$_6$GG] box sequences in both promoter and intronic regions regulates target genes. Two distinct VSMC gene programs are controlled by the transcriptional activity of SRF, depending on its interaction with specific cofactors (423). Binding of myocardin to SRF activates VSMC-specific contractile genes while binding of ETS-like transcription factor 1 (Elk-1) promotes the expression of growth-related immediate early genes (IEG such as *Fos*/c-*Fos* genes). It should be noted that SRF has a lower affinity for contractile gene promoters than for IEG promoters. Several mechanisms have been proposed to regulate the switch-directing contractile gene or IEG expression. Classically, competition between myocardin and Elk-1 for binding to a common site on SRF (568), downregulation of cofactors and repressors of SRF by microRNA(miRNA)-143/145 and miRNA-221/222 (323), and modifications of epigenetic histone marks of chromatin structure (5) are considered as major regulators of VSMC phenotypic switching. The phenotypic switch induced by PDGF works by its action on the transcriptions factors Elk-1 and Krüppel-like factor 4 (KLF4) and on the miRNA-221/222 leading to the disruption of the myocardin binding to SRF (462). Nucleo-cytoplasmic shuttling of SRF is another possible mechanism regulating VSMC differentiation (188). Phosphatase and tensin homolog (PTEN), a cytoplasmic lipid phosphatase, has been recently identified as novel actors in the cofactor interactions with SRF. The formation of nuclear protein complexes constituted by PTEN, SRF, and myocardin promotes selective binding of SRF to promoters of differentiation-associated genes and prevents SRF translocation out of the nucleus. In vascular diseases, stimuli disrupting the interaction of PTEN with SRF and driving their nuclear exclusion enhance the binding of available nuclear SRF to alternative growth-associated gene promoters (188).

The general process of inducible cell regeneration called autophagy is the recycling of cytoplasmic elements that result from lysosomal degradation (464). The occurrence of autophagy in VSMCs was first reported in atherosclerosis and hypertension (394). Cultured cells have yielded further insight into stimuli and mechanisms contributing to autophagy. Growth factors and cytokines, ROS, and metabolic stress have been reported to trigger autophagic programs through MAPK, AMP-activated protein kinase (AMPK), Akt, and endoplasmic reticulum stress signaling pathways. PDGF drives the degradation of contractile proteins via an autophagic process and the conversion to the synthetic VSMC phenotype via mitochondrial fragmentation (465). In cultured cells, autophagy induced by PDGF also prevented cell death (463). Of note, proteosomal activity is not required for the VSMC phenotypic switch, whereas it has a role in VSMC hyperplasia. Dedifferentiation of VSMCs thus is hallmarked by a dichotomy, i.e., the coupled removal of contractile elements mediated by autophagy and repression of contractile genes, which is discrete from the induction of the proliferative feature through the transcriptional machinery regulated by changes in mitochondrial morphology and activity.

The widely accepted concept (paradigm) that VSMC phenotypic modulation or plasticity underlies many vascular occlusive diseases has been recently challenged. The finding of media-derived multipotent vascular stem cells repopulating the tunica media and forming neointima after vascular injury is supportive of the hypothesis that multipotent vascular stem cell activation and differentiation rather than VSMC dedifferentiation of mature VSMCs contributes to vascular remodeling and disease development (530).

C. Vascular Tone, VSMC Stiffness, and Adhesive Properties of VSMCs Are Major Determinants of Arterial Stiffening

1. Vascular tone

Vascular tone, defined as an intrinsic spontaneous level of vasoconstriction, contributes to the dynamic regulation of blood flow and small artery diameter. Abolition of vascular tone with potassium cyanide increases arterial compliance in situ in the rat carotid artery (285). The active role of VSMCs is achieved by changing their position within the media and the attachments between themselves and with ECM proteins (337). Indeed, there is an optimal short-term distribution of wall stress within the vascular wall which contributes to more efficiently sustained vasoconstriction. A contractile cell in a soft ECM encounters little resistance to its contraction, whereas a stiff ECM material produces a large resistance to its contraction (571). In the long term, a sustained regulation to a smaller diameter is achieved via structural changes. Elastic arteries are characterized by

tonic (slow) contractions in contrast to muscular arteries where phasic (fast) contractions occur allowing the fine tuning of regional blood flow circulation (439).

Understanding the crucial role of the Ras protein superfamily, and in particular Rho family proteins, in the regulation of VSMC contraction required for BP control has progressed rapidly during the past 10–15 yr (304, 452). Phosphorylation of 20-kDa MLC has been identified as a key event of VSMC contraction **(FIGURE 2*B*)** (429, 509). MLC is phosphorylated by Ca^{2+}/calmodulin-activated MLCK and dephosphorylated by Ca^{2+}-independent MLC phosphatase (MLCP). When activated by ANG II, the ERK1/2 pathway exerts a hypertensive action by triggering MLCK and thereby MLC phosphorylation. Pharmacological data and genetic studies have revealed that RhoA activation exerts a major role in the pathogenesis of hypertension. This happens by stimulating target RhoK that phosphorylates myosin phosphatase target subunit 1 (MYPT1), a regulatory subunit of MLCP, and inhibits MLCP activity. RNA sequencing has revealed alternative splicing generating fast and slow variants of MHC, MLC, and MYPT1 responsible for velocity of shortening or cGMP relaxation (439). The RGS (regulator of G protein signaling)-containing guanine nucleotide exchange factors participate in RhoA activation induced by vasoconstrictors acting through GPCRs (153), while Rap1 downregulates RhoA activity via the increase in cAMP and cGMP. Activation of RhoA/RhoK signaling pathway results in Ca^{2+} sensitization of contractile proteins and thereby tonic VSMC contraction.

Myogenic tone is defined by vasoconstriction in response to elevated BP and contributes to autoregulation of blood flow. Myogenic tone may involve different mechanosensors that are cell membrane proteins (ion channels, GPCRs), cell-ECM interactions via integrins connected to the cytoskeleton, and intercellular junctions through cadherins-catenins complexes (178). Blockade of N (neuronal)-cadherin, which is a major cell-cell protein in VSMCs belonging to the type I cadherin family, prevents the pressure-induced myogenic response without changes in intracellular calcium in VSMCs (210). Nonselective stretch-activated cation channels are key players of the myogenic response via the opening of voltage-dependent calcium channels. In VSMCs, stretch-activated cation channels opening is negatively regulated by polycystin-2 (500). This inhibition works by actin cross-linking induced by binding of polycystin-2 to filamin-A, thereby reducing the tension applied on microdomains in the VSMC membrane for a given level of BP. Myogenic constriction occurs mainly in myogenically small-sized muscular arteries and is positively regulated by integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ and NADP oxidase (Nox)-induced production of ROS mainly from mitochondria (141, 338). At the single cell level, external forces applied to a fibronectin-induced FA site induced a micromyogenic event through interactions with $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins and Src activity (525). Fibronectin-induced Src activation is explained by phophorylation of the L-type Ca^{2+} channel. Such a myogenic response was not observed in response to forces applied to collagen type I, laminin, or vitronectin despite involvement of these two similar integrins, suggesting ECM specificity (525). Alterations in myogenic response have been well implicated in microvascular disorders (mainly cerebral and coronary vasospasms and diabetes) and may be enhanced by ECM stiffening (178).

ROS play a major role in microvascular remodeling. Noxs are the major source of superoxide anion in VSMCs (514). NADP is composed of five subunits: the catalytic subunit gp91phox and p22phox in the membrane, p47phox, as well as p40phox and p67phox in the cytosol. The redox status of VSMCs regulates Nox activity through the chaperone enzyme protein disulfide isomerase (211). The small G proteins interfere at different levels of Nox activation. Indeed, after stimulation, the cytosolic units form a complex that requires the presence of (GTP)ase Rac to interact with the units located in the membrane. The Nox family may be stimulated by GPCRs such as for ANG II, endothelin, and thrombin which are important modulators of vascular tone. The polymerase delta-interacting protein (Poldip2) through increased activity of Nox4 has been also shown to activate FA complexes via Rho-dependent pathways (330). ROS may be produced also by the endothelial NO synthase (eNOS) isoform of NOS in case of abnormalities of NO synthesis by reducing tetrahydrobiopterin (BH4) and L-arginine availability. Differential effects of ROS on cellular growth and apoptosis have been described. Although ROS increases proliferation, apoptosis as well as rarefaction of capillaries may occur also in response to specific ROS such as H_2O_2 in a dose-dependent manner (291). Production of ROS via positive interactions with GTPases and integrin activation plays a major role in vascular tone particularly in the microcirculation. Indeed, ROS generation in response to vasoconstrictive agents could exert a positive action in VSMC actin polymerization via the protein complex of the actin-related protein 2/actin-related protein 3 (Arp2/3) with the nucleation promotion factors (NPF) (4, 127, 227). This effect of actin polymerization has been shown to be associated with increased myogenic tone in response to VSMC stretching (282). In addition, expression and activation of ECM metalloproteinases (MMP-2 and MMP-9) participate in the vasoconstrictor-induced inward eutrophic remodeling of large elastic arteries (67). Regarding arteriolar remodeling, several reports have consistently implicated activation of transglutaminases. Recently, it was demonstrated that activation of transglutaminases in response to the topical application of serotonin that triggers inward remodeling is associated with an increase in cofilin phosphorylation, thereby directing the actin polymerization dynamics towards the formation of F-actin (130). Tissue transglutaminase (TG2) modulates also remodeling and stiffness in aorta via two different mechanisms, matrix assembly through crosslinking-dependent functions in particular during aging

(see below) and VSMC tone through endothelium dysfunction [activation of Ca^{2+}-activated K^+ (BK) channels and reduction of NO bioavailability] (220, 519).

2. VSMC stiffness

Cell stiffness and adhesion properties of VSMCs have been proposed as important determinants of the overall stiffness of the intact vessel. Atomic force microscopy (AFM) allows accurate assessement of cell topography, adhesion force (the force required to rupture the bonds between probes coated with ECM proteins and cell surface), and cell elasticity (Young's elastic modulus calculated from indentation-force relationship). F-actin connected to FA sites at the membrane represents the first model of cell stiffness (376). These mechanisms involve dynamic actomyosin interactions as well as the capacity of actin to rapidly depolymerize and repolymerize. The actomyosin interactions occur within a timescale of seconds, but full reorganization of FAs has a timescale of minutes. The increase in cell stiffness depends on the degree of stretch of the original F-actin and the recruitment of new F-actin. VSMCs subjected to a 10% cyclic equibiaxial stretch at 0.25 Hz induce a rapid peak of increase of cell stiffness and number of FAs at 2 min which returns to normal values after 5 min. Despite concomittant increases in FA-associated proteins (more paxillin than vinculin), it is not known whether this mechano-adaptation of cell stiffness in response to cyclic stretch is due to polymerization/depolymerization of F-actin or development/resolution of contractility (377). Stiffness of VSMCs and adhesion to ECM via integrins are increased during contraction and reduced during relaxation. In addition to FAs, there is experimental evidence suggesting that cadherin-mediated adherens junctions may regulate microvascular tone through the reorganization of F-actin in VSMCs (527). N-cadherin adhesion complexes and FA complexes share mechanosensing properties and exhibit similar responses to substrate stiffness. Common anchoring proteins, signaling molecules, and spatial distribution of these two complexes argue for an integrated regulation of VSMC tone. In response to outside-in signaling pathways, both cell adhesion and elasticity show similar oscillations with time characterized in terms of frequency and amplitude. ANG II increased and adenosine decreased their amplitude as well as the density of stress fibers in a coordinated manner, but the exact mechanisms and signification related to dynamic changes of cytoskeletal structures and synchronized contraction or relaxation responses are not known (184, 185).

The interaction between fibronectin and $\alpha_5\beta_1$ is the one that has been the most studied. Measured with AFM, the range of adhesion forces for a single bond is 34–43 pN (526). Multimolecular process are involved in the regulation of fibronectin-$\alpha_5\beta_1$-induced FA activation. It has been shown that PDGF-BB decreases fibronectin-$\alpha_5\beta_1$ binding, indicating that the proliferating effect of PDGF-BB is linked to this complex. In contrast, lysophosphatidic acid, a small phospholipid in the membrane of VSMCs, increased integrin-fibronectin adhesion via $\alpha_5\beta_1$ and $\alpha_v\beta_3$ activation. This effect, mediated through GPCRs (lysophosphatidic acid receptors 1–6), induces production of ROS (514). The main characteristics of $\alpha_5\beta_1$ binding are the rapidity of activation-deactivation cycles ranging from 2 to 25 h in serum-starved cells and the time dependency of the functional activity of $\alpha_5\beta_1$.

Combined with a higher elastin content, the smaller size of FAs in the thoracic aorta compared with femoral arteries may trigger higher distensibility assessed using a magnetic tweezer coupled with a RGD peptide and optical twisting cytometry (106). Proteomic data showing higher expression of proteins of both cytoskeletal structure and FA complexes in VSMCs from thoracic aorta are consistent with a higher elasticity if one assumes that changes in cytoplasmic rigidity can positively control VSMC stiffness. These findings also raise the question of the cell response to stretch, since higher levels of cyclic stretch increase VSMC elasticity in the entire arterial tree. The influence of this factor is prominent in the thoracic aorta where the coupling between cytoplasmic rigidity depending on FA plasticity and circumferential stress is optimal. The importance of cytoskeletal subnetworks linked to non-integrin receptors has also been demonstrated using a magnetic trap to apply a controlled force to cells via magnetic beads coated with fibronectin, anti-transferrin, or anti-dystroglycan antibodies (191). Cell stiffness was greater when there was a linkage between the cytoskeleton and a membrane receptor such as the dystroglycan receptor. The concept of cytoskeletal subnetworks linked to specific cell type receptors in the regulation of cell stiffness has been proposed.

Increased stiffness and adhesive properties of VSMCs of spontaneously hypertensive rats (SHRs) were found compared with Wistar-Kyoto normotensive controls using AFM in nanoindentation experiments (492). In SHR, VSMC stiffness is characterized by slower oscillations but of higher amplitude compared with normotensive rats, indicating a dynamic regulatory process of cytoskeletal proteins. VSMC stiffness increases with hypertension superimposed on aging (490). Stiffness of VSMCs, i.e., the Young's elastic modulus and adhesion, was significantly higher in cells harvested from the aorta of old versus young monkeys, in association with increased expression of α-SMA and activation of β_1 integrin (430, 599). The amplitude of the oscillations with time in adhesion was higher in old animals than in young animals. VSMC stiffness and the corresponding oscillations were strongly reduced after disruption of the actin cytoskeleton or inhibition of MLCK in both old and young monkeys, indicating the highly dynamic regulation of the VSMC function and structure. At this time, it is still very difficult to determine whether VSMC stiffness is a cause or a consequence of large artery stiffness. Recently, it has been reported that VSMC stiffness is

increased in SHR at the level of the large arteries but not in small arteries (248, 597). The proposed mechanism is a hypertension-induced increase in SRF and related transcriptional pathways in the thoracic aorta. Clearly, we are on the way to understanding the relative contribution of VSMC stiffness along the arterial tree in systemic arterial stiffness (FIGURE 3).

3. VSMC–ECM Interactions and Arterial Stiffness

The elastin and collagen network represents the classical mechanical scheme of arterial stiffness, since the elastic fiber network is the most distensible component of the arterial wall, whereas the (initially wavy) collagen fiber network lacks elastic properties but provides rigidity and strength of the arterial wall (TABLE 3) upon stretching. Elastin-deficient mice die a few days after birth and display reduced aortic compliance compared with control mice (79). The lack of elastin induces loss of cell-cell contacts leading to an extensive proliferation of VSMCs and arterial occlusion. Mice with haploinsufficiency for elastin develop severe hypertension and arterial stiffness associated with an increased number of lamellar units and similar values of tension per lamellar unit, indicative of adaptive arterial remodeling (123). In addition, the loss of elastin-induced proliferative response is the major cause of aortic stenosis in the Williams syndrome in humans (244) (see below).

Among proteoglycans, small leucine-rich proteoglycans (SLRPs) are crucial regulators of collagen fiber organization and fibrillogenesis. Proteome analysis of the nonatheroscle-
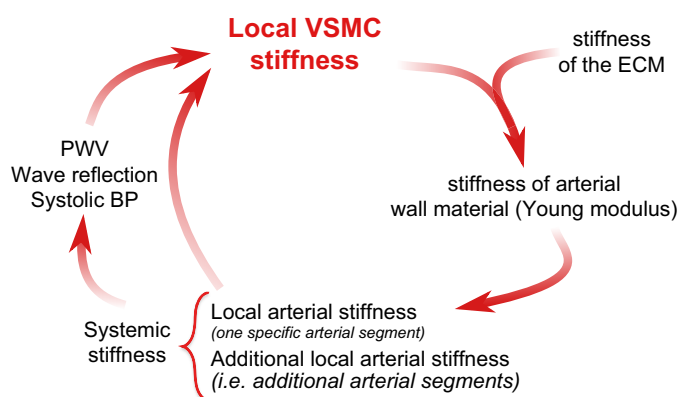
rotic mammary artery has identified three SLRPs (prolargin, mimecan, and asporin) significantly underexpressed in patients with high arterial stiffness (assessed by an elevated PWV, see below) without modification of large proteoglycans (318). Interestingly, none of these SLRPs is a determinant of high values of PWV, whereas basement membrane-associated collagen α-1 (IV) and collagen α-1 (VIII) expression are increased and predictive of high PWV. In addition, several intracellular proteins related to actin cytoskeleton organization, such as tropomyosin α-4 chain, are also determinants of increased PWV. It has been reported also that elastocalcinosis and accumulation of proteoglycans in the media induced large artery stiffness in *abcc6*-deficient mice, a model of pseudoxanthoma elasticum (226).

In VSMCs, desmin, the main component of the intermediate filaments, is associated with FA-associated proteins closely linked to actin filaments. Despite a slight reduction in BP, desmin-deficient mice exhibited a lower distensibility and mechanical strength of the carotid artery without changes in elastin and collagen content (245). Desmin is also required to control microvascular tone and flow-induced endothelium-dependent and -independent dilation (314). A strong mechanistic proof of the role of VSMC tone in arterial stiffness was given by experiments in mice invalidated for SRF in VSMCs. In this model, the mice exhibit a higher arterial distensibility (lower Young's elastic modulus) without modification of the collagen-to-elastin ratio (137). In these VSMC-specific SRF knockout mice, gene expression of contractile components (α-SMA and MLC), regulators of the contractile response [MLCK, MYPT1, and protein kinase C-potentiated myosin phosphatase inhibitor (CPI-17)] and integrins was reduced. Additional details of other VSMC molecular determinants of arterial stiffness using a classification according to their location are presented in TABLE 3.

FIGURE 3. Large artery stiffness: cross-talk between local and systemic stiffness. In large arteries, the stiffness of vascular smooth muscle cell (VSMC) is a key determinant of the stiffness of the arterial wall material. The stiffness of ECM plays a major additional role. The stiffness of the arterial wall material is often expressed as the value of Young or incremental elastic modulus for a given circumferential wall stress. These stiffness moduli, together with the relative wall thickness, determine the functional stiffness of the arterial segment. Systemic arterial stiffness is the complex result of the stiffness of all arterial segments. In turn, systemic arterial stiffness plays an important role in the local stiffness of the VSMCs, through the effects of pulse wave velocity (PWV), reflected waves and systolic BP.

In support of a role of integrins in arterial stiffness, it has been proposed that the increase in $\alpha_5\beta_1$ and $\alpha_v\beta_3$ with age observed in the mesenteric artery in SHR may in part determine arterial stiffening at high levels of circumferential wall stress via increase in cell-ECM attachments together with an increase in the collagen-to-elastin ratio (206). The increased expression of $\alpha_v\beta_3$ and activation of the signaling pathway in VSMCs in response to cyclic mechanical stretch argues also for integrin involvement in both cellular and arterial stiffness changes in hypertension (333, 525). The importance of cyclic mechanical forces on integrin adhesome in VSMCs has been highlighted using an RGT peptide, which disrupts the interaction β_3 cytoplasmic tail with Src (333). The collagen-binding α_1 subunit is required for mechanical strength of the arterial wall but not for arterial stiffness in the physiological range of BP. Genetic α_1 subunit knockout mice did not exhibit VSMC proliferation and arterial stiffness in response to ANG II, indicating that

Table 3. *VSMC molecular determinants of arterial stiffness*

Molecules	Role in Stiffness and Insights From Mouse Models With Genetic Manipulations	Reference Nos.
Extracellular		
Elastin	Elasticity	
	Loss of elastin yielded proliferation of VSMCs and occlusion. Eln$^{+/-}$ mice displayed high systemic blood pressure and increased elastic modulus at high pressure	123, 561
	Mice overexpressing LOX in VSMC exhibited increased aorta stiffness	339
	SSAO knockout mice displayed increased carotid diameter without modification of elastic modulus	353
Collagens	Rigidity and strength α2(I) collagen-deficient mice exhibited decreased breaking strength and elastic modulus	417
Cytokines-metalloproteinases	Inflammation and ECM remodeling	
	CT-1 null mice exhibited reduced carotid elastic modulus	310
	There was no age-related arterial stiffening in MMP-12-null mice	301
	In MMP-9$^{-/-}$ mice, arterial stiffness was increased in response to ANG II	134
	Increased MMP-2 and MMP-9 activities in ANG II-treated VSMC SirT1-deficient mice was associated with increased PWV	128
Proteoglycans	Adhesion	
	Mice lacking *N*-deacetylase-*N*-sulfotransferase1 in VSMCs displayed decreased sulfation of heparan sulfate and tangent modulus in aorta	1
	*Abcc*6-deficient mice displayed features of pseudoxanthoma elasticum and reduced distensibility	226
Membrane		
Glycosphingolipids	Cholesterol biosynthesis	
	Administration of an inhibitor of glycosphingolipid synthesis in apoE$^{-/-}$ mice reversed the increase in PWV induced by diet	74
G protein-coupled receptors	Contraction and proliferation	
	Mice carrying 3 copies of the angiotensinogen gene displayed decreased elastic modulus	47
Adhesion receptors	Focal adhesion and mechanotransduction	
	α_7 Integrin-null mice exhibited reduced vascular compliance	573
	Deletion of α_1 integrin in mice resulted in loss of ANG II-induced arterial stiffness	316
Intracellular		
Transcription factors	Gene expression and differentiation	
	VSMC-specific invalidation of SRF in mice decreased elastic modulus and vasomotor tone	137
	VSMC-specific invalidation of MR suppressed the aldosterone/high salt-induced increase in arterial stiffness	138
Intermediate filaments	Distensibility	
	A null mutation in the *Des* gene reduced in vivo carotid distensibility, in vitro mechanical force and mechanical strength	245
miRNA /LncRNA	Gene expression regulation	
	Distensibility of Dicer knockout mesenteric arteries was reduced	33
	Inhibition of miRNA-92a by an antagomir in aorta of old mice increased PWV	169

VSMC, vascular smooth muscle cell; LOX, lysyl oxidase; SSAO, semicarbazide-sensitive amine oxidase; CT-1, cardiotrophin-1; MMP, ECM metalloproteinase; SirT1, sirtuin-1; PWV, pulse wave velocity; SRF, serum response factor; MR, mineralocorticoid receptor; LncRNA, long non-protein-coding RNA.

