International Angiology 2024 December;43(6):563-90
DOI: 10.23736/S0392-9590.24.05339-2

REVIEW
VENOUS DISEASE

EDITOR'S CHOICE

Glycocalyx disruption, endothelial dysfunction and vascular remodeling as underlying mechanisms and treatment targets of chronic venous disease

Jose A. DIAZ [1], Sergio GIANESINI [2, 3], Raouf A. KHALIL [4] *

[1]Division of Surgical Research, Light Surgical Research and Training Laboratory, Surgical Sciences, Vanderbilt University Medical Center, Nashville, TN, USA; [2]Vascular Diseases Center, Translational Surgery Unit, University of Ferrara, Ferrara, Italy; [3]Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [4]Vascular Surgery Research Laboratories, Division of Vascular and Endovascular Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

*Corresponding author: Raouf A. Khalil, Division of Vascular and Endovascular Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA. E-mail: raouf_khalil@hms.harvard.edu

ABSTRACT

The glycocalyx is an essential structural and functional component of endothelial cells. Extensive hemodynamic changes cause endothelial glycocalyx disruption and vascular dysfunction, leading to multiple arterial and venous disorders. Chronic venous disease (CVD) is a common disorder of the lower extremities with major health and socio-economic implications, but complex pathophysiology. Genetic aberrations accentuated by environmental factors, behavioral tendencies, and hormonal disturbances promote venous reflux, valve incompetence, and venous blood stasis. Increased venous hydrostatic pressure and changes in shear-stress cause glycocalyx injury, endothelial dysfunction, secretion of adhesion molecules, leukocyte recruitment/activation, and release of cytokines, chemokines, and hypoxia-inducible factor, causing smooth muscle cell switch from contractile to synthetic proliferative phenotype, imbalance in matrix metalloproteinases (MMPs), degradation of collagen and elastin, and venous tissue remodeling, leading to venous dilation and varicose veins. In the advanced stages of CVD, leukocyte infiltration of the vein wall causes progressive inflammation, fibrosis, disruption of junctional proteins, accumulation of tissue metabolites and reactive oxygen and nitrogen species, and iron deposition, leading to skin changes and venous leg ulcer (VLU). CVD management includes compression stockings, venotonics, and surgical intervention. In addition to its antithrombotic and fibrinolytic properties, literature suggests sulodexide benefits in reducing inflammation, promoting VLU healing, improving endothelial function, exhibiting venotonic properties, and inhibiting MMP-9. Understanding the role of

glycocalyx, endothelial dysfunction, and vascular remodeling should help delineate the underlying mechanisms and develop improved biomarkers and targeted therapy for CVD and VLU.

(*Cite this article as:* Diaz JA, Gianesini S, Khalil RA. Glycocalyx disruption, endothelial dysfunction and vascular remodeling as underlying mechanisms and treatment targets of chronic venous disease. Int Angiol 2024;43:563-90. DOI: 10.23736/S0392-9590.24.05339-2)

Key words: Endothelium; Glycocalyx; Varicose veins; Vascular smooth muscle; Sulodexide.

The glycocalyx is composed of glycoproteins, proteoglycans, and glycosaminoglycans (GAGs), and acts as a molecular sieve, selectively allowing the passage of molecules based on their size, charge, and shape. Additionally, it serves as mechanosensory, transducing mechanical forces exerted by blood flow into biochemical signals that modulate endothelial function. Some conditions may alter the glycocalyx functions, and recently, it has been postulated that there is a potential involvement in venous diseases, including but not limited to chronic venous disease (CVD), which deserves our attention.