VSMC integrin receptors and FAK phosphorylation are key players for arterial wall remodeling (316). In cultured cells grown onto collagen I or fibronectin, the proliferative effect of ANG II via the angiotensin II, type 1 receptor (AT1R) and ERK activity has been reported to be dependent on both $\alpha_1\beta_1$ and $\alpha_5\beta_1$ integrins (57).
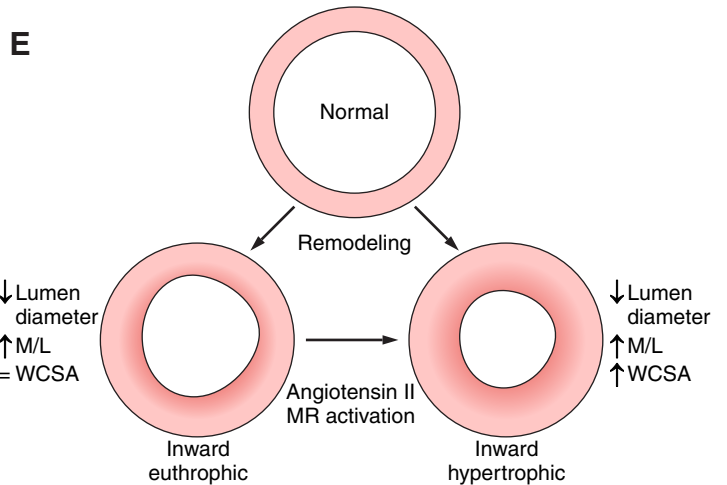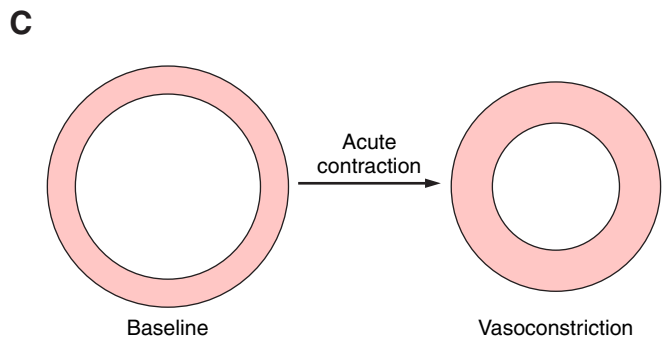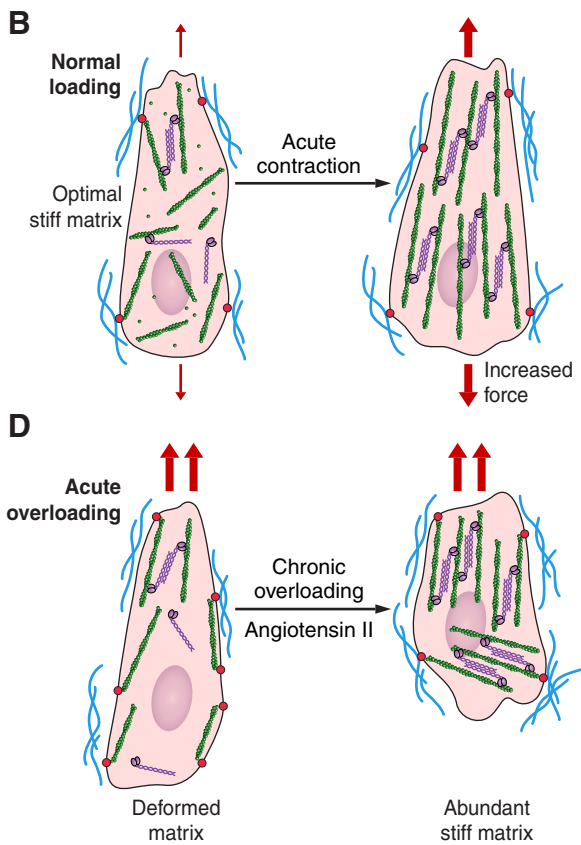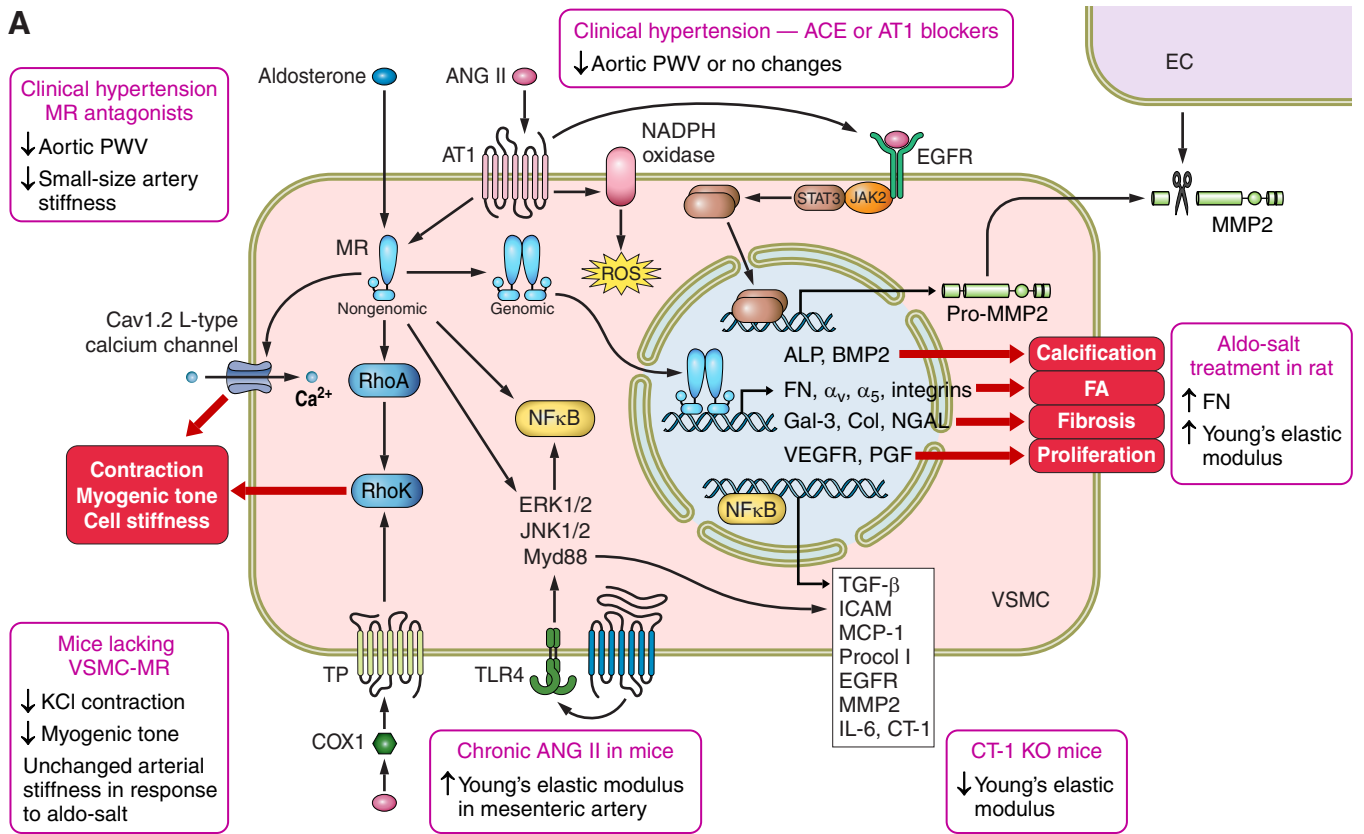
From a purely mechanical point of view, an increase in FA site number limits the arterial wall deformability and increases the stiffness of wall constituents. This hypothesis is supported by the SHR model in which a higher expression of fibronectin and $\alpha_5\beta_1$ integrin is present in the media (31, 32). The increase in the Young's elastic modulus in SHR is due to a higher level of BP and not to the increased stiffness of the wall materials indicating mechanical adaptation of the vascular wall through an increase in fibronectin and $\alpha_5\beta_1$ in the media. In sinoaortic denervated and chemically sympathectomized rats, two models of increased arterial stiffness without hypertension, an increased number of cell-ECM interactions contributed to large artery stiffness (40). There, nonetheless, remains a gap between our current understanding of in vivo changes in arterial stiffness and dynamics of FAs at the molecular level.

4. Application to the angiotensin–aldosterone–receptor system

Aldosterone and its mineralocorticoid receptor (MR) have been shown to play an important role in arterial stiffness in the course of primary aldosteronism (29, 521) or during arterial aging in human patients (297, 469, 475, 522) and in experimental models (380, 443). Selective and nonselective aldosterone blockers decrease PWV in hypertensive patients and patients with chronic kidney disease (CKD) (37,

233, 534). In rats, administration of aldosterone produced an increased Young's elastic modulus associated with an increase in fibronectin independently of BP (247). MR expression was increased in aortas and VSMCs from adult and aged Brown Norway X Fischer 344 (F344XBN) rats. MR signaling likely related to ANG II and epidermal growth factor receptor (EGFR) activation is implicated in upregulation of inflammatory marker expression (phospho-ERK1/2, ICAM-1, TGF-β, and procollagen 1) during arterial aging **(FIGURE 4A)** (242). MR antagonism is able to reduce arterial aging through a recovery of a young contractile VSMC phenotype (242). The group of Jaffe and co-workers (349, 350) have reported a mouse model with conditional inhibition of VSMC MR expression. They demonstrated a direct role for VSMC-MR at baseline in BP regulation and in myogenic tone without any modifications in arterial structure and distensibility in aged mice. However, in larger elastic arteries, like carotids, conditional inactivation of VSMC MR suppressed the aldosterone/high salt-induced increase in arterial stiffness and α_5 subunit of integrins, indicating that VSMC MR modulates directly large artery stiffness via contraction and reinforcement of cell/ECM interactions independently of major vascular structural changes. This is in agreement with previous work (348, 350) showing a key role of VSMC MR in controlling microvascular tone and remodeling effects of aldosterone in mice. It has been reported that invalidation of galectin-3 in mice which interacts with various integrins and ECM proteins such as collagen, elastin, and fibronectin inhibits aldosterone-induced collagen expression in VSMCs (310, 398). This mechanism also explained the action of VSMC MR on arterial stiffening. Cardiotrophin-1 is able also to stimulate expression of fibronectin and collagen particularly during aging. Absence of cardiotrophin-1 reduced carotid Young's

FIGURE 4. Role of smooth muscle cells in resistance artery remodeling. *A*: aldosterone and ANG II signaling pathway in arterial stiffness. Mineralocorticoid receptor (MR) activated by aldosterone exerts rapid nongenomic effects (seconds to minutes) leading to activation of Cav1.2 subunit of the L-type calcium channel, Rho-associated kinase (RhoK), and MAPK pathways. This signaling causes contraction, myogenic tone, and cellular stiffness. The genomic effects (minutes to hours) result in activation of genes involved in calcification, focal adhesion (FA) formation, fibrosis, and proliferation. NF-κB nuclear translocation occurs through both genomic and nongenomic effects. Pink boxes indicate the experimental and clinical data supporting the resulting effects of MR on arterial stiffness. ANG II acting through AT1R directly stimulates MR and increases production of ROS via NAPDH oxidase. ANG II increases pro-MMP2 protein expression via the EGFR-JAK2-STAT3 pathway. Final activation of proMMP2 is endothelial-dependent. TLR4 signals through Myd88/JNK activating NF-κB and an increased expression of pro-inflammatory genes. In resistance arteries, COX1 via ANG II stimulates contractile thromboxane receptor (TP). VSMC, vascular smooth muscle cell; EC, endothelial cell; AT1R, ANG II type 1 receptor; ROS, reactive oxygen species; EGFR, epidermal growth factor receptor; TP, thromboxane receptor; TLR4, Toll-like receptor 4; COX1, cyclooxygenase-1; MMP2, ECM metalloproteinase-2; HRE, hormonal response elements; ALP, alkaline phosphatase; BMP2, bone morphogenetic protein 2; FN, fibronectin; Gal-3, galectin-3; Col, collagen; NGAL, neutrophil gelatinase-associated lipocalin; VEGFR, vascular endothelial growth factor receptor; PGF, placental growth factor; TGF-β, transforming growth factor-β; MCP-1, monocyte chemoattractant protein-1; CT-1, cardiotrophin-1. *B*: acute contraction induces phosphorylation of myosin and remodeling of the actin cytoskeleton. *C*: acute inward remodeling of a small artery during functional vasoconstriction. External and lumen diameters are reduced in response to the contraction of the VSMCs. The number of VSMCs remains unchanged, since this is an acute phenomenon. However, the number of dense plaques (and focal adhesions) increases, to strengthen cell attachment to the ECM or between them. *D*: overloading elongates VSMCs and increases applied forces. Long-term effects of ANG II produce a fibrotic response and promote formation of larger focal adhesions and actin stress fibers. *E*: acute inward eutrophic remodeling of a small artery during functional vasoconstriction. The reduction in lumen diameter is associated with an increased media/lumen (M/L) ratio and no change in wall cross-sectional area (WCSA; i.e., eutrophic) since it is an acute phenomenon. Long-term effects of ANG II or mineralocorticoid receptor activation lead to further remodeling, characterized by acute inward hypertrophic remodeling, i.e., an increase WCSA associated with the reduction in lumen diameter and the increase in wall-to-lumen ratio. Acute inward remodeling can be also transformed into a chronic inward remodeling, for instance, during essential hypertension.

A

Clinical hypertension
MR antagonists
↓Aortic PWV
↓Small-size artery
stiffness

Clinical hypertension — ACE or AT1 blockers
↓Aortic PWV or no changes

Aldosterone

ANG II

AT1

NADPH oxidase

EGFR

EC

STAT3 JAK2

MMP2

MR
Nongenomic

Genomic

ROS

Pro-MMP2

Cav1.2 L-type calcium channel

Ca²⁺

RhoA

NFκB

ALP, BMP2 → **Calcification**
FN, α_v, α_5, integrins → **FA**
Gal-3, Col, NGAL → **Fibrosis**
VEGFR, PGF → **Proliferation**

Aldo-salt treatment in rat
↑FN
↑Young's elastic modulus

**Contraction
Myogenic tone
Cell stiffness**

RhoK

ERK1/2
JNK1/2
Myd88

NFκB

TGF-β
ICAM
MCP-1
Procol I
EGFR
MMP2
IL-6, CT-1

VSMC

Mice lacking VSMC-MR
↓KCl contraction
↓Myogenic tone
Unchanged arterial stiffness in response to aldo-salt

TP

TLR4

COX1

Chronic ANG II in mice
↑Young's elastic modulus in mesenteric artery

CT-1 KO mice
↓Young's elastic modulus

B

Normal loading

Optimal stiff matrix

Acute contraction

Increased force

C

Baseline

Acute contraction

Vasoconstriction

D

Acute overloading

Deformed matrix

Chronic overloading
Angiotensin II

Abundant stiff matrix

E

Normal

Remodeling

Angiotensin II
MR activation

↓Lumen diameter
↑M/L
= WCSA

Inward euthrophic

↓Lumen diameter
↑M/L
↑WCSA

Inward hypertrophic

elastic modulus and increased life span in mice caused by a reduction of the apoptosis response, NFκB pathway activity, and premature senescence in VSMCs (310, 311).

AT1R activation by systemic or local ANG II signaling is an important determinant of arterial stiffness since it induces collagen and fibronectin accumulation and MMP activation. In clinical hypertension, there is no clear evidence whether angiotensin converting enzyme (ACE) inhibitors and AT1R blockers decrease arterial stiffness independently of BP reduction (269, 319, 495, 534). However, at the level of VSMCs, ANG II increases indirectly the expression of latent pro-MMP-2 via EGFR and JAK2/STAT3 pathways, whereas in ECs it directly involves JNK1 signaling. In the aorta, the final activation of pro-MMP-2, mainly expressed by VSMCs, is more complex and likely requires the presence of endothelium (234). Acute administration of ANG II produces vasoconstriction (FIGURE 4, B AND C), whereas chronic administration of ANG II in rats decreased the wall-to-lumen ratio and distensibility and increased collagen accumulation and the Young's elastic modulus of small mesenteric arteries (FIGURE 4, D AND E). This occurs partly through Toll-like receptor 4 (TLR4) activation, a proinflammatory agent in VSMCs. In this model, the phosphorylation of JNK1/2, MAPK, and the myeloid differentiation factor 88 (MyD88)-dependent activation of NF-κB are reduced in response to TLR4 blockade, suggesting that TLR4 is a potential link between oxidative stress and hypertension-induced arterial stiffness (175). Another mediator of ANG II-induced structural alterations and stiffness of resistance arteries is the cyclooxygenase-1 pathway which produces contractile 6-keto-PGF$_{1\alpha}$, a metabolite of prostacyclin acting on prostanoid thromboxane receptors expressed on VSMCs (557). The production of pro-inflammatory molecules [IL-6, monocyte chemoattractant protein-1 (MCP-1) and TGF-β] induced by ANG II hastens leukocyte/macrophage recruitment and ECM turnover, thereby promoting fibrosis of the entire vessel wall including the adventitia (196) ANG II is considered a main regulator of the cross-talk between proximal and distal arteries. Increased aortic stiffness transmits higher pulsatile flow to the microcirculation. In the presence of high pulsatile flow, EC-induced ANG II production is able to increase the degree of VSMC muscularization with higher expression of differentiation markers such as α-actin and SM-MHC (483).

III. ARTERIAL STIFFNESS IN RELATION TO PULSATILE HEMODYNAMICS

A. Arterial Stiffness: Definition and Measurement

The stiffness of the arterial wall is determined by the intrinsic properties of its constituents, their relative proportions,

and their three-dimensional organization and interconnectivity. As the composition and organization of blood vessels varies over the arterial tree, so will their stiffness (FIGURE 5). The major ECM proteins are elastin and collagen, with Young elastic moduli in the range of 100–600 kPa and 10–100 MPa, respectively (27). When inflating an artery in an ex vivo setting over a large enough pressure range, the typical nonlinear pressure-area relation is found (254), with the slope representative of stiffness (FIGURE 6). At low strains, the relation is determined by the distensible elastin; it is only at higher strains that the very stiff collagen is progressively recruited (i.e., waviness in collagen fibers disappears) and stretched, progressively bearing the load and stiffening the vessel. In physiology, arterial stiffness is generally quantified by *functional* indices that integrate the intrinsic properties (e.g., the Young's elastic modulus for a linear elastic material or the incremental elastic modulus, Einc, characterizing the stiffness around a certain working point) of ECM and cellular arterial wall components and their organization, as well as geometrical factors such as the size of the vessel and its thickness.

At the local level, the most generic index of arterial stiffness (or rather its reciprocal) is the distensibility coefficient (DC) defined as $DC = \dfrac{dA/A}{dP}$, with dA being the (infinitesimal) change in lumen cross-sectional area from its value A, and dP the corresponding (infinitesimal) change in pressure. Defined as above, DC is dependent on BP and will typically decrease over the physiological BP range, as described above.

In clinical practice, DC is usually calculated as $DC = \dfrac{\Delta A/A_d}{\Delta P}$ with ΔA being the diastolic-to-systolic change in lumen cross-sectional area from its value at diastolic pressure (A_d) and ΔP the local PP. This yields one single value, which can be seen as an average value over the BP range. DC can be measured using any invasive (pressure catheter, intravascular ultrasound) or noninvasive techniques [applanation tonometry, ultrasound, MRI, computed tomography (CT)] that provide lumen cross-sectional area (or diameter) and BP at a sufficiently high resolution. In reality, DC is most often measured making use of ultrasound wall tracking that allows for accurate diameter (and distension) measurement: $DC = \dfrac{2\Delta D/D}{\Delta P}$ (note the factor 2!). DC is expressed in mmHg^{-1} or Pa^{-1}.

Because of the incompressibility of the blood and the distensibility of arteries, the pulse generated by the heart travels at finite wave speed (the PWV) along the arterial network. The locally measured DC is easily converted into a (local) PWV by means of the Bramwell-Hill formula: $PWV = \sqrt{\dfrac{1}{\rho DC}}$ with ρ being the density of blood ($\sim$1050 kg/m^3) (49). Other methods to determine local PWV exist. One class, the single point PWV
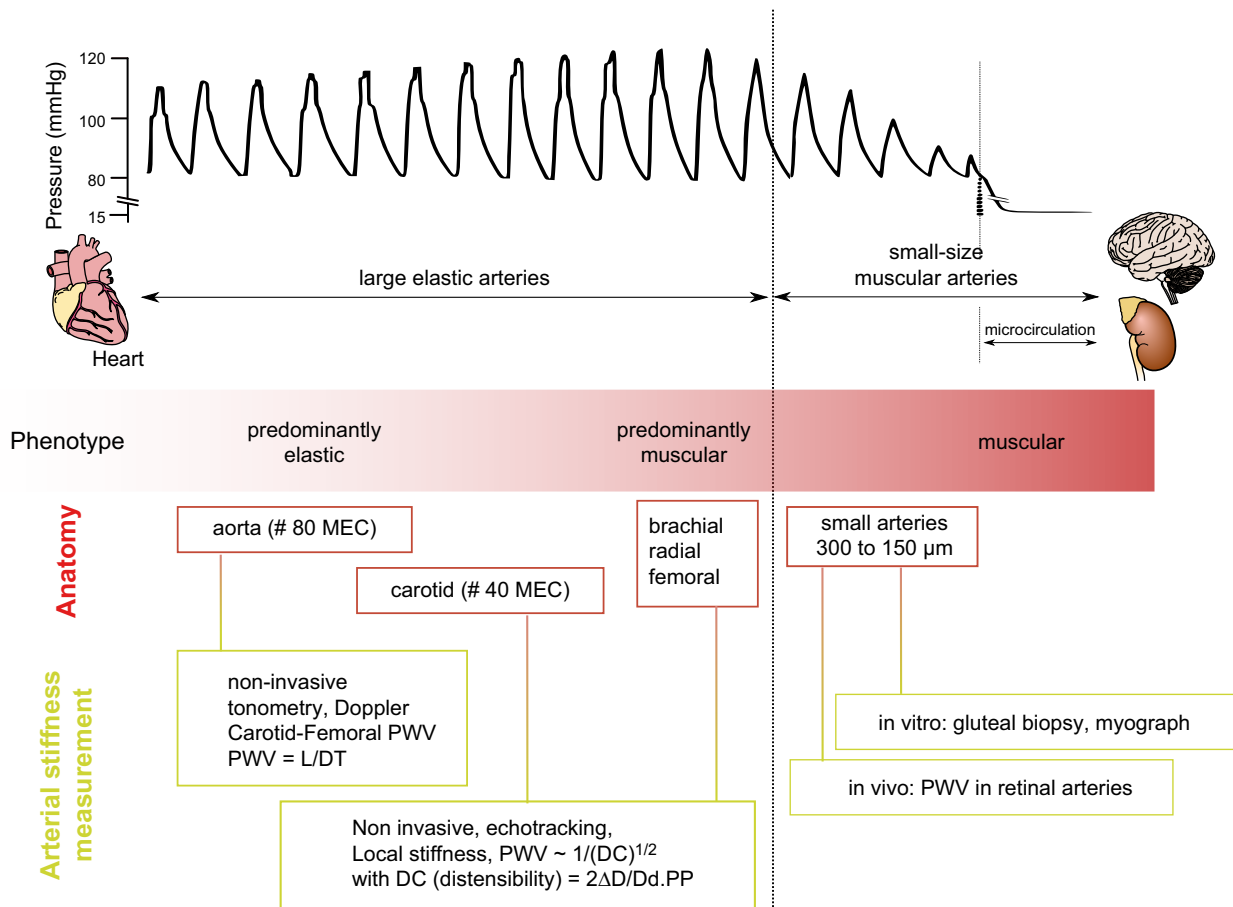
FIGURE 5. Arterial stiffness parameters. The methodology for measuring arterial stiffness depends on the arterial territory, i.e., the size of the artery and its location. The gold standard for the measurement of aortic stiffness is carotid-femoral pulse wave velocity (PWV). Local PWV can also be calculated as the inverse of the square root of distensibility [PWV = $1/(\text{dist})^{1/2}$]. MEC, musculo-elastic complex; ΔD; stroke change in diameter; Dd, diastolic diameter; PP, local pulse pressure.

measurement, is basically a variant of the Bramwel-Hill equation and is based on the simultaneous local measurement of cross-sectional area and flow, diameter and flow, or BP and flow velocity (230, 431). These methods are, however, very susceptible to local wave reflections and have been shown to be unreliable for the common carotid artery (489). Interesting advances are also taking place in the ultrasound community. On the one hand, ultrafast imaging modes are being explored to measure directly the propagation of the pulse wave in superficial vessels (though again with limited success for the common carotid artery) (174, 243). On the other hand, elastography methods are being explored that aim to track the propagation of shear waves in the tissue (90). The propagation speed of the shear waves is directly proportional to the shear and Young's elastic modulus of the tissue. Validation of this novel technique is pending, but when successful, it would provide a unique clinically applicable tool to measure stiffness directly.

PWV is also the gold standard index to quantify the stiffness of the aorta (269). In this case, PWV is not measured locally (although this can also be done) but regionally over a region

spanning the aorta (or parts of it). PWV is then derived from (minimally) two measurements along the aortic path as $L/\Delta T$, with L being the distance between the two measuring sites and ΔT the time it takes for the pulse to travel from site 1 to site 2. The arrival of the pulse can, in principle, be detected with any technique that allows to invasively or noninvasively measure BP (applanation tonometry), flow velocity (ultrasound, MRI), arterial distension (ultrasound, MRI, CT) or volume changes [(photo)plethysmography], or even pulse-induced mechanical perturbations at skin level (vibrometry, accelerometers). Despite the fact that the path traveled by the pulse wave is not unequivocal and that different distance measurements have been used, there is a consensus (at least in Europe and the United States) that the current reference method in clinical research is carotid-femoral PWV, with measurements at the carotid and femoral artery (549). Based on this technique and pooling data from nearly 17,000 subjects, reference values for PWV have been determined, demonstrating that even in normal adults with optimal BP, PWV practically doubles from a value of ~5 m/s at the age of 20 to over 10 m/s at the age of 80 (531a). Given the above
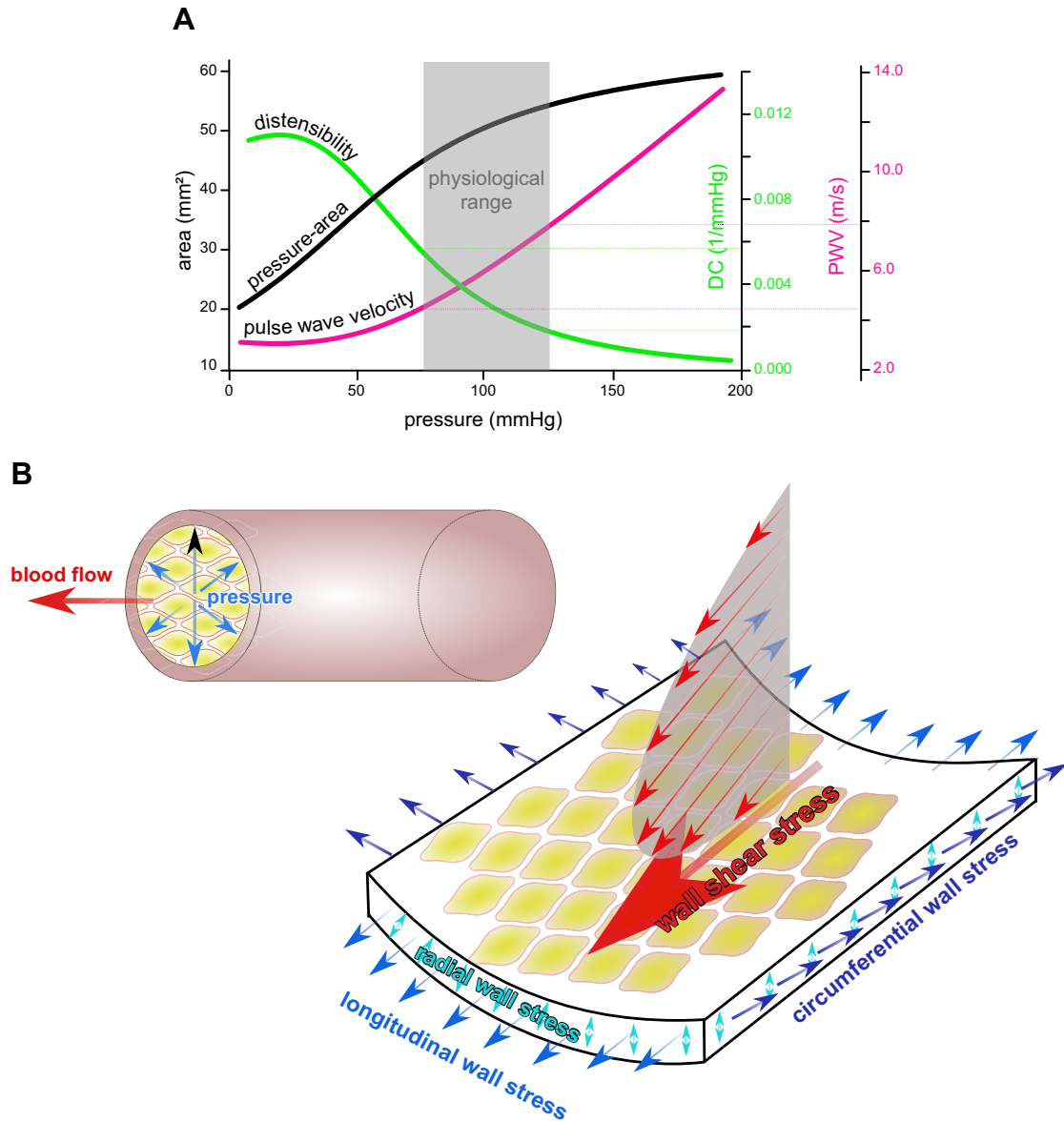
A

B

FIGURE 6. Arterial stiffness parameters and hemodynamics. *A*: the black curve illustrates the typical nonlinear relation between blood pressure and the lumen cross-sectional area of arteries when pressure varies over a large pressure range. Upon inflation, the load bearing of the artery is transferred from elastin to the stiffer collagen, explaining the nonlinearity. The green curve (with *y*-axis on the *right*) is the distensibility of the artery which decreases with pressure. Concomitantly, the pulse wave velocity (PWV, magenta curve, with *y*-axis on the *right*) increases with increasing blood pressure. It is explained in the text how distensibility and pulse wave velocity are calculated from the pressure-area relation. The gray shaded area indicates the in vivo physiological pressure range, the working point of the artery. *B*: blood flow exerts a tangential stress, the wall shear stress, on the endothelial surface (indicated by the red arrow). Blood pressure, on the other hand, leads to stresses inside the arterial wall (blue arrows). The stress field is composed of stresses in *1*) the circumferential direction (which can be approximated by Laplace law), *2*) the radial direction, and *3*) the longitudinal direction.

equation, this implies that the distensibility of the aorta is reduced by a factor of 4 over that age range.

In recent years, aortic PWV has also been measured with MRI. Different sequences and signal processing algorithms have been developed and explored, but the technique is accurate (151). MRI allows the derivation of segmental PWV. Recent studies demonstrated that it is possible to measure PWV over the aortic arch and that a loss in distensibility of the aortic arch is one of the earliest manifestations of arterial aging (436). It has been shown also that the ascending and thoracic aortas stiffen faster with age than the abdominal aorta in humans (101). Drawbacks of MRI, however, are the cost of the equipment, long scanning times, and limited access to and availability of scanners. At the other end of the spectrum, cuff- and sensor-based de-

vices have been developed for routine assessment of PWV-like measurements providing transit-time measurements between brachial and ankle, heart and ankle, finger and toe, etc . . . The accuracy and validity of these and other devices is under investigation and should be addressed with care. We refer to Reference 272 for a recent review.

All of the above methods pertain to large and medium-sized arteries. Unfortunately, the toolkit to assess the stiffness of smaller muscular vessels is much less developed. There are a few reports on PWV measurements in retinal arteries, showing elevated PWV in hypertension (239), but the field is limited. Study of the biomechanical properties of small resistance arteries remains ex vivo, where small resistance vessels obtained via gluteal biopsies can be studied on the pressure myograph (236).

B. Arterial Stiffness: A Passive Property Modulated by VSMC Tone

The modulating role of VSMC tone on arterial stiffness is easily demonstrated ex vivo by performing pressure inflation tests in organ baths. Experiments were, among others, conducted by Dobrin (109), testing contracted and relaxed cylindrical segments of dog carotid artery and human internal mammary artery. While VSMC contraction tended to stiffen the artery up to 150–250 mmHg, the contracted vessel exhibited decreased stiffness at higher pressures (109).

Gaballa et al. (136) determined the BP-radius relation in vivo in carotid arteries from 6- and 23-mo-old F344XBN rats. In 6-mo-old rats, activation of VSMCs reduced vessel diameter, but also enhanced the Young's elastic modulus measured at 200 mmHg, thus destiffening the vessel at high pressure. In 23-mo-old rats, the difference between active and passive properties was greatly reduced. VSMC tone thus modulates arterial stiffness differently during aging (136).

Seminal work on the impact of hypertension on the passive and active properties of arteries was done by Cox in rat models of renal hypertension (91) and later in dogs (92), demonstrating a considerable regional variability of changes in arterial wall in response to hypertension. More recent data were collected by Fridez et al. (132) in a rat (ligation) model of acute hypertension. Pressure-diameter curves were measured in vitro under normal, maximally contracted, and totally relaxed VSMCs. Basal VSMC tone was found to rapidly increase in the acute hypertension phase (2–8 days postsurgery), but decreased towards control values at 56 days postsurgery. It was postulated that VSMC contraction may act as a first, rapid defense mechanism of the arterial wall. As such, time can be gained for the slower geometrical and structural remodeling to restore the biomechanical environment and function of the arterial

wall to control VSMC tone levels (132). Computer model simulations suggest that such a vasoconstrictive response to increased BP can decrease the magnitude and transmural gradients of the BP-induced wall stresses and return the mean wall shear stress toward its homeostatic value (197). VSMC contraction has been also demonstrated to play a role in the overall rheological behavior of the arterial wall, with VSMC contraction inducing a large degree of hysteresis (viscous energy dissipation) in inflation-deflation tests and pressure-radius loops in rabbit (150) and human carotid arteries (11). In any case, the potential modulating role of VSMCs will depend on the relative amount of VSMCs (of the contractile phenotype) in the arterial wall. In large elastic arteries, the effect of VSMC contraction probably plays a role via a redistribution of tensile forces between elastin and collagen (149). Its action may unload collagen fibers and de-stiffen the artery, though it might require overall constriction to reach this unloading effect, which may be rare in vivo (110). Since VSMC contraction actively changes diameter, it is important to specify whether measurements are done under isobaric (constant pressure) or isometric (constant diameter) conditions. In vivo measurements at the brachial artery, for instance, demonstrated no impact on (incremental) Young's elastic modulus of VSMC contraction under isobaric conditions, while large changes were found under isometric conditions in thoracic aorta of conscious dogs (18) and in the human brachial artery (16).

From a biomechanical perspective, data are best interpreted in terms of stress-strain relations, although it is not easy to truly assess stress and strain in vivo due to the presence of initial and residual stresses in the arterial wall (87), and the impossibility to determine the unloaded configuration. It is also interesting to observe that biomechanical models, describing the constitutive behavior of arterial tissue, are extending from models accounting for the passive behavior of the arterial wall [e.g., strain-energy based models (140, 183, 579)] to models incorporating the contribution of VSMC tone (562, 600) and complex remodeling laws (548).

C. Arterial Stiffening in Relation to Systemic Hemodynamics and Pulsatile Load

The importance of the distensibility of the large arteries is often functionally translated to the "cushioning" function of the large arteries, where the aorta serves as a compliant, damping reservoir (a "windkessel"), converting the pulsatile flow from the heart into a more damped outflow towards the organs and tissues. While this interpretation of arterial hemodynamics provides an elegant way to get insight into cardiovascular pathophysiology, systemic hemodynamics are nowadays addressed more commonly in terms of wave physics, with BP resulting from the interaction of a forward wave generated by the heart, and waves reflected in the periphery. Both the windkessel and wave

interpretations are intricately related; they only provide two different paradigms to assess arterial hemodynamics and to interpret the arterial input impedance, which is the most general way to quantify the arterial load (354, 356, 374, 382, 392, 406).

As demonstrated by Elzinga and Westerhof (119) in isolated cat hearts loaded with a hydraulic model with independent control over arterial resistance and compliance, a decrease in total arterial (windkessel) compliance causes an increase in PP (i.e., an increase in pulsatile load), through mainly a decrease in diastolic and mean aortic BP, and only modestly via an increase in systolic BP. It is thus only through a concomitant increase in systemic vascular resistance, which is generally the case in vivo via compensatory mechanisms, that an increase in stiffness increases systolic BP. This mechanism has been confirmed repeatedly using computer simulations based on windkessel models (28, 488, 520).

Randall et al. (434) inserted a stiff tube in the aorta of six closed chest anesthetized dogs. Consistent with a 63% decrease in compliance (119), systolic BP increased by 18%, while diastolic BP decreased by 24%. However, mean BP did not change significantly but cardiac output fell by 21%, implying an increased vascular resistance. Decreased compliance mainly caused changes in the low-frequency range of the input impedance (434). Ioannou et al. (208) wrapped a Teflon prosthesis around the aortic arc to limit proximal aortic compliance in Yucatan miniature swine. Banding decreased compliance by 52%, with an increase in systolic (37%) and PP (87%). Diastolic BP, mean BP, cardiac output, and systemic vascular resistance did not change significantly (208).

In the above animal experiments, increased stiffening was induced via a local impediment of aortic distensibility, which is different from human pathophysiology, where arterial stiffening is generally not confined to a segment of the aorta. Using a one-dimensional computer model of the arterial tree, Reymond et al. (445) simulated the effect of local aortic stiffening with compliance reduced only in the region of the aortic arch, or globally, with an equivalent uniform decrease in compliance in all arterial segments. Both scenarios yielded the same increase in PP, but the underlying mechanisms are different. Local stiffening in the region of the aortic arch mainly augments the forward pressure wave through an increase in proximal characteristic impedance. Global stiffening, on the other hand, leads to an increased contribution of wave reflections. Which of the two mechanisms drives the increase in systolic BP with aging or in disease will depend on the topology changes in arterial stiffness and arterial geometry (445).

Invasive measurements of input impedance in humans demonstrated an increased peripheral vascular resistance by 37% over the age range of 20–60 yr, whereas characteristic impedance (a functional property of the proximal aorta depending proportionally on aortic stiffness and inversely proportional on aortic diameter) increased by 137%. The observed patterns of the input impedance were consistent with the ascending aorta becoming stiffer with age, accompanied by a decrease in the cross section of the peripheral vascular bed. These phenomena lead to an increased PWV and wave reflections with age (385). The changes in input impedance are mirrored in the shape of the pressure waveforms. In subjects younger than 30 yr, early systolic BP usually exceeds late systolic BP (the waveform is of the so-called type C) due to the arrival of the bulk of the reflected waves in late systole and early diastole. In subjects older than 50 yr, the impact of the reflected waves occurs earlier in systole, boosting late systolic BP (type A pressure waveform) (228, 374, 385). Similar effects, though more pronounced, play a role in patients with isolated systolic hypertension (383).

The increased load, obviously, also impacts the heart. Chronically increased arterial stiffness by aortic wrapping leads to left ventricular (LV) hypertrophy in pigs after 60 days (207). Three-month exposure to increased aortic stiffness in vitamin D_3-nicotine rats induces LV hypertrophy with moderate interstitial fibrosis and a shift in the MHC-isoform pattern, though maintaining LV performance (256). In humans, the increase in vascular load with aging may account for the observed decrease in stroke volume (23%, $P < 0.025$) and cardiac output (20%, $P < 0.005$) and the development of mild LV hypertrophy and prolonged relaxation with advancing age (385).

When considering the pulsatile load on the heart, the "sequence of events" is important, as the sensitivity to systolic load of the contracting left ventricle increases progressively throughout the ejection period. The relaxation rate of the left ventricle decreases more with late than with early BP increases (142). The timing of wave reflection during the cardiac cycle may thus have an important effect on LV relaxation and coronary flow (583). This was recently demonstrated in a large cohort of middle-aged subjects. Analysis of the timing of the sequence of loading events showed that subjects whose heart was experiencing prominent late systolic stress had a reduced longitudinal systolic function and a slower diastolic relaxation (84), despite the fact that peak myocardial stress occurs in early systole, before important contributions of reflected waves to central BP (83).

Attentive readers will have noticed that, in the above, the role of VSMCs in pulsatile load was not discussed. Pulsatile load can be modulated by vasoactive drugs such as nitrates, which act on VSMC tone. The response of arterial territories to nitrates is heterogeneous, with measurable vasodilatory effects on carotid, brachial, and femoral arteries which

are, however, not necessarily accompanied by a decrease in arterial stiffness (261). Nonetheless, nitroglycerin has a large effect on systemic hemodynamics. It was demonstrated in dogs that nitroglycerin led to a reduction in the amplitude of reflections from the periphery, and delayed the arrival of these reflections at the aortic root. These effects are visible in the impedance modulus and phase patterns (shifted to the left) and indicate a shift of the reflection sites towards the periphery (258). This is consistent with observations in humans, where vasoactive drugs drastically reduced aortic augmentation index in healthy men, independent of aortic PWV (229). About 25 yr ago, O'Rourke (405) stated that antihypertensive drugs have little or no direct effect on arterial stiffness. However, several pharmacological studies demonstrated that this is not the case. Drugs can reduce wave reflection via modulation of vascular tone, and hence decrease aortic BP augmentation (an effect which might not be picked up when measuring in a peripheral artery) (405). An acute direct effect, i.e., independent of BP reduction, of the calcium channel blocker diltiazem (515) on aortic stiffness was demonstrated through the BP-diameter curve. A delayed, long-term direct effect of 1) the ACE inhibitor perindopril (538) and 2) the AT1R blocker olmesartan (262) was demonstrated on carotid stiffness and aortic stiffness, respectively. In contrast, the beta-blocker celiprolol was able to stiffen the carotid artery wall, after 4 yr administration in patients devoid of hypertension (403).

D. Arterial Stiffness and Baroreceptor BP Control

The carotid and aortic baroreceptors are located in the wall of large proximal, elastic arteries, and "sense" the level of BP, possibly via stretch-sensitive neural pathways. As such, a loss of the artery's ability to stretch under BP, due to arterial stiffening, would directly impede baroreceptor functioning. Arterial stiffening and attenuation of baroreflex sensitivity (BRS) are processes that typically go hand in hand with aging or in patients with hypertension. The key question is whether there is any causal relation between both. As illustrated below, literature on the topic is somewhat mixed.

In a small-sized study in the early 1990s, Lage et al. (251) concluded that although both carotid arterial compliance is abnormal and arterial baroreflex regulation of heart rate is attenuated in patients with hypertension, reduced arterial compliance is not solely responsible for baroreflex dysfunction in these individuals. A stronger position with respect to the role of reduced carotid artery compliance in BRS was taken by Monahan et al. (362), who concluded that carotid artery compliance was the strongest independent physiological correlate of cardiovagal BRS, explaining 51% of the total variance. Regular exercise in previously sedentary humans increased both carotid artery compliance as well as BRS (363).