CVD is a common vascular disorder characterized by dilation and tortuosity of the lower extremity veins and varicose veins (VVs), with several stages of severity. If not treated, CVD progresses in ~10% of cases to chronic venous insufficiency (CVI) with venous complications, skin changes, and venous leg ulcer (VLU). Family history, genetic background, and environmental factors, including sedentary lifestyle, obesity, and sex hormones, influence the risk for CVD. A study of nearly half a million subjects (VVs and control), utilizing genome-wide association studies (GWAS) and machine learning, suggested advanced age, female sex, obesity, pregnancy, deep venous thrombosis (DVT), increased height, and leg bioimpedance as risk factors for VVs.[1] However, the pathophysiology of primary varicosis is not clearly understood. Theories ranging from the incompetence of the venous valves to structural changes in the vein wall have been proposed as essential events leading to CVD, and biochemical and functional studies support both theories.[2] Glycocalyx disruption and endothelial dysfunction have been observed in multiple vascular diseases, including hypertension, atherosclerosis, peripheral arterial disease, and coronary artery disease. Additionally, glycocalyx disruption and endothelial dysfunction could initiate events that trigger a cascade of proteolytic enzymes, metabolic products, and inflammatory factors, leading to venous remodeling and CVD and progressing to venous tissue damage and VLU. Management of CVD includes elastic compression stockings, sclerotherapy, endovenous therapies, and surgical removal. Venoactive drugs could limit the progression of VVs to CVI. Sulodexide has shown benefits in improving endothelial function, and could exhibit venotonic properties, and inhibit matrix proteinases and venous remodeling in CVD and VVs.[2-4]

This scoping review of scientific and clinical reports published in Pubmed and Medline from 1990-2024 as well as data from our laboratories will provide insights into the importance of the glycocalyx for normal endothelial cell (EC) function and how the glycocalyx disruption could contribute to endothelial dysfunction in different cardiovascular disorders, including CVD. We will first describe the role of the glycocalyx in EC biology and vascular physiology. Then, we will describe how the changes in the glycocalyx and EC bio-signaling observed in basic scientific research and animal models could help to understand the mechanisms of different vascular disorders encountered in clinical practice. In addition, we will discuss how understanding the role of glycocalyx and EC pathophysiology has helped delineate the underlying mechanisms of CVD. Lastly, we will summarize some of the medical and surgical strategies used for the management of VVs and discuss some of the reported benefits of sulodexide and its effectiveness in reducing inflammation and promoting VLU healing, as well as recent observations of its benefits in improving EC function and venous contraction and inhibiting MMP-9 and extensive venous remodeling in CVD.

Endothelium and glycocalyx in vascular physiology

The endothelium is a single layer of cells lining the interior surface of blood vessels, forming a critical interface between circulating blood and the vessel wall. This layer

plays multifaceted roles in vascular physiology, including regulating vascular tone, maintaining vascular homeostasis, and participating in immune responses. ECs dynamically respond to various physiological and pathological stimuli, orchestrating processes such as vasodilation, vasoconstriction, inflammation, and coagulation. Furthermore, the endothelium communicates with surrounding tissues and blood cells, regulating vascular permeability and angiogenesis.[5]

Central to the endothelial function is the glycocalyx, a carbohydrate-rich layer covering the luminal surface of ECs. Composed of glycoproteins, proteoglycans, and glycosaminoglycans (GAGs), the glycocalyx acts as a molecular sieve, selectively allowing the passage of molecules based on their size, charge, and shape. Additionally, it serves as mechanosensory structure, transducing mechanical forces exerted by blood flow into biochemical signals that modulate endothelial function. The glycocalyx is crucial for maintaining vascular integrity, regulating leukocyte-endothelial interactions, and preventing excessive platelet adhesion and aggregation.[6]

The glycocalyx also plays a pivotal role in vascular permeability by forming a barrier that restricts the passage of macromolecules and circulating cells from the bloodstream into the vessel wall and surrounding tissues. Disruption of the glycocalyx, whether due to pathological conditions or mechanical trauma, compromises endothelial barrier function, leading to increased vascular permeability and tissue edema. Moreover, shedding glycocalyx components into the circulation has been implicated in the pathogenesis of various cardiovascular diseases, including atherosclerosis, hypertension, and acute lung injury.[7]

Several factors influence the structure and function of the endothelial glycocalyx, including shear stress, inflammatory mediators, and oxidative stress. Physiological levels of shear stress exerted by blood flow promote glycocalyx synthesis and maintenance. In contrast, disturbed flow patterns associated with atherosclerosis and vascular stenosis can lead to glycocalyx degradation and dysfunction. Inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), stimulate glycocalyx shedding and disrupt endothelial barrier function by activating matrix metalloproteinases (MMPs) and other proteolytic enzymes.[8]