Classically, fluctuations in BP are used to assess global BRS, which is the result of both vascular (dependent to the arterial stiffness) and neural components of the baroreflex. However, baroreceptors respond to deformation and not to BP per se. Therefore, peripheral changes in BP might not accurately reflect changes in carotid bulb distension in subjects with increased arterial stiffness, making global BRS a poor indicator of the neural component of the baroreflex. Moreover, both vascular and neural components of the baroreflex can be jointly or singularly altered in several pathological conditions. To overcome these limitations, Kornet et al. (235) measured directly diameter distension using ultrasound and found that, rather than the absolute change in diameter, the rate of distension of the common carotid artery was a considerably more accurate predictor of R-R interval variability [more accurate also than the variability in systolic (finger) BP]. In that same study, the authors ascribed the reduced BRS in the elderly mainly to a deterioration of conduction by the neural baroreflex pathways (235). Using the same methodology, Zanoli et al. (588) showed that, compared with controls, subjects with metabolic syndrome had a lower neural component of the baroreflex and higher carotid stiffness than age- and sex-matched control subjects. The neural component of the baroreflex was positively associated with carotid stiffness in controls, but this association was lost in subjects with metabolic syndrome. The determining role of carotid distensibility as such in BRS was also questioned by Steinback et al. (517) in a head-up-tilt protocol study, who found maximal carotid distensibility, which occurs in early systole, to contribute to reduced cardiovagal BRS with head-up-tilting tests.

Important data sources on the topic are the Rotterdam study, including elderly people, and the Young Finns study based on young adults. In the Rotterdam study, subjects with higher arterial stiffness (quantified by carotid-femoral PWV) experienced a higher drop in BP during orthostatic intolerance testing, without a significant change of heart rate, than subjects with lower arterial stiffness. This observation led to the conclusion that arterial stiffness may explain, at least in part, the reduced baroreflex observed in older adults (346). In a follow-up publication on the same database which also included measurement of carotid distensibility, a stronger position was taken with the conclusion that arterial stiffness appears to be an independent determinant of impaired BRS (345). Carotid stiffness and BRS were also measured in the Young Finns study. In 1872, healthy 24- to 39-yr-old subjects, carotid distensibility significantly related with all measured components of heart rate variability, supporting the hypothesis that reduction in carotid artery wall elastic properties may lead to low vagal tone (237).

Baroreflex control runs via both the vagal and sympathetic branches of the autonomic nervous system. In a study focusing on the association between carotid stiffness and the response in sympathetic activity to changes in BP, Okada et

al. (401) found elderly women to have a lower sympathetic BRS than elderly men. Elderly women also exhibited higher carotid artery stiffness. The authors concluded that barosensory artery stiffness seems to be one independent determinant of sympathetic BRS in elderly men and women (401). The interaction between arterial stiffness and BRS was recently addressed in a computer model study, modeling the integrated cardiovascular system including baroreflex control. The model predictions were dependent on the stipulated relation between arterial distensibility and baroreflex signaling (i.e., BRS). It was demonstrated that arterial stiffening, via its effect on BRS, seems sufficient to explain age-related emergence of hypertension and the impaired capacity of hypertensive individuals to regulate short-term changes in BP (416).

E. Cross-Talk Between Large Elastic Arteries and Small-Sized Muscular Arteries

Small and large artery alterations in normal and accelerated aging are closely interdependent. A simple straightforward cause-effect relation is difficult to establish, and a cross-talk, by which large elastic artery alterations appear to influence a small-sized muscular artery phenotype, and conversely small artery alterations appear to influence a larger artery phenotype, is more likely (267).

With normal aging, there is a moderate increase in the level of peripheral resistance (see above), despite a likely increase in lumen diameter and media thickness in small-sized muscular arteries without a change in media-to-lumen ratio, indicative of outward hypertrophic remodeling (FIGURE 7) (129, 372, 400). The increase in brachial and central systolic and PPs with aging is due to the stiffening of large arteries that increases the speed of reflected waves, and to the increase in geometric taper that generates more wave reflection (384). Indeed, normal aging exerts opposing effects on proximal elastic arteries that enlarge, and distal muscular arteries (common femoral, brachial, and radial arteries) that do not (43, 45, 60, 209). Thus normal aging increases the geometrical taper in large arteries which in turn aggravates the difference of impedance between small and large arteries (impedance mismatch), generates wave reflection, and limits transmission of pulsatile energy into the microcirculation. There is, however, a parallel reduction in the stiffness gradient between proximal large elastic
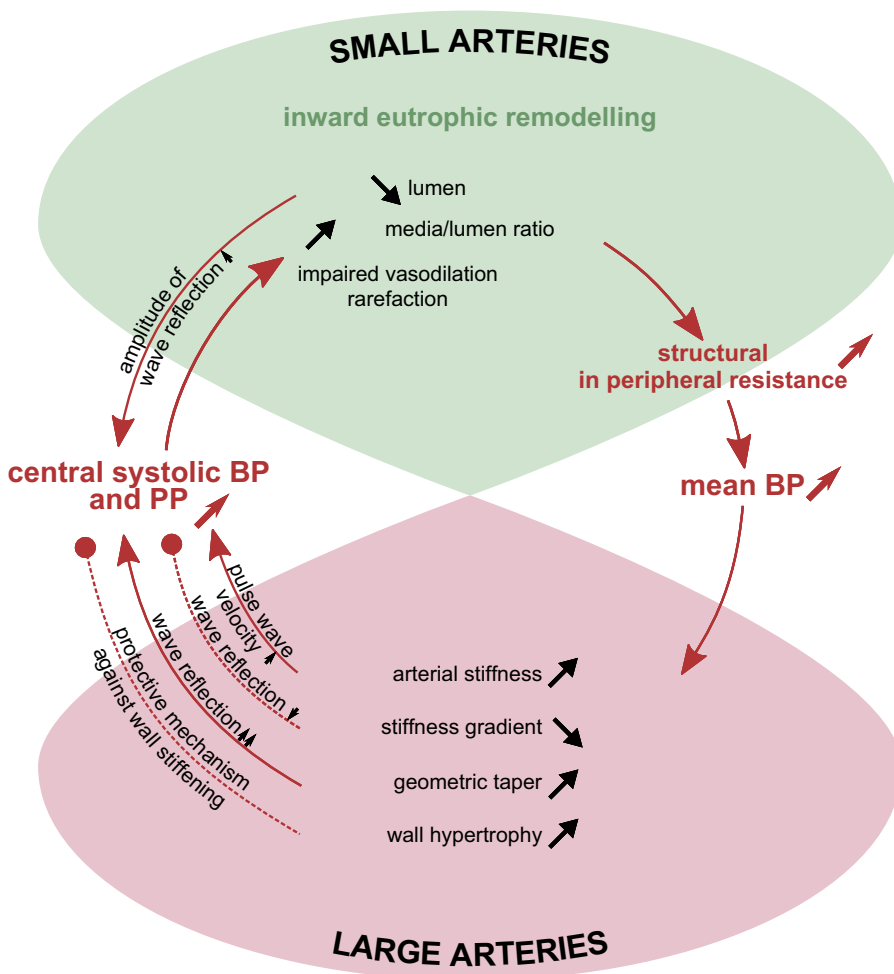

FIGURE 7. Cross-talk between the large artery and microcirculation. Reduced lumen diameter of small arteries and impaired vasodilation contribute to increased peripheral resistance to blood flow which increases mean blood pressure (BP). The increase in brachial and central systolic pressure as well as pulse pressure (PP) with aging are due to the stiffening of large arteries (that increases the speed of reflected waves) and to the increase in geometric taper (that generates more wave reflection). The reduction in the stiffness gradient between small and large arteries and the increase in arterial wall hypertrophy, as an adaptative mechanism in essential hypertension, tend to limit the increase in central pulsatility.

arteries (stiffening more rapidly) and distal medium-sized muscular arteries, which tends to reduce the impedance mismatch (43, 45, 60, 209, 257, 270, 550), and exerts opposite effects on transmission of pulsatile energy that is exaggerated. Altogether, the balance between these opposite mechanisms leads to a higher central pulsatility, which when transmitted to target organs could damage the kidney, the brain, and the heart, and maintains the outward hypertrophic remodeling of small-sized muscular arteries (129, 372, 400).

In primary (essential) hypertension, the cross-talk between small and large arteries is transformed into a vicious circle of aggravation (FIGURE 7). Starting the cross-talk from small-sized muscular artery damages, vasoconstriction and impaired vasodilation, reduced lumen diameter associated with increased wall-to-lumen ratio indicative of inward eutrophic remodeling and rarefaction of small arteries are major causes of the increase in total peripheral resistance and mean BP (129, 170, 372). The loading of stiff components of the arterial wall, mainly collagens, is responsible for the increase in large artery stiffness at high mean BP (266). The rise in central systolic BP and PP results from the synergistic action of the increase in large artery stiffness and structural alterations of small arteries contributing to an increase in the amplitude of wave reflection (384). The increased central PP is correlated with increased media-to-lumen ratio of subcutaneous small-size muscular arteries (370). The wall-to-lumen ratio of retinal arteries is also positively correlated with 24 h systolic BP (466). Interestingly, in hypertensive patients, changes of subcutaneous small-sized muscular arteries and carotid-femoral PWV are both independent determinants of central systolic BP (370).

F. Tensile Pulsatile Circumferential Wall Stress Is a Major Determinant of VSMC Differentiation and Arterial Remodeling: Bioengineering Concepts Applied to Cell Biology

Physical forces, ECM, and cell structure play a key role in the control of normal development, as well as in the maintenance of tissue form and function (200). Cardiovascular cells, including VSMCs, adjust the expression and synthesis of ECM molecules to adapt their environment to these changes (34, 156). Cyclic mechanical strain profoundly influences cultured VSMC orientation, growth, and phenotype and increases the secretory function of VSMCs leading to increased ECM protein production (574).

Wall stress is one of the lesser tangible mechanical quantities, which unfortunately cannot be measured, but needs to be estimated via biomechanical models (192). Most often, circumferential wall stress (σ_θ) is deduced from a continuum mechanics approach using Laplace's law. For an isotropic, homogeneous thin-walled cylinder (ratio of radius to wall thickness less than 10), $\sigma_\theta = Pr/h$, with P being the internal pressure, r the vessel radius, and h the wall thickness (FIGURE 6). With VSMC contraction leading to a reduction in vessel radius and an increase in wall thickness, VSMC tone can actively modulate the stress level to which the vessel is exposed. A factor that is commonly overlooked in vivo (as it cannot be measured) is the fact that arteries are also exposed to a longitudinal stress component (135, 192). Indeed, when arteries are excised from the circulation, they shorten significantly due to the unloading in the longitudinal direction (with values up to 50% for, e.g., canine and porcine aorta) (160). It may therefore be clear that the Laplace's law is an extremely crude way to estimate the (circumferential) stress component. It does not account for residual stresses, and the assumptions behind the formula are never met in biology. It cannot be used to obtain reliable estimates of absolute stress levels, but the general principle holds that circumferential stress is directly proportional to internal pressure and vessel radius, and inversely proportional to its thickness.

Considering that the organism is a highly regulated dynamic system, a basal level of VSMC tone would keep the vessel in a position from where the diameter can be quickly up- and downregulated, which is a mechanism that is especially important for small-sized muscular arteries regulating BP. Although the level of myogenic tone can be quite different from vessel to vessel (the smaller the diameter, the higher the tone), it is thought that active (VSMC) stress levels are quite constant throughout the arterial network (50). In a theoretical model study, Rachev and Hayashi (433) showed that basal VSMC tone reduces also the strain gradient across the thickness of the arterial wall and yields a near uniform stress distribution. During temporary changes in BP, the increase in myogenic tone induced by elevated BP tends to restore the distribution of circumferential strain in the arterial wall, and to maintain the flow-induced wall shear stress at normal level (433).

In the earliest bioengineering models described by Rachev (432), the active stress generated by VSMCs was simply added as an extra stress component in the stress balance equations. Later on, models gradually increased in complexity, integrating the vascular smooth muscle as a structural element into the models (e.g., via a pseudo strain energy function). The contribution of vascular smooth muscle to load bearing is then modulated by the contraction, which, for instance, also allows to integrate vascular smooth muscle myogenic tone in response to local increases in stretch into the models (600). Models also more and more account for the multi-layered nature of arteries, with properties that vary from the intima towards the media and adventitia, which provides additional insight into residual stress-related opening angle and the axial prestress (225). The most advanced models are now capable of predicting growth and remodeling of arteries, with models validated

most often using experiments in hypertension-induced experimental models (7, 600). These experiments and models have suggested that increasing VSMC tone is a first defense mechanism of the vessel against an acute increase in BP which lowers wall stress, and "buys time" for the vessel to respond to the increase in wall stress in a more structural way, by an increase in cell protein synthesis (hypertrophy) and ECM secretion (132). VSMCs also interplay with stress distribution over the arterial wall and the level of residual stress which builds up in remodeling vessels (7, 225, 433) [and which, e.g., can be visualized when cutting a ring segment of an artery, which will open up to a certain opening angle, which is a measure of internal residual stresses (87)]. These residual stresses are highly variable and change as the vessel remodels in the circumferential and longitudinal direction.

It is, however, important to understand that all of the above models are based on continuum mechanics, i.e., considering the arterial tissues as a continuous medium (though with complex anisotropic properties that are based on the ultrastructure of the arterial wall). Vessel remodeling responses are mediated by complex inter- and intracellular signaling pathways (249), which are likely to depend on the mechanobiology of the individual cell. How stresses and forces are transmitted to the level of the individual cells and its intracellular structures and components is likely to depend on the three-dimensional organization and interconnection of the ECM component and cytoskeleton (see mechanobiology above). A relatively popular conceptual model of cell biomechanics is the tensegrity structure [a structure composed of elements being either under compression (microtubules) or under tension (actin filaments)] (201). The tensegrity hypothesis implies that cell stiffness must increase in proportion with the level of the tensile stress (the prestress) (567), but the same behavior is also explained by the exponential stress-stretch relation of isolated actin filaments, without any prerequisite on the structure of the cytoskeleton (193). Other cellular bioengineering models consider the cell as a pressurized cytoplasm surrounded by a membrane under tensile stress (493, 596), but models confining all structural strength to the membrane cannot adequately capture in vivo observations during micro- and nanomanipulation of cells (331). We refer the reader to References 193, 202 for more complete discussions on the biomechanics of cells and their subcellular organelles and structures and bioengineering models of cell cytoskeleton with their respective strengths and limitations.

These cellular biomechanical models have, at present, little to no application in vivo. Nonetheless, it is possible to determine, using nonlinear models, the in vivo mechanics of common carotid arteries in humans. Thus it is possible to compare treated hypertensive patients with normotensive subjects, for wall stress and the contributions of wall microconstituents. Using a well-accepted theoretical three-dimen-

sional model of arterial mechanics, Masson et al. (340) obtained in vivo data from the human carotid artery, under noninvasive conditions and assuming an anisotropic, hyperelastic, active-passive, and residually stressed wall. To solve the quasistatic boundary value issue, a semi-analytical software was used over a cardiac cycle. Surrounding perivascular tissue was also accounted for. A nonlinear least-squares method estimated model parameters (intramural fibrillar collagen, elastin, and VSMCs) using the best-fit values. Temporal changes in intraluminal BP were captured by the model, as well as the estimated wall stress fields (reflecting age and disease effects) and possible changes in microconstituent mechanics. For instance, in normotensive subjects, age was positively and significantly correlated with residual stress and altered fibrillary collagen. These results thus indirectly validated the microconstituents in the model. In treated hypertensives, the level of stresses was higher, as was vascular tone. Stiffer elastin fibers were the main changes in ECM. These data, which were expected in response to aging and hypertension, have helped to increase our understanding of the contribution of microcontituents at the molecular levels of the cell-ECM relation to the mechanics of the arterial wall in vivo.

IV. VASCULAR SMOOTH MUSCLE CELLS AND LARGE ARTERY STIFFNESS DURING DEVELOPMENT AND NORMAL AGING

A. Shear Stress and Tensile Stress as Mechanical Factors for Arterial Development

In this section, we briefly touch upon biomechanical factors involved in large artery development. We refer to dedicated embryological literature for details on the development of the cardiovascular system. Biomechanical stimuli include the blood flow-induced tangential wall shear stress, sensed by the ECs, and tensile stress and strain (FIGURE 6). The wall shear stress (τ_w) is calculated as $\tau_w = \mu \dfrac{\partial u}{\partial y}$ with μ being the viscosity of the blood and $\dfrac{\partial u}{\partial y}$ the velocity gradient calculated at the endothelial interface (i.e., the slope of the velocity profile near the wall).

The primary vascular network that sets the basis for further development comprises nascent EC tubes (embryonic capillary plexus). This EC tube network is formed and grows in the absence of any blood flow and pressure, but blood flow is critical for the patterning and arteriovenous differentiation (218, 275). This is not surprising, given that blood flow and the resulting wall shear stress are key biomechanical stimuli in mechanotransduction. ECs cultured in the presence of unidirectional steady flow, for instance, will alter

their cytoskeleton and align in the direction of flow (102). Expression of growth factor genes have been demonstrated to be regulated differentially by fluid shear stress in the vascular EC (326). In embryological development, pulsatile shear patterns may be central for supporting arterial identity, with arterial gap junction alpha-5 protein (Gja5, also known as connexin 40) expression being suggested to play a functional role in arteriogenesis (59).

Concomitant with the establishment of blood flow, primordial VSMCs are recruited to align with the EC tubes (79, 198). The embryonic origin of these cells is not the same for the arterial tree (as described above). The recruitment of these cells and the phenotypic maturation are likely to depend on shear stress (79). At the level of the microcirculation, pericytes and VSMC may differentiate into each other in relation with arterial development. With the use of AFM, the pericytes of the microvasculature have been demonstrated to exert a direct mechanical stimulus at the EC-basement membrane interface through their effective actomyosin-mediated contraction influencing physiological angiogenesis (277). Such measurements have not been performed in adjacent VSMCs.

In embryonic development, pulsatile BP is generated as soon as the heart starts beating. Along with the gradual increase in BP, the VSMCs organize in layers around the vessel, increasing its thickness. In mouse embryos, this process is complete around day 14. The VSMCs then start expressing structural ECM proteins, so wall thickness increases through the addition of elastin and collagen between the cell layers (79).

Mechanical stretch is the most important biomechanical stimulus of vessel organization. In vitro experiments have demonstrated stretch-induced upregulation of the production of elastin and collagen (79), which can also be taken advantage of in tissue engineering (232). The mechanical stimulus resulting from the pulsatile stretch is detected by the VSMCs through multiple sensing mechanism. The stimulus is translated, via mechanosensing pathways, into intracellular signals that modulate the function of cells and the expression of certain genes, leading for instance to cell proliferation, migration, apoptosis, and vessel wall remodeling. (FIGURE 2) (157). Mechanical stretch has an impact on numerous signaling molecules (79), among which the Notch pathway, a key regulator of vascular morphogenesis, controlling growth of the blood vessel network, cell proliferation, and the differentiation of arteries and veins (451).

B. Normal Aging of VSMCs

Aging of VSMCs includes DNA damage and telomere attrition, epigenetic modifications, defects in protein processing, reduced nutrient sensing, mitochondrial dysfunction, and reduced stem cell availability (312, 547). These hallmarks of aging cause an inflammatory response and thickening of intima by VSMCs. The consequences are the progressive loss in the immune privilege of the medial layer and increased aortic stiffness which define arteriosclerosis, thereby promoting the development of vascular diseases including atherosclerosis (see below). Progenitor cells such as multipotent stem cells are present into the adventitia and may represent another source of VSMCs migrating into the intima with aging (539). Increase of proteasome activity and endoplasmic reticulum stress characterize the process of unfolded protein response in VSMCs also observed in monocytes and ECs (484).

Canonically, VSMCs undergo phenotypic switching towards proliferation, migration, apoptosis, and senescence with aging as reviewed previously (364). Regarding proliferation, the mechanisms are an increased expression of milk fat globule protein epidermal growth factor-8 (MFG-EGF8) together with an enhanced VSMC responsiveness to PDGF. However, contradicting results have been reported regarding age-related proliferation and migration of VSMCs. These discrepancies are related to in vitro/in vivo culture conditions, animal models of aging, methodologies to assess VSMC changes, or types of vessels. Similarly, variations in signaling pathways such as cell cycle activators (cyclin-A, cyclin-D1, cyclin-dependent kinase-2) or inhibitors (P27Kip1) as well as Akt or telomerase activation may also occur. Increased levels of ANG II, MCP-1, calpain-I/C-C chemokine receptor type 2 (CCR2), MGF-EGF8, and MMP-14 associated with the decrease of MMP tissue inhibitor of MMP-2 (TIMP-2) lead to VSMC migration. All of these key molecules are organized in the positive-feedback loop combining the effects of colocalized MCP-1, MMP-2, and TGF-β1 (566). In addition, production of MMPs by VSMCs and alterations of cell-ECM interactions reduce VSMC plasticity and the capacity of tissue repair. The contribution of mature VSMC and progenitor cells may explain some of the conflicting results for the age-related differences in the phenotypic switch during aging. The controversy may also be explained by the critical role of endothelial aging on VSMC functions. Proliferation and migration of VSMCs is controlled by Notch receptors and their ligands, including Jagged1, through MAPK and PI3K/Akt pathways. Age-associated downregulation of Jagged1 endothelial expression known to control VSMC expression of Jagged 1 enhances VSMC proliferation and migration, independently of NO release (578). Thus the loss of endothelial regeneration capacity aggravates intimal thickening in aging. There is compelling evidence that vascular wall proteoglycans modulate the effects of growth factors. Production of sulfated proteoglycans by VSMCs from large and small arteries contributes to reduce proliferation in response to ANG II (447, 448), but the question still remains open in the context of the aging-induced proinflammatory state.

In aging, VSMCs undergo an inflammatory phenotype that does not necessarily require the involvement of inflammatory cell infiltration. Activation of proinflammatory and oxidative pathways in VSMCs occurs via several receptors including those for advanced glycation end products (AGE), AT1R, lectin-like oxidized LDL receptor (LOX-1), TLRs, and Nod-like receptors (NLRs) (295). ANG II and aldosterone represent the main factors in inflammatory VSMC behavior leading to chronically elevated levels of low-grade inflammatory molecules such as MCP-1/CCR2 receptor, adhesion molecules (ICAM-1, VCAM-1, MMPs), various cytokines (IL-6, cardiotrophin-1), chemokines (CCL2), and calpain-I. An excessive production of ROS mainly by increased expression and activity of NAD(P)H oxidase is not downregulated by an increased activity of antioxidant enzymes. The production of AGEs and the cross-talk between calpain-I and MMP-2 influence markedly the mineralization of VSMCs and the development of calcification (see below). The proinflammatory state of VSMCs reinforces the effects of inflammatory cells on VSMC senescence. Oxidative stress represents the main cause of epigenetic modifications with aging (see below) such as global DNA hypomethylation of VSMCs in patients with atherosclerosis (421) or more specifically of the anti-oxidant enzyme superoxide dismutase gene (259).

It appears more difficult to assume a unique effect of aging of VSMC apoptosis. One of the key modulators is the activation or not of the cGMP-specific phosphodiesterase type 5 (PDE-5)-mediated cGMP degradation which depends on NO signaling which can be differently affected by the aging process. The maintenance of a high activity of PDE-5 caused an increased level of VSMC apoptosis with age. In aged rats, it has been reported that larger, less mobile, and highly elongated mitochondria may hasten these VSMC functional changes, in particular at the level of cerebral small arteries (71). The loss of mitochondrial function may be caused by only mitochondrial DNA damage or together with oxidative stress (587). ACE2 resulting in generation of Ang 1 to 7 (126) exerts a protective effect on small-sized muscular artery remodeling and arterial stiffness with age by reducing ANG II-induced VSMC apoptosis, increased MMP activity, and oxidative stress. The loss of ACE2 in mice leads to an excessive aortic dilation and aneurysm in response to ANG II (412).

VSMC senescence induced either by replication or stress induction and revealed by nonspecific β-galactosidase staining is characterized by the loss of arterial tissue repair and regeneration. Endogenous sirtuin-1 (SirT1) deacetylase expression, one of the main causes of dysregulated nutrient sensing, was shown to be reduced in older human donor cultured VSMCs compared with young donors, as well as in VSMCs from atherosclerotic-diseased arteries compared with VSMCs from a non-diseased section of the same artery (146, 532). This was associated with decreased capacities to proliferate and to migrate in response to stimulation. The molecular signature is an upregulation of γH2AX, p27/p21, and acetylated p53, markers of DNA damage and telomere shortening, cell cycle inhibition, and cellular stress response, respectively. Likewise, shorter telomeres and low level of telomerase have been reported in VSMCs in atherosclerotic plaques (347, 399). It has been reported that telomerase activation, maintenance of telomere length, and decrease of p53 tumor suppressor protein expression promote aortic VSMC proliferation and suppress apoptosis in SHR (65). In the context of accelerated vascular aging, it has been reported that VSMC SirT1 can reduce arterial stiffness in diet-induced metabolic syndrome in mice (133). Invalidation of VSMC SirT1 also promotes abdominal aortic aneurysm formation through VSMC senescence and NF-κB-mediated transcription of MCP-1 chimiokine (75).

Age-related VSMC stiffness accelerates aortic stiffness observed in hypertension (430, 490). However, if VSMC stiffness similarly increases with age in both the thoracic and the abdominal aortas, the stiffness increases more in the abdominal than in the thoracic aorta in monkeys. This result contrasts with previous studies in humans (101) and is attributed mainly to the highest values of collagen-to-elastin ratio and a marked increase of structural disarray of elastin and collagen fibers in the abdominal compared with the thoracic aorta both in young and old monkeys (592). Decreased expressions of cytoskeletal desmin and a shift towards calpain I-mediated vimentin cleavage together with an increase of MHC contribute to reduced VSMC integrity and contractile competence with age (36, 215). In aorta and iliac arteries, the expression of genes coding for MYL9, integrin α_1, B cell leukemia/lymphoma 2 (Bcl2), VCAM-1, and NOX4 is increased, whereas expression of collagens and VEGF-A are downregulated with age. The overexpression of MYL9 occurs mainly in the EC layer, suggesting its implication in EC contractility and the subsequent increase in vascular permeability. It is only in response to vascular injury that an increased expression of VSMC MYL9 is observed during the early steps of cell proliferation (501).

Stimulation of α_1-adrenoceptor or depolarization with elevated extracellular potassium increased in vitro isometric contraction, more in small-sized muscular arteries than in large elastic arteries from adult mice (280). These discrepancies are related to higher production of basal NO in the elastic arteries which are also less sensitive to the L-type Ca^{2+} channel blocker. The hypothesis of different isoforms of L-type Ca^{2+} channels together with endothelial dysfunction has been suggested to explain the higher level of age-related stiffness in elastic arteries. The ECM crosslinking enzyme tissue-transglutaminase (TG2) present in ECs and VSMCs contributes to the arterial stiffness increase with age. TG2 may also control the activation of TGF-β complex. It has been reported in mouse aorta that its activity is normally maintained at a low level via a NO-dependent

S-nitrosylation action. The reduction of NO bioavailability in aging leads to a higher level of TG2 activity, increasing large arteries stiffness. High levels of transglutaminases and TGF-β activation as well as accumulation of AGE in aged humans are in agreement with this hypothesis (467). The role of the TG2/NO interaction has been confirmed using eNOS knockout mice (220).

C. Arterial Wall Mechanical Properties in Normal Aging

Normal aging exerts opposing effects on proximal large elastic arteries and distal small-sized muscular arteries **(FIGURE 8)** (26, 45, 209, 253). High-resolution echo-tracking systems (26, 45, 209) and MRI (435) have shown that age-induced enlargement predominates in humans on proximal elastic arteries, such as the common carotid artery or the aorta (aortic root and aortic arch) where it is associated with a decrease in aortic arch curvature. This enlargement is generally attributed to load-bearing degradation of elastin fibers. Indeed, age, heart rate, and carotid PP are independent determinants of carotid lumen diameter (43).

Similarly, age-induced arterial stiffening predominates on proximal elastic arteries, with no effect on distal medium-sized arteries, e.g., brachial, radial, and femoral arteries (26, 45). The total amount of VSMCs and ECM (especially elastin) is much higher in the media of large proximal elastic arteries than in medium-sized distal muscular arteries (108). In addition, the amplitude of stroke change in diam-

eter is 10-fold higher at the site of the carotid artery than at the site of the radial artery (26, 45). The influence of mechanical stretch (see above) on growth and apoptosis of VSMCs is involved strongly (279, 444). Structural alterations that occur with aging are associated with changes in both active (reduced number of VSMC nuclei) and passive stiffness (reduced elastin-to-collagen ratio) (136).

The age-induced arterial stiffening attenuates the stiffness gradient throughout the arterial tree. The stiffness gradient is characterized by the fact that, in middle-aged healthy humans, PWV increases from 4–5 m/s in the ascending aorta to 5–6 m/s in the abdominal aorta, thence to 8–9 m/s in the iliac and femoral arteries (257); concomitantly cross-sectional distensibility decreases from 40 kPa$^{-1} \times 10^{-3}$ in the thoracic aorta (209) to 15–25 kPa$^{-1} \times 10^{-3}$ in the carotid (26, 45, 556) and brachial (550) arteries, 10–15 kPa$^{-1} \times 10^{-3}$ in the common femoral artery (26, 45, 556), to 5 kPa$^{-1} \times 10^{-3}$ in the radial artery (270). The impedance mismatch between large elastic and small-sized muscular arteries at the origin of partial wave reflections has been detailed above. Altogether, the balance between the opposite effects of age-induced geometrical tapering and reduction in stiffness gradient leads to a reduction in central BP and pressure amplification (brachial systolic BP - central systolic BP) with aging, that plateaus after the age of 30 (172).

The age-induced geometric tapering may be larger than the sole balance of the loss of stiffness gradient. Indeed, Segers
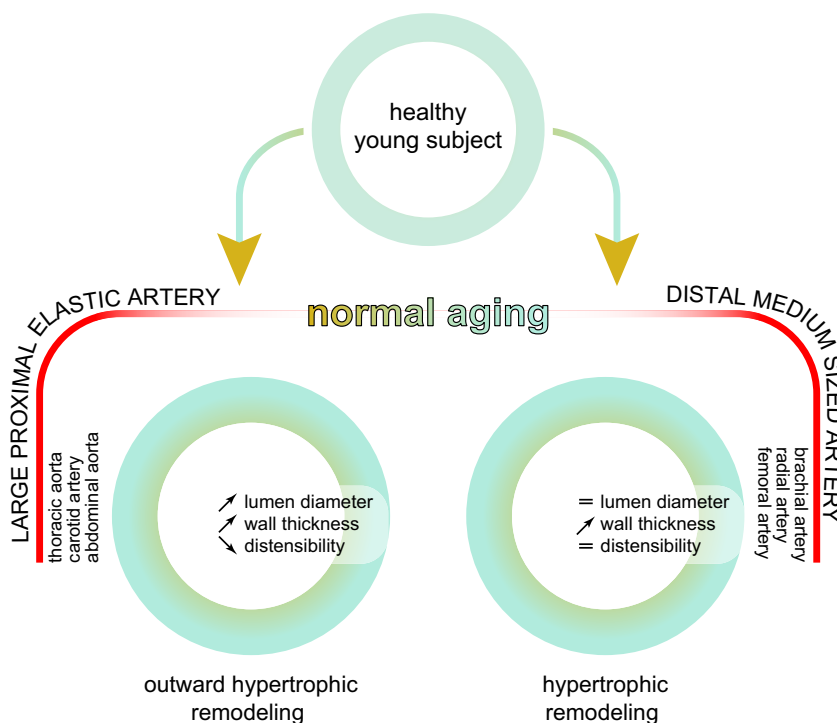

FIGURE 8. Different types of arterial remodeling with normal aging according to the location and size of large arteries. Large proximal elastic arteries, such as the thoracic aorta, the carotid artery, and the abdominal aorta, enlarge (outward remodeling) and stiffen with aging. Large proximal arteries may contain up to 80 musculo-elastic complexes (ascending aorta), whereas distal muscular medium-sized arteries contain a smaller number. Distal muscular medium-sized arteries do not enlarge with aging, and their distensibility remains unchanged. At both sites, normal aging is associated with media hypertrophy (hypertrophic remodeling). =, no change.

et al. (487) showed an increase in PWV of 15% in healthy middle-aged subjects, between the age of 35 and 55, as well as an increase in augmentation index, reflection coefficient, and reflection magnitude and no change in characteristic impedance. They concluded that the increase in aortic stiffness was not accompanied fully by an increase in arterial impedance and suggested that there was a role for age-dependent modulation of aortic cross-sectional area.

D. Age-Related Phenotypic Changes in VSMCs and Medial Calcifications

Arterial calcification is recognized as one of the main causes of arterial stiffness and considered as an independent risk factor in heart failure (99). The pathogenesis of calcifications is multifactorial, implicating factors inducing and those opposing it, along with plasma constituents maintaining minerals in solution and inhibiting tissue mineral deposition (190, 381, 498). Elevated extracellular inorganic phosphate (P_i) affects multiple signaling pathways leading to VSMC mineralization. Complex changes in miRNA expression are often referred to as master regulators mediating VSMC transdifferentiation to osteoblast-like cells (reviewed in Ref. 281). Calcium and P_i are synergistic to induce calcification. The primary mechanism by which P_i enters VSMC is type III Na-dependent P cotransporters (PiT-1 and 2). The major mechanism whereby elevated extracellular calcium and P_i drives VSMC calcification is via release of phospholipid-bound matrix vesicles recently identified as exosomes originating from intracellular multivesicular bodies (222). The most reliable markers of exosomes are tetraspanins CD9 and CD63. Elevated extracellular calcium induces expression of sphingomyelin phosphodiesterase 3 (SMPD3) and cytoskeletal remodeling that regulates exosome biogenesis and VSMC calcification (223). These matrix vesicles containing alkaline phosphatase and annexins provide nucleation complexes for crystalline hydroxyapatite deposition within the ECM in both media and intima. An increase in P_i leads also to an early expression of MMP-9 responsible for ECM degradation. Exosomes contain also miRNA-143 regulating SRF and FAs thus potentially controlling migration and proliferation of VSMC. Inhibitory factors such as the vitamin K-dependent γ-carboxyglutamic acid protein (MGP) and fetuin-A loaded in vesicles limit calcium deposits. Circulating fetuin-A taken up by VSMC and subsequently loaded into ECM vesicles stabilizes the mineralization process. Osteoprotegerin exerts also a protective effect on vascular calcifications by blocking the binding of the receptor activator of NF-κB ligand (RANKL) to its receptor RANK on the osteoblastic precursor cells (231). Binding of RANKL to RANK activates both canonical and alternative NF-κB pathways as well as increases bone morphogenetic protein (BMP)-4 production (411). An imbalance in the RANK/RANKL/osteoprotegerin axis orchestrates a cross-talk between bone metabolism and vascular calcifications (576).

Whatever the type of calcification, high levels of osteo/chondrogenic markers including BMP-2, osteopontin, and Runx2/Cbfa1-dependent alkaline phosphatase characterize VSMC transdifferentiation. The Wnt/β-catenin and the cAMP signaling pathways are known also to be key regulators of osteo/chondrogenic differentiation. Similarly to VSMCs in the media and aortic intima, pericytes in microvessels and myofibroblasts in the adventitia can differentiate in osteoblasts.

Vascular calcification is classified into intimal atherosclerotic calcification or medial calcification independently of atherosclerosis or calcific uremic arteriolopathy in arterioles. In atherosclerotic calcification, environmental factors such as inflammatory cytokines, monocyte-macrophages, oxidative stress, and oxidized lipids initiate mineralization of subpopulations of VSMCs. The ability of monocytes to ingest hydroxyapatite crystals may per se accentuate the inflammatory response (378). The time course of nodule formation is governed by interaction between BMP-2 and its inhibitor MGP. The question of the use of warfarin, a vitamin K antagonist in coronary diseases but which also acts as an MGP inhibitor in VSMCs, is not well solved. Medial calcification is associated strongly with type 2 diabetes mellitus, CKD, and aging. Calcifications are preferentially located along elastin fibers surrounding VSMCs in aorta and peripheral small-size muscular arteries. VSMCs in calcifications express markers of senescence including, prelamin-A, BMP-2, and IL-6 that accelerate the osteogenic differentiation. miRNA-mediated overexpression of Runx2 suppressing myocardin/SRF regulation of VSMC contractile proteins is the basic mechanism leading to arterial stiffening and decreased compliance related to arterial calcifications (576).

E. Coupling Between Vascular Inflammation and Remodeling

Chronic low-grade inflammation is well accepted as a major determinant of large elastic artery and small-sized muscular artery remodeling, particularly in hypertension (205, 325). This relation between vascular inflammation and remodeling is partly dependent on activation of the renin-angiotensin-aldosterone and the endothelin systems (see above).

Remodeling of the small artery wall, which mainly targets the ECM, is triggered by increased oxidative stress and production of growth factors, such as TGF-β, PDGF, IGF, and basic fibroblast growth factor (213, 476). Adhesion molecules contribute to the inflammation-induced remodeling of the small-sized muscular arteries, particularly by reorganizing ECM-VSMC interactions and influencing the phenotypic modulation of VSMCs (206). A large number of cellular components of both the innate and adaptive immune systems mediate this type of remodeling: monocytes, macrophages, mast cells, natural killer cells (13), lympho-

cytes and the cytokines they produce (470). Schiffrin et al. (474) showed that the ANG II-induced remodeling of small arteries involved effector T cells such as T-helper (Th) 1 [producing interleukin (IL)-2, tumor necrosis factor-β, and interferon-γ] and Th2 lymphocytes (producing IL-4, IL-5, IL-6, and IL-10), as well as Th17 and T suppressor lymphocytes.

Chronic low-grade inflammation is also well accepted as a major determinant of the remodeling of large arteries. Large artery stiffening has been reported during various diseases associated with chronic low-grade inflammation, such as rheumatoid arthritis (325, 453), systemic lupus erythematosus (453), systemic vasculitis (38), human immunodeficiency virus (HIV) (485), and inflammatory bowel disease (589). Various mechanisms have been suggested, including endothelial dysfunction, activation of VSMC-MR related to ANG II, cell release of a number of inducible MMPs, elastocalcinosis and accumulation of proteoglycans in the media, and finally adventitial immune cells and cytokines released from the vasa vasorum in response to vessel ischemia (263). Interestingly, in untreated patients with essential hypertension, aortic stiffness (assessed through carotid-femoral PWV) was significantly correlated with high-sensitivity C-reactive protein and IL-6 (321).

F. Early Vascular Aging: Concept and Measurement

The concept of EVA was elaborated in 2008 (391) and further developed in additional publications (388, 389). The main idea is that increased arterial stiffness and PP are major independent determinants of arterial aging and cardiovascular risk. Recently, O'Rourke et al. (383) included this notion in the "cardiovascular ageing continuum" (397).

In contrast to optimal aging, which can be considered as a balance between the damaging effects of mechanical, metabolic, and chemical stresses and the repair mechanisms, EVA is rather a defect of repair mechanisms in face of various stresses. EVA reinforces the cross-talk by which small artery alterations influence large artery phenotype, and conversely large artery alterations influence small artery phenotype, as described above, into a vicious circle of increased peripheral vascular resistance (structural part), increased large artery stiffness, increase in central BP, mean levels and variability of 24 h ambulatory brachial BP (478), and ultimately target organ damage. EVA is observed typically in young hypertensive patients who display an increased Young's elastic modulus compared with older hypertensive patients or normotensive individuals (60).

The additive effects of adult life risk factors along with fetal programming caused by intrauterine growth retardation, which is often followed by rapid catch-up growth (17, 158), a hallmark the EVA process, also named the "early life developmental origins" of disease (390). This concept has been called also the "mismatch" hypothesis (144), which better depicts a mismatch in the environmental conditions in utero versus at birth (preprogramming of the fetus in utero is a major challenge in research at the moment). Fetal growth retardation leads to multiple dysfunctions, for instance, on glucose metabolism based both on changes in insulin sensitivity and β-cell function (163, 351), hemodynamic control (480), neuroendocrine regulation (486, 569), and kidney function (177). With regard to embryonic vascular development and the adult vascular system, endothelial dysfunction including capillary rarefaction (159, 418) and reduced aortic diameter and diastolic BP (287) are associated with impaired fetal growth when compared with normal fetal growth.

Vascular aging and more specifically EVA is increasingly investigated in humans using high-resolution noninvasive measurements of arterial stiffness indexes such as carotid intima media thickness (IMT), central BP, and endothelial damage parameters (389, 391). Particularly, normal and reference values of arterial stiffness, measured by carotid-femoral PWV, have been established in 16,867 subjects and patients originating from 13 different centers within several European countries (531a). PWV increases with age. The increase with age is more pronounced (i.e., EVA) for higher BP categories and more cardiovascular risk factors (531a).

These above indices may serve as arterial "tissue biomarkers." Their predictive values compared with classical "circulating" biomarkers, such as high-sensitivity C-reactive protein used to assess inflammation, are not definitely established. The consensus today focuses more on the use of mixed biomarkers to improve their individual predictive value (46, 591). However, the tissue biomarkers reflect the integration of several events but not the contribution of specific cell types of the vascular wall. Particularly, in an individual participant meta-analysis of prospective observational data from 17,635 subjects (25), arterial stiffness measured by carotid-femoral PWV proved to be a significant predictor of coronary heart disease, stroke, and cardiovascular events, independent of classical cardiovascular risk factors. Moreover, there was a significant interaction with age: the younger the subjects, the higher the predictive value (25). Altogether, these data on arterial stiffness validate the concept of EVA and its implementation in clinical practice.

V. GENE EXPRESSION PROFILING IN ARTERIAL STIFFNESS FOCUSED ON VASCULAR SMOOTH MUSCLE CELLS AND ECM

Heritability studies indicate a moderate to substantial genetic contribution to carotid artery structure or arterial stiffness, with estimates ranging from 0.18 to 0.62 (357, 393, 472). Additional evidence in support of a substantial genetic predisposition for accelerated arterial stiffening

comes from recent twin studies revealing that the genetic predisposition for accelerated arterial stiffening dominates over shared and unshared environmental components and age contributions (69, 70, 352).

A. Transcriptional Biomarkers

The correlation between specific patterns of genes and a quantitative trait of arterial stiffness, i.e., PWV, has first been investigated in aorta biopsies originating from coronary heart disease patients undergoing coronary artery bypass grafts (116). The functional analysis of genes expressed differentially in patients with higher versus lower aortic stiffness and/or correlated with PWV revealed that most annotated transcripts were related to the mechanical regulation of vascular structure, cell signaling/communication, or gene expression. In stiff human aortas, upregulation of the gene encoding the phosphoinositide-3-kinase regulatory subunit polypeptide 1 (p85α) and downregulation of genes coding for protein phosphatase-1, catalytic subunit, β isoform (PPP1CB), or A kinase (PRKA) anchor protein (yotiao) 9 (all involved in VSMC signaling driving contraction) support a role of VSMC tone in arterial stiffening (116). With the aim to unravel the genetic components of hypertension-associated arterial remodeling, transcriptional profiling of aortic media after N^G-nitro-L-arginine methyl ester (L-NAME) administration identified three biologically relevant patterns of gene expression changes. The first pattern related to VSMC proliferation including CDC-2, CKS-2, cyclin A, and the transcription inhibitors Id1, Id2, and Id3. The second pattern comprised genes coding for components of ECM such as osteoadherin, periostin, osteopontin, fibronectin, thrombospondin-1, the latent TGF-β binding protein-2, SMAD6, and SMAD7. The third group of genes belonged to the cell signaling/communication class and orchestrating the control of VSMC tone, in particular the genes coding for the soluble guanylate cyclase and RGS-2 (115).

Further investigations provided genome-wide screening of mRNA expression in various clinical settings where VSMCs play a crucial role. An overexpression of genes involved in VSMC migration and proliferation, in particular lumican (LUM) and ornithine decarboxylase (ODC1), was reported in patients with CKD (122). However, the contribution of these two genes to molecular pathways in VSMCs leading to arterial stiffening needs to be confirmed. A transient increase in MLC gene (MYL9) expression with age in VSMC layers in mechanically injured arteries suggested a role for Myl9 protein phosphorylation state in age-related alterations of vascular contractility (501). Actin-binding Rho activating protein (ABRA) has been identified as a regulator of arteriogenesis based on an overexpression of this gene triggering VSMC proliferation via Rho signaling in a model of fluid shear stress-induced collateral artery growth (536).