Glycocalyx – biochemistry

The glycocalyx is an important macromolecule composed of glycoproteins with acidic oligosaccharides and terminal sialic acid, proteoglycans (heparan sulfate proteoglycan, syndecans and glypican core proteins), and glycosaminoglycan side chains that are sulfated (chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, and heparin), and non-sulfated (hyaluronic acid).[9]

The glycocalyx has a complex and dynamic structure. Central to its composition are glycoproteins, which are proteins covalently linked to carbohydrates. In addition to glycoproteins, the glycocalyx contains proteoglycans, proteins attached to long chains of repeating disaccharide units called glycosaminoglycans (GAGs). Proteoglycans confer unique properties to the glycocalyx, such as its ability to trap water molecules and form a hydrated gel-like matrix. This matrix regulates vascular permeability, mechano-transduction, and molecular sieving within the endothelial glycocalyx.[10]

Among the glycosaminoglycans found in the glycocalyx, heparan sulfate is particularly abundant and contributes significantly to its structure and function. Heparan sulfate chains interact with various growth factors, cytokines, and enzymes, modulating their bioavailability and activity. These interactions regulate diverse physiological processes, including cell proliferation, angiogenesis, and inflammation. Furthermore, heparan sulfate is a binding site for anticoagulant molecules such as antithrombin III, inhibiting coagulation cascade proteins.[11]

Another essential component of the glycocalyx is hyaluronic acid, a non-sulfated glycosaminoglycan that forms long, unbranched chains. Hyaluronic acid contributes to the hydration and lubrication of the glycocalyx, promoting its mechanical resilience and anti-adhesive properties. Moreover, hyaluronic acid interacts with cell surface receptors, such as CD44, to modulate cell adhesion, migration, and differentiation. Dysregulation of hyaluronic acid metabolism has been implicated in various pathological conditions, including cancer metastasis and inflammatory diseases.[12]

Sialic acids represent another class of carbohydrates present in the glycocalyx, serving as terminal residues on glycoproteins and glycolipids. These negatively charged molecules confer anti-adhesive properties to the glycocalyx by repelling negatively charged particles and cells in the bloodstream. Additionally, sialic acids participate in cell-cell recognition and immune responses by interacting with lectin receptors on leukocytes and pathogens. Alterations in sialic acid expression have been observed in inflammatory disorders and cancer, highlighting their importance in modulating cell adhesion and signaling.[13]

Furthermore, the glycocalyx contains a variety of glycosphingolipids, which are lipid molecules decorated with

carbohydrate moieties. Glycosphingolipids are enriched in lipid raft domains of the plasma membrane, where they play roles in cell signaling, membrane organization, and cholesterol metabolism. Within the glycocalyx, glycosphingolipids interact with cholesterol and membrane proteins to regulate cell surface dynamics and receptor function. Aberrant glycosphingolipid metabolism has been implicated in neurodegenerative diseases and lysosomal storage disorders.[14]

Mechanical signal transduction

The glycocalyx serves as a crucial mechanosensor in vascular physiology, transducing mechanical forces exerted by blood flow into biochemical signals that modulate endothelial function. Shear stress, generated by the frictional force of flowing blood on the endothelial surface, induces deformation of the glycocalyx, leading to the activation of mechanosensitive signaling pathways. Key mechanotransduction mechanisms involve the activation of ion channels, such as transient receptor potential (TRP) channels and piezo channels, which respond to changes in membrane tension and cytoskeletal deformation. Additionally, integrin-mediated signaling pathways play a role in mechanotransduction, as mechanical forces transmitted through the glycocalyx can trigger conformational changes in integrin receptors, initiating intracellular signaling cascades that regulate gene expression, cell proliferation, and cytoskeletal remodeling.[10, 15]

Moreover, the glycocalyx acts as a reservoir for mechanosensitive molecules, including growth factors, cytokines, and enzymes, which are released in response to mechanical stimulation and contribute to the regulation of vascular function and homeostasis. For example, shear stress-induced deformation of the glycocalyx promotes the release of nitric oxide (NO) from ECs, leading to vasodilation and inhibition of platelet aggregation and leukocyte adhesion. Additionally, mechanical forces applied to the glycocalyx stimulate the secretion of vasoactive peptides and molecules such as endothelin-1 and prostacyclin (PGI_2), which regulate vascular tone and blood pressure. These findings underscore the pivotal role of the glycocalyx in translating mechanical cues into physiological responses that govern vascular health and disease.[16]

Endothelial bio-signaling: from basic science to clinical practice

The endothelium can be considered an "organ" because of its multiple actions on vessel contraction, cell and nutrient trafficking, coagulation balance, and permeability regulation.[17] Such organ replies to the hemodynamic forces acting on itself by expressing different phenotypes that could be summed up in an anti or pro-inflammatory kind, based on the physiological or pathological flow, respectively flushing them. The phenomenon is regulated by flow-sensitive proteins and gene expression modulation, leading first to vascular remodeling and then to pathological manifestations like vessel wall inflammation, thrombosis, and atherosclerosis.[18]

Not only shear but also stretch stress (transversal force) is involved in the phenomenon. This is an important consideration, particularly if related to vessels with significant caliber variations, such as those veins behaving as capacitance reservoirs.[19] Turbulent flow is associated with a venous pro-inflammatory, pro-thrombotic phenotype, similar to how endothelial expression becomes atherogenesis in the arteries.[20] Conversely, laminar flow leads to anti-inflammatory signaling, demonstrating even atheroprotective properties.[21] Different types of flow were demonstrated to change even the shape of the same ECs. For example, in the presence of turbulent flow, ECs were found to be more rounded with random and short actin filaments, mainly at the periphery of the same cell.[22]

Turbulence modulates the expression and permeability of intercellular junctional proteins such as connexins and vascular endothelial cadherin.[23] A reliable way to investigate the specific phenotype expression of ECs exposed to different types of flow is the vascular ECs culture. The comparison between *ex vivo* human ECs from CVD patients *vs.* healthy controls demonstrated how venous reflux leads to an increased surface expression of CD146, CD31/PECAM-1, and intercellular adhesion molecule-1 (ICAM-1). The CD146 elevation justifies monocyte transendothelial migration alterations associated with venous hypertension.[24] Similarly, CD146 involvement explains the angiogenesis promotion in this condition.[25] CD31/PECAM is involved in the leukocyte trans-endothelial migration, endothelial integrity protection, and the responses to shear stress.[25]

ICAM-1 recruits leukocytes, platelets, and erythrocytes, an action that aligns with the pathogenesis of venous disorders.[26] Pathological ECs show higher proliferation and survival in starvation rate, but decreased migration properties. CVD endothelium also demonstrates an increased release of osteoprotegerin (OPG, a bridge between leukocytes and ECs) and vascular endothelial growth factor (VEGF). Interestingly, in varicose patients, OPG and VEGF levels have been found to increase as well, therefore paving the way for venous inflammation biomark-

ers.[27] While it is well known that CEAP Clinical Class is not a severity score.[28] Interestingly, OPG was found to be more elevated in C3 than in C2 ECs than in controls.[27] This finding is of particular importance considering also that OPG high levels have been found to be correlated with an increased risk for cardiovascular disease.[29] This is also in line with the increased risk of cardiovascular disease in CVD patients.[30] The suppression of the venous reflux leads to the normalization of several inflammatory mediators in an interesting timing: during the first 2 months after the procedures IL-8, PDGF, EGF, VEGF, and Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES) are further increased, possibly as a consequence of the surgical insult and the vascular remodeling, but then, after 6 months, the inflammatory panel normalizes. Patients presenting a CVD recurrence also showed a recurrence in the elevation of EGF, PDGF, and RANTES.[31] Interestingly, venous reflux time correlated with the spontaneous release of PDGF-BB, which could also explain the thrombogenic action of the CVD state.[32] In terms of both morphology and inflammation mediators release, endothelial phenotype is, therefore, the expression of the interaction between the living vessel wall and the hemodynamic forces acting on it. Translating such mechanical force into a biochemical message is known as "mechano-transduction" or bio-signaling.[33]

A fundamental aspect of the endothelial "organ" correct expression is the ability of its cells to sense changes in the bloodstream and signal-related biochemical messages. This is made possible by receptors on its surface that act like real antennas decoding the physical force into a pro or anti-inflammatory message. In this sense, blood flow acquires morphogenetic properties as it can shape the endothelial lining phenotype and expression, affecting transcriptional processes and governing cellular specification. Biosignaling is made possible by an intricate machinery on the endothelium lining and inside the cytoskeleton and the extracellular matrix (ECM). This process involves components like actomyosin stress fibers, microtubules, scaffolding proteins, phosphatases, and several kinases.[33] Different endothelial monolayers present different types of cells and related structures based on their location in the vascular tree. This finding has a rationale in the different hemodynamic solicitations acting on the vessel wall in the heterogeneous anatomical regions of the body.