Two independent research groups have demonstrated differentially expressed apoptosis-related genes in atherosclerosis. Martinet et al. (336) found that nine genes were upregulated and eight were downregulated in human carotid endarterectomy specimens compared with nonatherosclerotic mammary arteries. Among these differentially expressed genes, the death-association protein kinase (DAPK) gene was upregulated approximately fivefold and predominantly in VSMC-derived foam cells. Although DAPK is a proapoptotic cytoskeleton-associated serine/threonine kinase that belongs to the calmodulin-regulated kinase superfamily, the induction of type I (apoptosis) or type II (autophagy) programmed VSMC death by DAPK overexpression in atherosclerotic plaques remains unknown. Marchetti et al. (334) found higher B cell CLL/lymphoma 3 (BCL3) mRNA levels in VSMCs cultured from atherosclerotic carotid arteries than in nonatherosclerotic segments. Bcl-3 is a member of the inhibitor of NF-κB (IκB) family involved both in the positive and negative regulation of NF-κB target genes and is endogenously located in the cytoplasm and nucleus of VSMCs. In addition to its role in cell death, Bcl-3 is also involved in VSMC proliferation since deubiquitination of Bcl-3 via the enzyme CYLD (cylindromatosis), thereby preventing its nuclear translocation and subsequent activation of the NF-κB-dependent cyclin D1 pathway (341), inhibited VSMC proliferation (528).

Gene expression profiling using cDNA array analysis focused on cell cycle gene was also used to identify novel genes or pathways that may contribute to VSMC proliferation. A marked downregulation of genes encoding minichromosome maintenance (MCM) proteins 6 and 7 through peroxisome proliferator-activated receptor γ (PPARγ) activation allowed elucidation of a molecular pathway leading to regulation of DNA replication in VSMCs by this nuclear factor (56).

B. Genetic Components of Quantitative Traits by Genome-Wide Association Studies

Based on prior pathophysiological knowledge of arterial stiffness, the search for candidate genes associated with the structure and the function of the arterial wall has pointed to numerous common polymorphisms in genes encoding molecules of the renin-angiotensin-aldosterone system, elastic fiber structural components, MMPs, inflammatory cytokines, β-adrenergic and endothelin receptors, and the NO pathway, all involved in VSMC phenotypic modulation (TABLE 4) (246). However, most of these genes except CYP11B2 (14, 422) and NOS3 (78, 357) were not positioned within the chromosomal regions identified by genome-wide linkage studies as being associated with arterial stiffness. Genome-wide association studies (GWAS) which do not rely on any prior biological hypothesis represent the most relevant genetic approaches since arterial stiffness and

Table 4. *Gene polymorphisms associated with arterial stiffness and VSMC functions*

Gene	Polymorphism or Marker	Chr	Type of Genetic Study	Number of Subjects	Arterial Parameter Associated	P Value	Role of the Encoded Protein in VSMC Phenotype or Function
CYP11B2	rs2717594	8	GWAS (286)	644	Carotid-femoral PWV	0.003	ANG II-stimulated VSMC proliferation (580)
	rs1799998		GWLS (14)	441	Pulse pressure	Suggestive	
NOS3	rs3918226	7	GWLS (357)	590	Forward waves	Suggestive	VSMC proliferation (273)
MEF2A	rs3138597	15	GWLS (357)	590	Forward and reflected waves, carotid-femoral PWV	Suggestive	VSMC proliferation, migration, and senescence (595)
CHSY1	122 cM	15	GWLS (357)	590	Forward and reflected waves, carotid-femoral PWV	Suggestive	VSMC apoptosis (73)
ADD2	94 cM	2	GWLS (357)	590	Carotid-femoral PWV	Suggestive	To be elucidated
PACE4	rs900414	15	GWLS (357)	590	Forward and reflected waves, carotid-femoral PWV	Suggestive	Unknown
FURIN	rs6227	15	Gene-centric array (139)	61,619	Mean arterial pressure	3.65×10^{-9}	VSMC migration and apoptosis (545)
	rs4702		Genome-wide expression quantitative trait loci (542)	1,428	Systemic vascular resistance index	0.005	
TACR1	94 cM	2	GWLS (357)	590	Carotid-femoral PWV	Suggestive	To be elucidated for VSMCs
ADRA2B	94 cM	2	GWLS (357)	590	Carotid-femoral PWV	Suggestive	VSMC contraction (35)
IL6	29 cM	7	GWLS (357)	590	Carotid-femoral PWV	Suggestive	VSMC migration and proliferation (276)
MEF2C	rs770189	5	GWAS (286)	644	Carotid-brachial PWV	Suggestive 2.53×10^{-6}	Increase in VSMC differentiation (410)
SYNE1	rs1322512	6	GWAS (286)	644	Mean arterial pressure	Suggestive 7.76×10^{-6}	Marker of VSMC contractile phenotype (593)
COL8A1	rs792833	3	GWAS (286)	644	Reflected waves	Suggestive 6.01×10^{-6}	VSMC migration and apoptosis, focal adhesion formation (81)
PREX1	rs6063312	20	GWAS (286)	644	Reflected waves	Suggestive 2.09×10^{-6}	Rac-1-mediated fibronectin-dependent synthetic phenotype of VSMC (505)
TNFSF9	rs348384	19	GWAS (286)	644	Forward waves	Suggestive 1.16×10^{-5}	Collagen synthesis and VSMC proliferation (402) and VSMC apoptosis (219)
TNFSF11	rs10507514	13	GWAS (286)	644	Reflected waves	Suggestive 1.28×10^{-5}	VSMC calcification (411)
TGFBR2	rs3773643	3	GWAS (286)	644	Mean arterial pressure	Suggestive 1.99×10^{-7}	ECM synthesis and VSMC differentiation (594)
COL4A1	rs3742207	13	GWAS (531)	4,221	PWV	5.94×10^{-5}	Cell-basement membrane interactions of VSMCs (531)
BCL11B	rs7152623	14	Meta-GWAS (358)	20,634	Carotid-femoral PWV	1.0×10^{-11}	To be elucidated
ADM	rs11042717	11	GWAS (30)	4,155	Reflection index	$<10^{-4}$	VSMC migration and calcification (63)
ADAMTS7	rs3825807	15	GWAS (481)	80,849	Coronary artery disease	1.07×10^{-12}	VSMC migration (428)

Continued

Table 4.—*Continued*

Gene	Polymorphism or Marker	Chr	Type of Genetic Study	Number of Subjects	Arterial Parameter Associated	*P* Value	Role of the Encoded Protein in VSMC Phenotype or Function
INO80D	Ser818Cys	2	WES (496)	5	Hypoplasia		To be elucidated
CLEC16A	rs2903692	16	Exome array (427)	3,681	Internal diameter	4.3×10^{-7}	Unknown

See text for a full list of genes. Reference numbers are given in parentheses. GWAS, genome-wide association study; GWLS, genome-wide linkage study; WES, whole-exome study; cM, centimorgan; PWV, pulse wave velocity.

VSMC phenotypic changes are common complex traits influenced by genomic and environmental factors. Framingham Heart Study data provided the first demonstration of linkage regions for carotid-femoral PWV and identified potential candidate genes in these regions: the myocyte-specific enhancer factor 2A (*MEF2A*), insulin-like growth factor-1 receptor (*IGF1R*), chondroitin synthase (*CHSY1*), β-adducin (*ADD2*), proprotein convertases (*PACE4* and *FURIN*), neurokinin-1 receptor (*TACR1*), α_{2B}-adrenergic receptor (*ADRA2B*), and IL-6 (*IL6*) (357).

More contemporary studies are now focused on the analysis of genome-wide single-nucleotide polymorphism (SNP) associations using dense panels of common SNPs or whole-exome sequencing to target the human genome that is protein coding and identify rare variants with important phenotypic effects **(TABLE 4)**. The first GWAS of arterial stiffness, performed using a 100K panel of common SNPs and vascular/hemodynamic phenotypes (carotid-brachial PWV, forward and reflected pressure waves and mean BP), has identified some interesting candidate genes involved in arterial wall structure and function (286): myocyte enhancer factor 2C (*MEF2C*), spectrin repeat containing, nuclear envelope 1 (*SYNE1*), collagen type VIII α1 (*COL8A1*), phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1 (*PREX1*), tumor necrosis factor (ligand) superfamily member 9 (*TNFSF9*), tumor necrosis factor ligand superfamily member 11 (*TNFSF11*), and TGF-β receptor II (*TGFBR2*). *MEF2C* encodes a transcriptional regulator required for myocardin expression and VSMC differentiation (410). *SYNE1* codes for nesprin-1 which is a marker of differentiated, contractile VSMCs (593). *COL8A1* codes for type VIII collagen, a short-chain collagen upregulated in atherosclerosis and known to promote FA formation and VSMC migration to the intima while reducing VSMC apoptosis and to contribute to plaque stabilization (309). Type VIII collagen expression within carotid arteries is increased by oxidized phospholipids, and in particular 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine, through Sp1-induced activation of KLF4 (81). *PREX1* codes for a guanine nucleotide exchange factor for the Ras homologous (Rho) family of small GTP-binding proteins that bind to and activate Rac1 which is able to regulate the fibronectin polymerization-induced downregulation of α-actin and calponin and

enhancement of VSMC growth (505). *TNFSF9* encodes CD137 ligand which acts synergistically with proinflammatory cytokines to reduce collagen synthesis in VSMCs (402) and to induce the apoptosis of VSMCs, thus increasing the vulnerability of advanced atherosclerotic plaques (219). *TNFSF11* encodes RANKL, which is upregulated by the osteogenic transcription factor Runx2 (523). *TGFBR2*, directly downregulated by the miRNA-145 in VSMCs (594), encodes a receptor which plays a role in the regulation of ECM synthesis. Heterozygous mutations in *TGFBR2* are associated with decreased expression of contractile proteins causing a predisposition to aneurysms and dissections (199).

Further studies have been performed with a larger sample size and using more informative arrays. They identified new candidate genes, some of them being potentially involved in VSMC function **(TABLE 4)**. A nonsynonymous SNP in the collagen, type IV, α1 (*COL4A1*) gene encoding a major structural component of basement membranes that interferes with cell-ECM interactions and VSMC differentiation has been reported to be associated with PWV in the SardiNIA study (531). A meta-analysis of GWAS data in the AortaGen Consortium revealed an association between carotid-femoral PWV or an increased risk for coronay artery disease and a common genetic variation in a locus in the B cell CLL/lymphoma 11B (*BCL11B*) gene desert (358). This locus spans ~97.7 MB in which is located a linkage disequilibrium harboring a cluster of overlapping, spliced expressed sequence tags. *BCL11B* codes for the chicken ovalbumin upstream promoter transcription factor-interacting protein 2, a zinc finger protein that interacts directly with SirT1 to enhance transcriptional repression of various target genes, some of them could potentially be relevant to VSMC phenotype and function. A GWAS performed in subjects enrolled in the Gutenberg Health Study identified a SNP associated independently with adrenomedullin (*ADM*) gene expression and reflexion index, a marker of vascular tone of small-size resistance arteries (30). Because adrenomedullin, which is produced by a wide range of cells including VSMCs, has potent vasodilator and hypotensive effects (48) and is able to inhibit VSMC migration and calcification (63), this finding argues for a causal involvement of the encoded protein in the regulation of vascular tone. Several GWAS resolve the existing evidence of a non-

synonymous SNP at the ADAM metallopeptidase with thrombospondin type 1 motif, 7 (*ADAMTS7*) locus associated with coronary atherosclerosis (428). The causal involvement of this genetic variation in VSMC function is supported by its association with reduced VSMC migration and thrombospondin-5 cleavage, a substrate of ADAMTS7 disintegrin produced by VSMCs and involved in VSMC migration. Furthermore, variants associated with PP have been extensively studied and are the focus of several recent meta-analyses (535, 563).

More recently, whole-exome next generation sequencing, focusing on coding regions or exons, has proven to be an effective alternative to locus-specific and gene-panel tests in the research of new genetic bases of several diseases **(TABLE 4)**. Exome chips offers great potential for the identification of rare variants which may have a greater effect. Such an approach has already been developed to annotate a rare missense mutation in the inositol requiring 80 (INO80) complex subunit D gene (*INO80D*) as the causal variant for a syndrome of accelerated arterial aging (496). The INO80 complex is an adenosine triphosphate-dependent chromatin remodeling complex controlling cardiac gene expression during development (161), but its role in VSMCs remains to be elucidated. More recently, a common polymorphism of the C-type lectin domain family 16 member A (*CLEC16A*) gene was reported to be associated with the carotid internal diameter (427). Although the major identified biological function of CLEC16a is to control mitophagy/autophagy in pancreatic β-cells (508), the role of Clec16a in VSMC function is unknown.

C. Epigenetics

Master regulators of VSMC plasticity, including transcriptional cofactors, modulate the pattern of gene expression (409). More recently, epigenetic regulators have emerged as additional on-off switches. These include chromatin regulatory elements and pathways as well as non-protein-coding RNA (ncRNA) (511).

Most of the experimental evidence in support of a VSMC-specific epigenetic signature has been provided by studies in cell culture systems, although such systems do not compile environmental cues that regulate VSMC differentiation in vivo. PDGF-BB-induced phenotypic changes of cultured VSMC was associated with histone modifications (300, 586). The recent development of single-cell epigenetic assays allowing reliable tracking of VSMC-derived cells within artery lesions provides further arguments in favor of a stable and VSMC-specific enrichment of the methylation of histone 3 lysine 4 (H3K4me2) on the VSMC marker genes. Indeed, this epigenetic mark is acquired during VSMC differentiation and is retained during phenotypic switching or transition to other phenotypes (145). Emphasis has also been made on DNA demethylation governed by

members of the ten-eleven-translocation (TET) family of proteins. TET2 has been identified as a master regulator of VSMC plasticity that drives epigenetic changes both in differentiation and de-differentiation-associated VSMC genes (299).

Canonically, ncRNA can be divided into short ncRNAs including (<200 nucleotides long) miRNAs that control gene expression and long ncRNAs (lncRNAs) (length from 0.2 to 2 kb) which can target entire regulatory networks via the transcriptional and posttranscriptional regulation of gene expression, depending on their location in the genome. There are exciting recent reviews available on the role of ncRNAs as fine-tuners of the plasticity of VSMCs and as contributors to cardiovascular diseases (21, 320, 533, 543). Pertinent to the present review is the identification of miRNAs regulating arterial stiffness or lncRNAs in vascular cells controlling VSMC-related pathways involved in arterial stiffening. RNA sequencing uncovered a human-specific, vascular cell-enriched 5′ overlapping antisense lncRNA named smooth muscle and EC-enriched migration/differentiation-associated long noncoding RNA (SENCR) (23). Silencing of this lncRNA, which is preferentially localized in the cytoplasm and exists as two spliced variants, resulted in a reduced contractile gene signature and an increase in two promigratory genes in cultured human coronary artery VSMCs. As the list of identified miRNA controlling VSMC functions grows, miRNA-29 has emerged as a multifaceted regulator that may be involved in vascular stiffness through posttranslational repression of genes coding for key components of the ECM including type III collagen $\alpha1$ chain (see sect. VIB), type IV collagen $\alpha5$ chain, elastin, and MMP-2 (85). Age-related epigenetic hypomethylation of the miRNA-203 promoter is associated with a decrease in FA signaling proteins Src, caveolin-1, and paxillin, which impair dynamic FA signaling and actin sytoskeleton remodeling pathways, thereby increasing VSMC stiffness (386). The new concept of SNPs located within a miRNA binding site in critical protein-coding genes contributing to arterial stiffening is very exciting. In support of this theory is the detrimental effect on arterial stiffness of a minor allele of a SNP enhancing the binding of the miRNA-765 to the 3′-untranslated region of the apelin gene (*APLN*) resulting in the downregulation of apelin expression and thereby in an increased vascular tone by reducing eNOS activity via the inhibition of ERK/Akt/AMPK signaling (293). Chromatin modifications related to histone H3-lysine-4 trimethylation (H3K4me3) and histone H3-lysine-36 trimethylation (H3K36me3) reveal in the rat genome a novel lncRNA dynamically upregulated by ANG II. This lncRNA may serve as the host transcript for miRNA-221 and miRNA-222, both known to enhance VSMC proliferation (284).

Evidence for an in vivo contribution of epigenetic mechanisms in arterial stiffness has been accruing steadily over the

last two years. In old mice, endothelium-specific overexpression of SirT1 improved endothelium-dependent relaxation to acetylcholine and reduced arterial stiffness assessed by PWV measurement via the epigenetic downregulation of plasminogen activator inhibitor-1 (PAI-1) expression since the binding of SirT1 to the PAI-1 promotor decreased the acetylation of histone H4 lysine 16 (565). In the Na-exposure-induced stroke-prone Dahl salt-sensitive rat model, the increase in PWV at 6 wk of age is paralleled with a huge increase in epigenetic regulators of histones (e.g., E1A binding protein p300, a histone acetyltransferase, the histone deacetylase 3, and the protein arginine N-methyltransferase 5 isoform c, a histone methyltransferase) in all vessel layers (176). The demonstration that gene-network changes assessed using integrative omic strategies combining genomics, transcriptomics, and epigenomics could unveil novel mechanisms contributing to arterial stiffness was recently provided with the TwinsUK cohort (329). In this female population, common variants associated with PWV were identified in the *CIB2* gene encoding for calcium and integrin-binding protein-2 (CIB2), and one of them was associated with lower PWV values and increased *CIB2* expression caused by hypomethylation of the promoter region. Because CIB2 is believed to regulate intracellular calcium, this finding points toward a genetic component in the association between vascular calcification and arterial stiffness.

VI. VASCULAR SMOOTH MUSCLE CELLS AND LARGE ARTERY STIFFNESS IN MONOGENIC DISEASES

A. Marfan Syndrome: Loss of Cell-ECM Connection, VSMC Dedifferentiation, and Arterial Stiffening

The Marfan syndrome (MFS) is an autosomal dominant genetic disease affecting the skeletal, ocular, and cardiovascular systems (540). Many mutations in the gene encoding fibrillin-1 (*FBN1*) lead to subsequent elastic fiber abnormalities (540).

The clinical complications and the major cause of death in MFS is aortic root dilation and associated aortic regurgitation, dissection, and rupture (373, 450, 540). The likely mechanisms of excessive dilation involve both abnormal elastic fibers, aortic stiffness, as well as steady and pulsatile stresses **(TABLE 5)**.

Table 5. *Vascular changes in monogenic connective tissue disorders*

Monogenic Disease	Marfan Syndrome	Vascular Ehlers-Danlos Syndrome	Williams-Beuren Syndrome
Genetic defect	Mutations in the gene encoding for fibrillin-1	Mutations in the gene encoding for type III procollagen	Deletion of one allele of the elastin gene
Target protein	Fibrillin-1	Type III procollagen	Elastin
Initiating event	Loss of VSMC attachment to elastic laminae	Abnormal collagen I fibrillogenesis, reducing the load-bearing ability of the arterial wall	Primary defect in elastin inducing proliferation of VSMC
VSMC phenotype	Dedifferentiation; Synthetic phenotype; Overexpression of contractile markers; Increased VSMC stiffness	No major change	Proliferation; Migration; Mature contractile phenotype
Extracellular ECM	Deficiency in connecting filament; Loss of VSMC attachments to elastic laminae; Elastic fiber calcification; Excessive deposition of ECM elements	Lack of VSMC signaling in response to wall stress	
Inflammation	Yes	Yes	No
Arterial stiffness	Reduction in cross-sectional distensibility limited to the thoracic aorta	Increase in cross-sectional distensibility	Increase in cross-sectional distensibility
Arterial remodeling	Outward remodeling limited to the thoracic aorta	Hypotrophic remodeling; Increased tensile wall stress	Hypertrophic remodeling; Reduction in tensile wall stress
Arterial complications	Aortic root dilation, dissection, and rupture	Arterial dissection and rupture	Hypertension; Arterial stenosis

The impaired crosslinking in elastin alters the load-bearing capacity of the aortic wall and predisposes to degeneration and microdissections of the elastic network and fibrosis of the media (373, 450). By combining observations in patients with MFS and mice homozygous for a targeted hypomorphic allele (mgR) of *Fbn1*, Dietz and co-workers (58, 415) detected early loss of VSMC attachments to internal elastic lamina leading to calcification and late ECM disorganization in the aorta and medium-size muscular arteries. Calcification of the elastic network as well as malformation of the same network are the triggers for VSMC proliferation in the intima. The main utrastructural changes revealed in mgR mice are the fragmentation of elastic fibers and disruption of fibrillary bundles attached to VSMCs. This elastolysis process enables inflammatory cells to infiltrate the vascular wall together with the fibrotic response (presence of myofibroblasts and collagen accumulation). VSMCs adjacent to elastic laminae retain expression of VSMC markers. The synthetic repertoire of these morphologically abnormal VSMCs in early vascular lesions include also MMP-9, a known mediator of elastolysis, which ultimately leads to structural collapse and stiffening of the vessel wall. An increased wall stiffness of the thoracic aorta has been reported from invasive (585) and noninvasive studies in adults and children (180, 212, 437, 471).

More recently (94), analyses of dilated aortas from Marfan patients showed that overexpression of collagen I and contractile protein markers was caused in part by enhanced activation of the canonical TGF-β signaling pathway and phosphorylation of its downstream effectors SMAD2/ SMAD3. In addition, upregulation of myocardin RNA and thereby TGF-β-mediated increased expression of its targets such as calponin as well as overexpression of RhoA, which is known to regulate both myocardin and SMAD activities, are likely responsible for the increase in both VSMC and EM stiffness measured by AFM. The increase of adhesome proteins such as FAK, paxillin, and vinculin and the subcellular localization of FA in Marfan VSMCs also contributes to increase arterial stiffness. These abormalities may add to the loss of cell attachments, seen above, both contributing to the synthesis and remodeling of a stiffer elastic ECM, leading thus to the known aortic rigidity that precedes or accompanies MFS aneurysm.

The increased aortic rigidity is also observed in preclinical small animal model studies. Thoracoabdominal PWV was higher in the mgR/mgR mouse, and aortic wall stiffness (Young's elastic modulus-to-wall stress ratio) was increased fourfold. A severe fragmentation of the elastic network was observed with no change in cross-linking, together with aortic dilation (335), suggesting that it is the fragmentation of the medial elastic network and not a defect in early elastogenesis which drives aortic dilation in MFS.

There is little information concerning PP at the site of the ascending aorta in patients with MFS. The high pulsatile stress (due to the dilated aorta) at the site of the aortic root further aggravates aortic dilation and explains why the initial aortic size in MFS patients is an independent predictor of aortic dilation (454). Only carotid PP adjusted to age and body surface area is positively correlated with ascending aorta diameter, whereas brachial BP and PP are not associated with aortic complications (217).

A putative sequence of events can be suggested from the above observations, leading patients with MFS to aortic dilation, dissection, and rupture. At the site of the ascending aorta, an abnormal fibrillin-1 makes connecting filaments more fragile under repeated pulsatile stress. When connecting filaments break, the VSMC-ECM connection is lost and VSMCs dedifferentiate and acquire a synthetic phenotype; more robust actin stress fibers and rearrangement of FAs increase stiffness of VSMCs and the ECM. In parallel, the abnormal synthetic repertoire of abnormal VSMCs increases the production of MMPs, leading to elastolysis. These changes translate into a vicious circle, through which arterial wall stiffening exaggerates the fatiguing effect of repeated pulsatile stress on wall components, favoring not only the breakage of connecting filaments at the origin of VSMC dedifferentiation, but also increasing the vulnerability of the damaged aortic wall to dilation, dissection, and rupture.

B. Ehlers-Danlos Syndrome: VSMC Hypotrophy and Increased Circumferential Wall Stress

Ehlers-Danlos syndrome (EDS) type IV is a vascular type (vEDS) that results from mutations in the gene for type III procollagen (22, 414), including the *COLA3A1* mutation **(TABLE 5)**. It is a rare inherited autosomal dominant connective tissue disorder typified by four main clinical features: a characteristic facial appearance, easy bruising, thin translucent skin with a visible underlying venous pattern, and arterial, hollow organ and uterine fragility (22, 414, 518). Diagnosis is often ascertained on the basis of spontaneous arterial dissections and ruptures which are the common cause of death, colonic perforation, or organ rupture. Thoracic and abdominal aorta are the predominant sites of arterial rupture. Despite the identification of the causative genetic defect, limited progress has been made in understanding the pathogenesis of vascular lesions in vEDS, and the prevention of arterial complications remains challenging in young adults (117).

Type III collagen is composed of homotrimers with 3 $\alpha1$ (III) chains folded into a triple helix. The $\alpha1$ (III) chains contain a glycine residue in every third position resulting in an ~330 (glycine-X-Y) repeating amino acid sequence, which is a prerequisite for the assembly into a triple helical

structure. The kinetics of the triple helical folding plays a critical role in the pathogenesis of vEDS (426). Missense mutations interrupting the (glycine-X-Y) sequence account for two-thirds of disease-causing mutations and are responsible for a delayed folding or misfolding of the collagen helix in the VSMC endoplasmic reticulum and accumulation of seven-eighths of abnormal type III collagen in intracellular compartments. These dominant mutations thus reduce mature type III collagen secretion (61). Unlike the missense mutations, nonsense and frameshift mutations in COL3A1 lead to premature stops in translation and nonsense-mediated mRNA decay, reducing by 50% the production of structurally normal type III collagen, which typically produces a milder clinical phenotype (482). Mice with mutations in type I and type III collagen exhibited premature death because of rupture of large blood vessels (302, 303).

Very few reports are available concerning the elastic and geometric properties of conducting arteries in vEDS patients (44, 131, 510). François et al. (131) estimated aortic stiffness from PWV measurement in a family with ecchymotic EDS (which actually corresponds to vEDS) and reported abnormally low PWV values in five relatives. Sonesson et al. (510) failed to demonstrate any alteration in carotid stiffness in EDS patients compared with control subjects, but the study enrolled patients with various subtypes of EDS and few patients with vEDS. Mean circumferential wall stress was 43% higher in vEDS patients than in age-, gender-, and BP-matched control subjects (44). Carotid pulsatile circumferential wall stress was also significantly (22%) higher than in control subjects. The higher circumferential wall stress in vEDS was due mainly to the hypotrophic remodeling, characterized by significantly lower IMT and wall cross-sectional area, and normal internal diameter. The pathophysiological mechanism underlining the lack of wall thickening, leading to the increase in wall stress, is unclear. Very likely, in the large artery wall of vEDS patients, the abnormal collagen ECM does not exert normal cell signaling for migration, adhesion, and proliferation through specific downstream signal transduction pathways. The role of the two major classes of receptors for collagen [the β_1 family of integrins and members of the discoidin-domain receptor (DDR) family], produced by VSMCs, is not yet understood in vEDS.

Another explanation is suggested by the lack of significant difference in arterial remodeling, between vEDS and controls, at the site of a distal medium-sized muscular artery, the radial artery. Indeed, unlike the carotid artery, the radial artery wall is able to thicken in vEDS. Although there is a significant relation between carotid IMT and radial IMT in control subjects, there is none in vEDS. Abnormal VSMC signaling (55) caused by abnormal type I collagen and leading to the lack of wall thickening despite high wall stress could be unmasked under conditions of high cyclic strain

occurring at the site of the carotid artery. Indeed, the stroke change in diameter is 18-fold higher at the site of the carotid artery than at the site of the radial artery. In support of this hypothesis, numerous in vitro studies have shown a greater impact of cyclic strain on VSMC phenotype and growth compared with static load, as well as the identification of local PP as a significant determinant of carotid but not radial wall thickness (42). Abnormal type I collagen fibrillogenesis may reduce the ability of the arterial wall to withstand mechanical loads, resulting in an excessive weakness of the artery and a propensity to rupture at high circumferential wall stress. However, various distal muscular arteries, such as the radial artery, are also affected by arterial dissections and ruptures in vEDS, despite a normal circumferential wall stress. Thus an abnormally high wall stress may not be a mandatory condition for the occurrence of arterial dissection and rupture.

Recently, Morissette et al. (367) hypothesized that tissue fragility may not be the sole mechanism involved in arterial dissection and rupture and that inflammation could play a major role. They reported that many of the established biomarkers of vascular inflammation, including markers of endothelial dysfunction, such as VCAM-1, ICAM-1, and MCP-1, and an acute phase reactant, C-reactive protein, were increased in patients with vEDS. In addition, circulating levels of TGF-β1 and TGF-β2 were also elevated. TGF-β1 is abundant in platelets. Vascular damage may lead to platelet degranulation and thereby release of this growth factor into the circulation.

In the BBEST study (403), celiprolol, a β_1-adrenoceptor antagonist with a β_2-adrenoceptor agonist action, prevented arterial dissections and ruptures in patients with vEDS, most likely through the reduction of "wearing and tearing" of the arterial wall. Of note, common carotid artery stiffness increased in response to celiprolol (i.e., lower distensibility and increased Young's elastic modulus). The mechanism involved more likely the positive loop between β_2-adrenoceptor agonist properties and activation of the TGF-β pathway. Stimulation of the β_2-adrenoceptor in response to celiprolol leads to increased production of collagen through activation of the canonical TGF-β-induced phosphorylation of SMAD2/SMAD3. There are strong associations between β-adrenergic receptors and the TGF-β pathway reported at the level of cardiac and skeletal muscle. Chronic stimulation of β_2-adrenoceptors would probably enhance collagen synthesis and cardiac hypertrophy through increased expression of TGF-β in mice (456). Indeed, in the hypertrophied rat masseter muscle, mRNA expression of TGF-β1, TGF-β2, TGF-β3, and PDGF-BB was upregulated in response to clenbuterol-induced β_2 stimulation (3). The interaction between α_1- and β_2-adrenoceptor stimulation and baroreflex stimulation may also contribute to TGF-β stimulation (395) and collagen production, thus increasing the mechanical strength of the vascular wall

(118, 554, 555). Treatment of Ehlers-Danlos syndrome by celiprolol is the first clinical trial showing a significant preventive effect against arterial mechanical complications. Whether this upregulation of TGF-β specifically affected the abnormal type III collagen is not known, but it is likely that both types I and III were concerned.

C. Williams Syndrome: Unregulated VSMC Proliferation Explains the Paradoxical Reduction in Arterial Stiffness

Williams–Beuren syndrome (WBS) is a complex medical and neurodevelopmental disorder with a characteristic constellation of problems but also considerable phenotypic variability (419). In brief, it is characterized by mental and statural deficiency, elfin face, infantile transient hypercalcemia, and cardiovascular disorders (TABLE 5). The complexity arises from the deletion of more than two dozen genes in the WBS chromosome region, whereas the variability may be due to their interaction with products from other genes outside this region. Although little progress has been made in drawing connections between aspects of the neurodevelopmental profile and specific genes within the WBS chromosome region, this is not the case for the cardiovascular abnormalities (121, 419). The main cardiovascular abnormalities are aortic supravalvular stenosis (70% of patients), narrowing of large arteries and arterial hypertension (50% patients, often in absence of aortic/renal narrowing). These features have been related to the deletion of one allele of the elastin gene, which occurs in ~90% of cases (121, 387).

Transgenic animal studies indicated that elastin was not only required for ensuring the elastic properties of the arterial wall, but elastin was also a major determinant of the terminal differentiation and quiescence of VSMCs (288, 289, 360, 420). Using VSMC from mice lacking elastin (Eln$^{-/-}$), Karnik et al. (224) showed that elastin inhibits the proliferation of VSMCs, induces a mature contractile phenotype in VMSCs, regulates migration of VSMC, and signals via the G protein-coupled pathway.

In elastin-null mice (Eln$^{-/-}$), increased VSMC proliferation both in vivo and in organ culture occurred during development (288). The aortic lumen became smaller and the aortic wall became thicker, with the arterial lumen eventually obliterated, and animals died soon after birth. The cellular mechanism underlying these changes was subendothelial accumulation of arterial smooth muscle, a process that involved cell proliferation, migration, and reorganization (288). In contrast, elastin haploinsufficiency in mice (Eln$^{+/-}$), a model closer to WBS in humans, resulted in living animals, with a stable 25–45 mmHg increase in mean BP compared with their wild-type counterparts (123). Aortic stiffness was higher in Eln$^{+/-}$ than in controls, ascribed to a higher collagen-to-elastin ratio, and this finding has been confirmed in several reviews and analyses (240, 255,

420). Importantly, however, arterial mechanics in Eln$^{+/-}$ mice have been analyzed at the physiological mean BP of each group, and the reduced distensibility may at least be partially explained by the higher mean BP (123). Indeed, when the aortic diameter-pressure curve was carefully analyzed over the full range of BP (123), and comparing parameters at similar BP, there was no difference in terms of Young's elastic modulus between animal groups in the pressure range of 0–125 mmHg, confirming that the reported elevated stiffness is due to the higher operating BP in these animals.

In addition, aortic and carotid wall thickness at physiological mean BP were lower in Eln$^{+/-}$ than in Eln$^{+/+}$, which at first glance, is in contrast to the arterial wall hypertrophy reported in Eln$^{-/-}$ mice. Importantly, on a structural level, Eln$^{+/-}$ mice have an increased number of elastic lamellae without major medial hypertrophy. As developed below, the concept generally accepted in hypertension remodeling (60, 260, 270) is that distensibility is increased only when an adapted arterial wall hypertrophy occurs. Thus it is likely that the limited arterial wall hypertrophy fully compensated for the arterial stiffening induced by the reduction in elastin-collagen ratio in hemizygous mice at low BP, and only partially at high BP.

The hyperproliferative phenotype was associated with decreased stress fiber and FA formation and increased migration of cultured VSMCs from Eln$^{-/-}$ pups. The critical regulatory role of elastin was demonstrated by inhibition of VSMC migration in response to the addition of exogenous tropoelastin, the monomer precursor of elastin polymers (224). Additional results supporting the new concept of direct involvement of the elastin network in proliferation came from the demonstration that aortic VSMCs and dermal fibroblasts from WBS patients or patients with familial aortic supravalvular stenosis exhibited the same inverse relation between elastogenesis and proliferation. As for mice, addition of insoluble elastin rescues a normal proliferative rate (546). Thus the occurrence of segmental obstructive lesions is thought to be a two-step process, consisting of the formation of an increased number of lamellar units and vessel wall thickening during fetal development, leading to a uniformly altered vascular tree, followed by postnatal injury-mediated inward remodeling (289).

Extrapolations from the Eln$^{+/-}$ mouse suggest that affected people may also have stiff arteries. In addition, according to physics laws, arterial wall hypertrophy, i.e., an increased wall thickness, is theoretically associated with a stiffer artery if the stiffness of the wall material remains unchanged. This is why arterial wall thickening seen with intravascular ultrasound imaging in humans with WBS has led to the hypothesis that hypertension could be related to a reduced compliance of the arterial tree (440). However, although an increased wall thickness has been confirmed at the site of

the carotid artery in WBS children (2, 529) and young adults (244), compared with age- and sex-matched controls, reliable clinical data on arterial stiffness showed no reduction in arterial compliance.

Indeed, the carotid wall was abnormally distensible in young WBS adults when WBS and controls were matched for age, sex, and mean BP (244). This was associated with a reduction in Young's elastic modulus. A reduction in carotid stiffness (2) or no change in aortic stiffness (328) have been also reported in WBS children, when they were compared with age-, sex-, and mean BP-matched controls. These clinical data suggest that, in contrast to what has been hypothesized in the 1990s, a primary defect in elastin leads to VSMC proliferation, arterial wall hypertrophy, and hyperdistensibility. Therefore, the main factor responsible for hyperdistensibility observed in WBS patients is arterial wall hypertrophy caused by the primary defect in elastin, which induces major changes in the phenotype of VSMCs. This also highlights the importance of the micro-structural organization and architecture of the arterial wall for its function: although the disease impairs the most distensible protein of the artery, the artery as a whole has become a more distensible structure.

VII. VASCULAR SMOOTH MUSCLE CELLS AND LARGE ARTERY STIFFNESS IN POLYGENIC DISEASES

A. Arterial Stiffness and Remodeling in Essential Hypertension: Isobaric Arterial Stiffness Does Not Increase Despite Arterial Wall Hypertrophy, i.e., VSMC Plays a Compensatory Role

Arterial wall hypertrophy resulting from the sustained heightened BP in essential hypertension compensates ideally for increased circumferential wall stress even if there is some degree of cellular hypertrophy. The laws of physics prompt us to anticipate that any increase in wall thickness, which results in the juxtaposition of materials with identical mechanical properties, should increase arterial stiffness for a given BP level. Surprisingly, several studies have pointed to a reduced Young's elastic modulus associated with arterial wall hypertrophy measured by carotid IMT in hypertension with no decrease in arterial distensibility under isobaric conditions (isobaric arterial stiffness) or identical wall stress (168, 271) in the carotid artery (268) or the radial artery (270). Similar findings were observed in SHRs and stroke-prone SHRs (SHR-SPs) in the carotid artery and the abdominal aorta, when compared with Wistar-Kyoto rats (32, 41). The similar or even increased arterial distensibility in hypertensive subjects or SHR documents the involvement of pulsatile stress rather than static conditions in arterial stiffness (294). At the level of mesenteric arteries, reduction

of stiffening of wall components has been also observed in essential hypertension (203). Altogether, these findings mean that hypertension-induced arterial wall hypertrophy is not associated with an enhanced isobaric arterial stiffness, but rather with structural changes in the arterial wall leading to its mechanical adaptation to an elevated BP.

Whether hypertension-induced arterial wall hypertrophy is associated with a reduced or increased VSMC tone has not yet been determined. A reduced VSMC tone would allow normalizing isobaric arterial stiffness despite wall hypertrophy. An increased VSMC tone could redistribute the mechanical load towards elastic materials (108), in synergy with a higher number of cell-ECM attachments and smaller fenestrations of the internal elastic lamellae. In response to potassium cyanide, the increase in compliance of the in situ isolated cartotid artery is higher in SHR than in normotensive rats, indicating that the activation of VSMCs plays a causal role in arterial stiffness independently of endothelium (285). Ultimately, these changes can be envisioned as adaptive mechanisms to compensate for the deleterious effects of wall hypertrophy and prevent excessive arterial stiffening at high BP levels (250, 266).

B. Arterial Stiffness and Remodeling in Diabetes: Increased Arterial Stiffness Is Primarily Due to ECM Alteration, i.e., VSMCs Lag Behind ECM

Type 2 diabetes (T2D) damages the large artery wall through its two major features: hyperglycemia and insulin resistance (516). Both factors may act at the structural and functional levels by a variety of mechanisms. Chronic exposure to hyperglycemia induces VSMC proliferation and enhances the production of AGE and collagen cross-linking (98) that stiffens the arterial wall material. In addition to the increase in expression of MMP-2 and -9, accumulation of ANG II is increased in vascular tissue (274). In VSMCs from small-sized muscular arteries, adhesion of glycated proteins, particularly glycated fibronectin, via binding to receptor for AGE (RAGE) is independent of integrin receptors and involves NF-κB signaling (104). Endothelial dysfunction and a shift to a pro-inflammatory phenotype of macrophages are associated to these phenomena.

Insulin resistance augments collagen synthesis, and increases the expression of several genes involved in the inflammatory processes (39). Arterial stiffening and thickening are thus likely the consequence of these changes. In addition, insulin resistance is associated with reduction of NO synthesis, increased release of ROS, very-low-density lipoprotein synthesis, and cholesterol transport into VSMCs (516). The high circulating levels of free fatty acids released from adipose tissue contribute to impair endothelial function and induce a low-grade inflammation. Altogether these mechanisms contribute to large artery wall

stiffening, thickening, and remodeling, which may favor atherosclerotic plaque development (516).

Clinical research shows that T2D is associated with accelerated stiffening of large elastic (e.g., carotid, ascending aorta), small-sized muscular (e.g., femoral), and mixed elastic-muscular (e.g., abdominal aorta) arteries (516). Increased carotid-femoral PWV was independently associated with cardiovascular and overall mortality in a glucose tolerance-tested sample of the community (95). In this cohort, mortality risk doubled in subjects with diabetes or glucose intolerance compared with controls, and a 1 m/s increase in carotid-femoral PWV was associated with a hazard ratio increase of 8%. Recent data suggest that stiffening of the carotid and the femoral arteries may have prognostic value independent of aortic carotid-femoral PWV (553). Arterial wall hypertrophy was also reported to be higher in T2D and hyperglycemic patients than in age- and BP-matched controls (120, 537). In hyperglycemic patients, either with impaired fasting glucose or T2D, glycemia proved to be a major independent determinant of carotid IMT, whereas local PP was not. In contrast, carotid PP, but not glycemia, was a significant determinant of carotid IMT in control subjects. It is likely that, above a certain glucose threshold (6.1 mM), glycemia may attenuate the mechanical influence of local PP on carotid IMT, through changes in the mechanotransduction pathways involved in the response of the arterial wall to pulsatile load.

A recent study has compared the stiffness of VSMCs in diabetic patients subjected to coronary artery bypass surgery and controls (105). Female sex and smoking, but not diabetes, were independent predictors of stiffening of VSMCs from thoracic aorta, assessed using the optical magnetic twisting cytometry. Confirmation by clinical investigation of carotid mechanics in diabetic patients is required to conclude that VSMC stiffness plays a smaller role than ECM protein changes in the stiffness of the arterial wall material, or, in other words, that changes at the level of VSMCs lag behind changes of the ECM.

C. Arterial Stiffness and Remodeling in Chronic Kidney Disease: Maladaptive Arterial Wall Remodeling Parallels the Decline in Kidney Function, i.e., VSMCs as a Target of Kidney Dysfunction

Patients with CKD demonstrate EVA (264, 389), characterized by an accelerated arterial enlargement and stiffening which occurs in parallel with the decline in glomerular filtration rate (51). The relationship between central hemodynamics (either arterial stiffness or central BP) and glomerular filtration rate decline is complex and depends mainly on both the level of BP and the stage of the disease [early CKD, advanced CKD, or end-stage renal disease (ESRD)] (52, 53).

Arterial remodeling is already observed in early stages and with progression of CKD (52). In comparison with normotensive and hypertensive controls, patients with CKD stage 2–5 had a significantly larger internal carotid artery diameter with no significant difference in IMT, resulting in a significant increase in circumferential wall stress, indicating inadaptive or inadequate arterial remodeling of large arteries in CKD. Carotid Young's elastic modulus increased with progression of CKD but was not different from hypertensive controls matched for BP. A word of caution is necessary here. Indeed, these calculations are approximate and may be misleading, as discussed above, in case of severe remodeling with build-up of residual stresses and stress redistribution through elongation of the vessel. In contrast to carotid stiffness, the carotid-femoral (aortic) PWV of CKD patients was significantly higher than in hypertensive and normotensive controls, suggesting that carotid and aortic stiffness could progress differently in this population. Consistent with this hypothesis, a prospective study has revealed that reduction of aortic stiffness independently of BP decreases all-cause and cardiovascular mortality in ESRD (152).

In opposition with observations made in nonuremic atherosclerosis where carotid IMT increases with the burden of atherosclerosis and the rate of increase is limited by lipid-lowering treatment (89), a study has shown that carotid IMT decreased during CKD progression (53). In this cohort, circumferential wall stress was the only arterial parameter independently associated with CKD progression and the onset of ESRD. Renin-angiotensin system blockers, often prescribed to CKD patients, could play a role in the defect of thickening because of their antiproliferative properties (308, 544). Another hypothesis is an excess of VSMC apoptosis. Indeed, in children with ESRD, Shroff et al. (506) showed apoptosis related to the reduced number of VSMCs compared with patients without CKD. In addition, increased ECM turnover with high MMP activity could also participate in the observed phenotype. MMPs are involved in flow-induced outward vascular remodeling (407) and in cardiovascular remodeling such as LV hypertrophy, atherosclerosis, or aortic aneurysm (162, 455). In CKD patients, several studies showed variations in serum levels of MMPs and their inhibitors (86, 189).

Finally, damage to large arteries may be related to bone disease not only in ESRD but also in earlier stages of CKD. In 107 CKD patients, in whom bone evaluation was performed by bone densitometry and the measurement of the bone-specific alkaline phosphatase (BSALP), bone disease was associated with the carotid outward remodeling in parallel with the decline of renal function in this population (54). This association existed only in patients with glomerular filtration rates ≤ 38 ml·min^{-1}·1.73 m^{-2}. BSALP was independently and positively correlated with carotid internal

diameter and explained 13% of the variance. These results suggest a crosstalk between kidney, arterial wall, and bone.

An inverse relation between arterial calcification and stiffness with bone density or bone turnover was observed in CKD and ESRD patients (306). Arterial calcification is a common complication of CKD and ESRD (167). Several studies have shown that low serum levels of the soluble calcification inhibitor fetuin-A is an independent predictor of aortic and carotid stiffness (173). Studies in ESRD and in the general population have shown a strong association between vitamin D deficiency, increased arterial stiffness, and deficient endothelial function (305).

In conclusion, these data suggest that VSMCs of large arteries are targets of kidney dysfunction, implying various mechanisms leading to maladaptive arterial remodeling. However, these mechanisms have not been studied very much until now, and data on apoptosis and reorganization of the ECM are lacking.

D. Arteriosclerosis Versus Atherosclerosis: VSMCs Are Directly Involved as Primary Events

Undoubtedly, the clinical relevance of the interaction between arterial stiffness and atherosclerosis has been well established in large, independent population-based cohorts. Carotid-femoral PWV was reported to increase while the common carotid distensibility coefficient consistently decreased with increasing IMT and the severity of plaques in the Atherosclerosis Risk in Communities (ARIC) study, the Rotterdam study, and the Multi-Ethnic Study of Atherosclerosis (MESA) study (298, 446, 552). In addition, an independent correlation between intrarenal vascular resistance and both aortic stiffness and carotid atherosclerotic lesions has been demonstrated, suggesting the involvement of small-sized muscular and large elastic arteries in theses connections (64). While PWV was associated with echogenic (fibrosis and calcification) plaques independently of age, gender, and hypertensive status, no association was reported with echolucent plaques (69, 601), suggesting that fibrosis and calcification may be more important than intimal lipoprotein deposition in linking atherosclerosis to arteriosclerosis. In support of the importance of plaque morphology in this link is the reported association between PWV and intraplaque hemorrhage resulting from a proangiogenic phenotype of VSMCs leading to neovascularization of advanced atherothrombotic lesions (181, 494).

One of the hallmarks of atherosclerosis is the involvement of multiple cell types, ECs, VSMCs, fibroblasts but also extravascular cells, and a large number of signaling pathways have extensively been reviewed previously (187). For arteriosclerosis, it appears more simple since it is primarily

mediated by structural changes of the media which is a privileged site, being avascular, and devoid of leukocytes. There are, nonetheless, two common factors in arteriosclerosis and atherosclerosis, i.e., hemodynamic factors and VSMC plasticity and calcification, and several steps in atherosclerosis, in particular inflammation, intimal thickening, fibrosis, thrombosis, and vascular remodeling are also directly relevant to arteriosclerosis.

The contribution of VSMCs to plaque, which is mainly driven by their phenotypic modulation, is complex and has probably been underappreciated in the past (187). VSMC marker expression and VSMC lineage tracing have revealed that excessive VSMC proliferation and transdifferentiation into macrophages and mesenchymal stem cells contribute to atherosclerotic plaque development (125). The reduced expression of the ATP-binding cassette transporter A1 (ABCA1) in VSMC-derived foam cells in atherosclerotic lesions (8) may have a role in arterial stiffening since ABCA1-mediated serum cholesterol efflux capacity measured ex vivo by incubation of serum from healthy subjects with macrophages was inversely correlated to PWV (124). Recently, the dual role of VSMCs in the plaque was highlighted, with a deleterious effect by transformation into foam cells contrasting with a positive effect at later stages by production of ECM proteins to maintain plaque stability (216). A proposed mechanism contributing to plaque instability is through KLF4-dependent VSMC phenotypic transition activating the proinflammatory properties of VSMCs (497). Recently, AMP-activated protein kinase (AMPK)α2 deletion was shown to promote plaque instability via NF-κB activation, resulting in binding of NF-κB p65 to the KLF4 promoter and thereby increasing transcriptional upregulation of KLF4 expression in VSMCs (107). In addition to this anti-atherogenic effect, activation of AMPKα reduced arterial stiffening in *Klotho*-deficient mice (296) or old mice (283), providing evidence for VSMC APMKα as a novel therapeutic target in preventing both atherosclerosis and arteriosclerosis.

Undoubtedly, animal models recapitulating atherosclerosis, mainly apolipoprotein E (apoE) and LDL receptor (LDLR)-deficient mouse models, have added clear evidence supporting the role of VSMC in linking atherosclerosis to arteriosclerosis. Young's elastic modulus of the thoracic aorta is increased in apoE-null (apoE$^{-/-}$) mice (238) or LDLR-deficient mice (LDLR$^{-/-}$) (112). Additional deficiency in osteopontin in LDLR$^{-/-}$ mice further increases aortic PWV (499). Mechanisms for increased arterial stiffening include increased expression of several ECM proteins and collagen as well as increased activity of lysyloxidase (LOX) producing crosslinks of collagen (238). The role of apoE-containing HDL was demonstrated in a cellular model of VSMCs. The suppressive effect of apoE on mechanically driven VSMC collagen I and fibronectin gene expression is specific to dedifferentiated VMSC and is mediated by the cycloox-

ygenase-2-prostaglandin I_2-prostacyclin receptor (Cox2-PGI_2-IP) pathway while miRNA-145 transduces LOX mRNA repression (238). Another proposed pathway includes regulation of noncanonical Wnt signaling by the LDLR-related protein 6 (LRP6). Indeed, mutation imparing LRP6 activity results in diminished transcription factor 7-like 2 (TCF7L2)-dependent inhibition of Sp1-mediated VSMC differentiation and increased atherosclerotic lesions (512), and deletion of LRP6 in the vascular smooth muscle lineage promotes upregulation of osteopontin via the upstream stimulatory factor 1 (USF1) protein-DNA interactome together with increased arterial stiffening (80). Accumulation of the glycosaminoglycan hyaluronan in the aorta that promotes the VSMC switch towards a synthetic phenotype has also been proposed as a common denominator of arterial stiffening and formation of plaques, but the mechanisms involved remain unclear (313).

A key question is the temporal dynamics of increased arterial stiffness and development of atherosclerotic lesions. In support of the concept of a causal role of elastic fiber fragmentation in arterial stiffening is the increase in local PWV assessed by MRI correlating with elastin fractures at both 18 wk and 30 wk of age in apoE$^{-/-}$ mice (147). Interestingly, the formation of atherosclerotic lesions became detectable only at the age of 30 wk. Additional insight has emerged from a study focusing on glycosphingolipids in apoE$^{-/-}$ mice. Glycosphingolipids are major regulators of lipid homeostasis, and these signaling lipids are critically involved in superoxide radical generation and atherosclerosis development. Pharmaceutical inhibition of glycosphingolipid synthesis (with D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) resulted in a dose-dependent reduction in atherosclerosis and a marked improvement in arterial elasticity in apoE$^{-/-}$ mice fed a Western diet (74). Of note, the reduction in PWV occurred whatever the stage of plaque development, at the moment of intimal thickening at 20 wk, and with advanced calcified plaques at 36 wk. Altogether, these results point in the direction that an increase in arterial stiffness precedes age-related full plaque development.

There is clear evidence that extracellular signals linked to intracellular signaling pathways via cell receptors are key players in the development of atherosclerosis and arterial stiffening. GPCRs and integrins represent two main classes of cell surface receptors involved in atherosclerosis and arteriosclerosis. In the first study reporting a causal role of Rho signaling pathways in atherosclerosis, administration of a RhoK inhibitor decreased the size of plaques by 30% in LDLR$^{-/-}$ mice, possibly by reducing NF-κB activation (327). However, assignment to VSMC-specific signaling was difficult because of the presence of GPCR signaling in all cells present in the plaque, in particular platelets and leukocytes. In fact, the role of large G proteins in atherosclerosis is far more complex. The two major heterotrimeric G proteins $G\alpha_q/G\alpha_{11}$ and $G\alpha_{12}/G\alpha_{13}$ exert an-

tagonist regulation of VSMC differentiation at sites of vascular injury, while their downstream signaling synergistically regulates vascular tone (9). Smooth muscle deficiency of $G\alpha_{12}/G\alpha_{13}$ in apoE$^{-/-}$ mice promoted atherosclerosis, accompanied by a reduced RhoA-mediated SRF-dependent transcription of VSMC differentiation markers. In contrast, smooth muscle deficiency of $G\alpha_q/G\alpha_{11}$ blocked the upregulation of early response genes and attenuated the downregulation of differentiation marker genes induced by vascular injury as well as neointimal hyperplasia.

Adhesion between cells and ECM endows integrins with relevant signaling pathways potentially related to both atherosclerosis and arteriosclerosis. While the role of several integrins in cell signaling altering intimal and medial functions in atherosclerosis has been extensively studied, their involvement in arterial stiffening is being increasingly identified. Invalidation of the collagen-binding integrin $\alpha_1\beta_1$ in apoE$^{-/-}$ mice reduced leukocyte migration, plaque area, and increased VSMCs and collagen contents in advanced plaques (473). Invalidation of the α_1 gene alone did not result in any modification of ECM composition, VSMC differentiation or proliferation or in carotid stiffness but reduced mechanical strength of the arterial wall (316). Thus loss of attachments between cells and collagen thereby producing a softer ECM provides a mechanism for controlling both atherosclerosis and arteriosclerosis. VSMCs express high levels of $\alpha_v\beta_3$ integrin, and this integrin promotes many functions such as VSMC proliferation and migration (62). Therefore, logically its presence has been shown to be increased in atherosclerotic plaques using in vivo imaging in double knockout mice deficient in LDLR and apolipoprotein B-48 (252). Similarly, inhibition of $\alpha_v\beta_3$ limits the recruitment of VSMCs into early atherosclerotic lesions in diet-fed apoE$^{-/-}$ mice, whereas inhibition of $\alpha_5\beta_1$ integrin does not prevent this recruitment (76). A similar differential role of these two fibronectin-binding integrins in VSMC functions related to arterial stiffening is less clear. During development, specific deletion of both α_5 and α_v integrins in VSMCs is required to prevent cell attachment to fibronectin and the formation of mature FAs and to disrupt TGF-β signaling (541). These observations would suggest that the process of FA maturation represents a most interesting target to understand the relations between arteriosclerosis and atherosclerosis. The recruitment and lifetime of FA, which depend on the equilibrium between association and dissociation rates governed by ECM stiffness and applied forces, are relevant in arteriosclerosis because they control the level of VSMC-ECM attachments, and in atherosclerosis in which variations of shear stress and wall stress correlate with the location of lesions in regions of turbulence and low shear stress.

VIII. CONCLUDING REMARKS

Position statements and recommendations on arterial stiffness have been introduced into clinical practice since arterial stiffness has been referred to as an independent cardiovascular risk

LACOLLEY ET AL.

and a predictor of future events (269, 534). In parallel, more accurate and validated devices have been developed to determine both local and systemic measurements of arterial stiffness, limited to large conduction arteries for technical reasons. Thus we have a fragmented understanding of the relative importance of stiffening between elastic and muscular arteries and the cross-talk between them. Moreover, the clinical features of arterial stiffness are not necessarily identical considering that arterial stiffness may be a cause or a consequence, or both, in multiple pathologies (195). One consistent lead is that many of the pathological conditions associated with arterial stiffness affect both ECM and VSMCs whatever their location along the arterial tree. The importance of VSMCs and molecular signaling have been established in rat models of hypertension and through the multiple effects of genetic manipulations in mice. Experimental rodent models develop systolic hypertension, intimal thickening, increased arterial contraction, decreased relaxation, and arterial stiffness with aging. The comparison between rodents and humans is only reliable if we consider the relative quantity of elastic lamellae and VSMCs for a species-specific artery site.

Intense efforts have been directed towards a basic understanding of cellular and molecular determinants of arterial stiffness. In the most current view as depicted in **FIGURE 9**, all actors claim a role, that is, hemodynamic factors and VSMCs together with ECM in which they reside and specific cell types in the vascular wall involving ECs, inflammatory cells, fibroblasts, and pericytes/progenitor cells. Our goal has been to focus on the dual cell and tissue mechanobiology and to bring together new physiological pathways and current clinical statements on arterial stiffness. For example, the deciphering of the cellular/molecular proinflammatory mechanisms driven by SRF at the level of elastic arteries versus resistance muscular arteries could provide future advances on the contribution of cellular stiffness to vascular wall stiffening.

As the fields of vascular biology, signaling, biomechanical phenotyping of arteries, and central hemodynamics mature **(FIGURE 10)**, advancing our knowledge is entirely dependent on data-driven computational models which will define mechanistically-driven hypotheses. The prevailing view
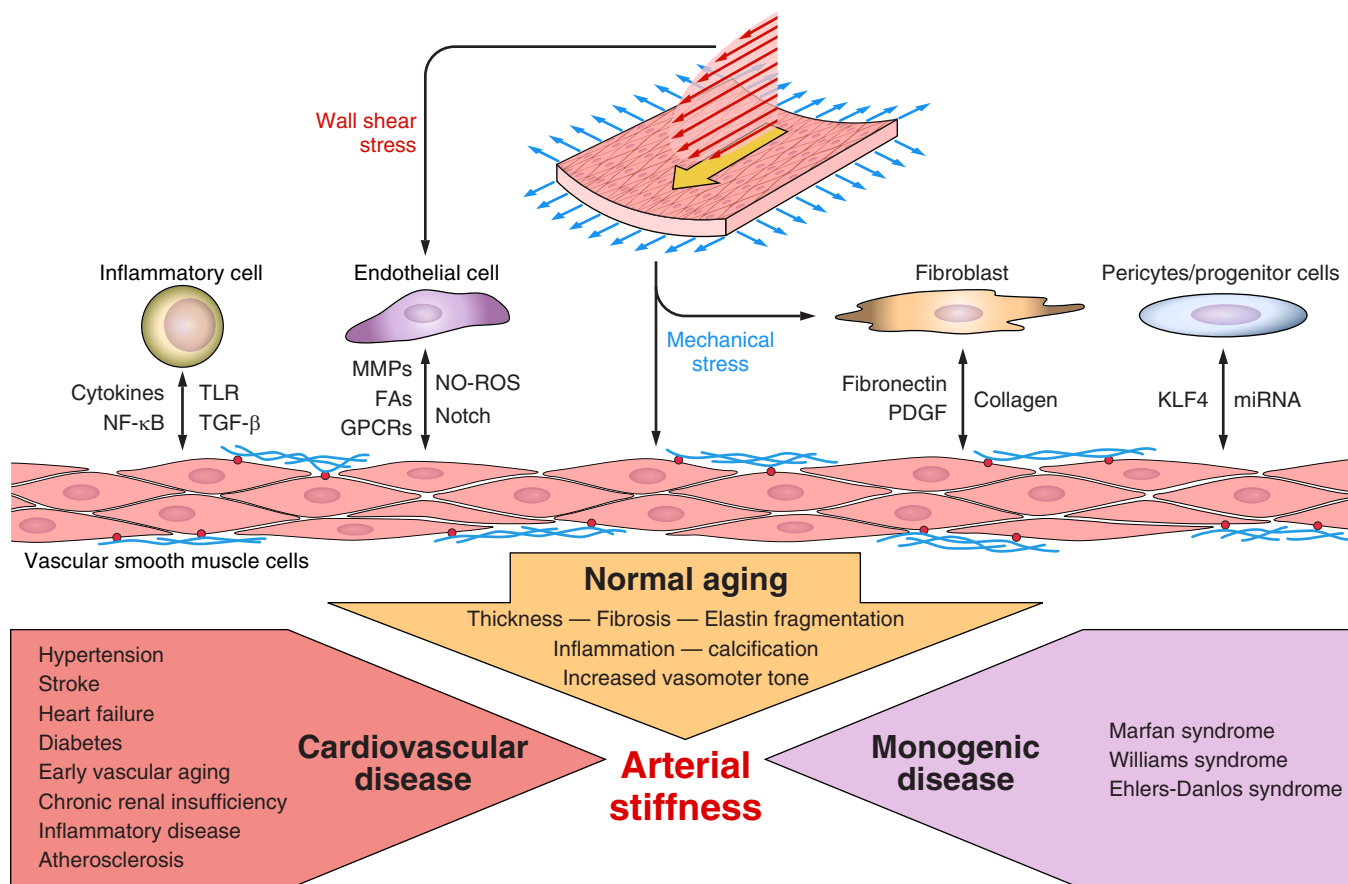

FIGURE 9. Multicellular nature of arterial stiffness. Strong interactions between endothelial (EC), inflammatory, and vascular smooth muscle (VSMC) cells are represented schematically in the *top left panel* with several molecules modulating these interactions. Interactions between fibroblasts, progenitor cells/pericytes, and VSMCs are in the *top right panel*. Expression of specific molecules by these cells and their roles are discussed in the text. VSMC investment in developing vascular and clinic phenotypes of arterial stiffening mainly through these cell interactions is highlighted in the *bottom panel*. Abreviations are as in the text.
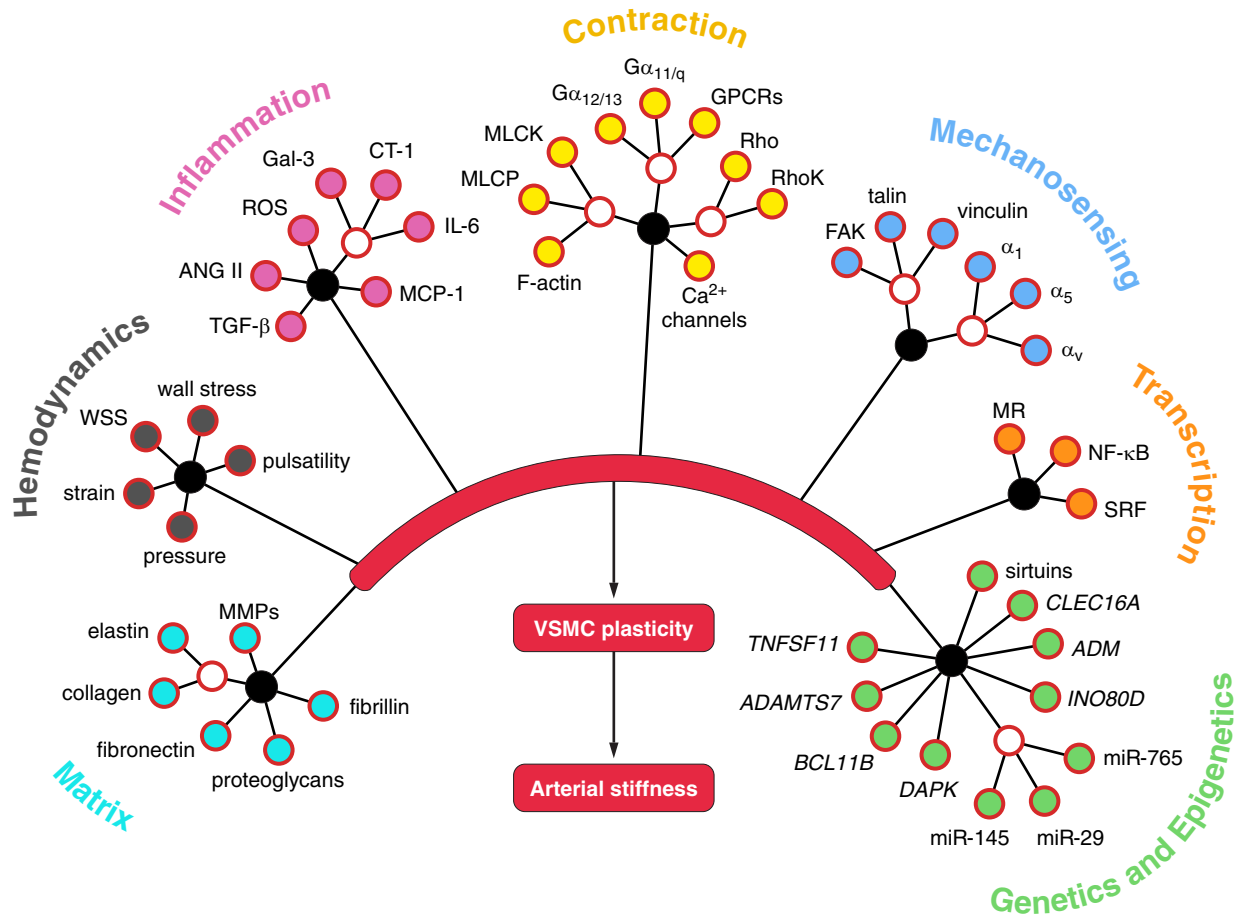
FIGURE 10. An integrative network of VSMC molecules subdivided into different functional families defined by their degree of relevance to arterial stiffness. The arcs define known significant interactions within the same family. Abreviations are as in the text.

that arterial stiffness may be represented as separate, hemodynamic, structural, or signaling cascades has to be embedded in a unique complex network in which interactions across the differents elements are ordered in a dynamic early (reversible) to late (irreversible) process. In addition, we are on the way to benefiting from high-resolution microscopy, such as AFM or velocity protein mapping, to be able to visualize in real time specific locations and movements of individual proteins, or clusters, in FAs and actin biomechanics.

Another challenge will be to correlate data from genetic analysis together with basic understanding gained from VSMC mechanotransduction studies, for example, how common genetic variations in a locus in the *BCL11B* gene interfere with VSMC differentiation and viscoelastic responses. The immuno-inflammatory balance may also enter into the game via genetic mutations. The emergence of large genetic approaches will allow the identification of loci or exomes regulating sets of common and rare variants involved in a complex trait such as arterial stiffness. At present, there are no studies identifying rare variants associated with arterial stiffness. In addition,

another level of control lies in epigenetic marks and in noncoding RNAs.

At present, the cornerstone for preventing and treating arterial stiffening remains the transfer of information from VSMC-ECM interactions and genetic analyses in biomarkers to assess tissue mechanical homeostasis. Redundancy as well as feedforward and feedback signaling make the search for new biomarkers difficult. Nevertheless, the explosion of molecular imaging of the arterial wall and proteomics opens up new dimensions and possibilities. Gaining an integrated understanding of the mechanisms that initiate and sustain VSMC phenoconversion (quiescent-to-activated cells) impacting on the continuum of arterial stiffnening will give way to targeted therapies to halt or even reverse progression. The development of open-access biobanks and relevant clinical populations will strengthen translational and reverse-translational research in the field.

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DISCLOSURES

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