Different sites also present different ECM compositions, with even specific elasticity and, therefore, reaction to the stretch and shear acting on the vessel. Literature shows how flow can influence even the nuclear architecture by means of a connection between the nuclear lamina and the cytoskeleton network.[34] The same physical force can act on the cytoskeleton and then on the nuclear lamina to stretch the chromatin and facilitate the binding of RNA polymerase II with the transcription factors.[35] In a metaphor, it's possible to imagine the different kind of vegetation populating the different lands of different regions around the earth in order to adapt to the different climatic conditions. In a similar way, along the different anatomical vascular beds, the endothelium adapts to the heterogeneous types of hemodynamics flowing over it by expressing different phenotypes. Such forces are transmitted on the endothelial surface receptors as well as on the cytoskeleton.[36]

This aspect is particularly evident in the ciliary structures over the endothelium. The ciliary length is correlated with the kind of flow acting on it: in the case of low-force fluids, the length is higher compared to regions where high forces are impacting on the endothelium structures. Ciliary structures are fundamental in sensing the flow and in translating it into a biochemical message by means of ions movement associated with the same ciliary bending.[37] The glycocalyx can be depicted as a cilia-like structure responsible for regulation of the endothelial permeability and integrity, vessel tone control, and coagulation balance, among other functions. The glycocalyx is the first point of contact of the vessel wall with the blood and its function is considered pivotal in vascular pathophysiology. Indeed, the glycocalyx alteration is associated with conditions such as atherosclerosis, hypertension, stroke, CVD, thrombosis and even sepsis.[38, 39] Its critical role in maintaining endothelial physiology and in stimulating thrombo-inflammation in case of malfunctioning has been observed during COVID-19 pandemic when restoration of the glycocalyx reported potential systemic benefits.[40, 41] Loss of glycocalyx volume has been correlated with increased hyperglycemia-induced endothelial damage. In a similar way, loss of glycocalyx volume has been correlated with atherosclerosis manifestation.[42]

The structure of the glycocalyx is shaped by the hemodynamics acting on it. Shear stress induces clustering of glypican-1, a cell membrane heparan sulfate proteoglycan representing a main glycocalyx component.[43] Sphingosine-1-phosphate (S1P) is another glycocalyx component involved in endothelial barrier function and vascular tone, therefore potentially explaining the vasomotion effect demonstrated in association with the same glycocalyx restoration.[44] A recent publication confirmed the role of the glycocalyx in the vasomotion control,

showing also a dose-dependent venocontractile action of sulodexide (glycocalyx restorer) in human veins.[45] The glycocalyx negative charge forms an electrostatic barrier whose degradation facilitate turbulent flow and the related vicious circle on the same endothelium phenotype expression. Interestingly, the knockdown of glypican-1 blocks the activation of NO synthase (NOS), therefore pairing the glycocalyx presence with the endothelial pathophysiology.[46] Syndecan is another glycocalyx component related to endothelial correct functioning as it is involved in EC survival and correct proliferation. A pathological flow altering syndecan presence is, therefore, leading to an altered endothelium turn-over.[47] Syndecan is also one of the main players in mechano-transduction as it senses the flow direction.[48]

The carotid bifurcation is a perfect example of the glycocalyx derangement associated with turbulence, as indeed, only in the regions where the laminar flow is lost glycocalyx shows a related alteration.[49] High shear stress for a prolonged time has been associated with enhanced endothelial NO synthase (eNOS) activity and NO production, and a role of glypican-1 has been suggested in enhancing EC sensitivity to laminar flow, mediating the eNOS activation under shear stress, protecting endothelial function against disturbed flow and preventing the progress of atherosclerosis.[50] In experimental models, the selective degradation of glycocalyx heparan sulfate or the silencing of glypican-1 genes showed a consequent inhibition of the shear-stress induced activation of eNOS and the production of NO.[50, 51] The glycocalyx modifications caused by the different hemodynamics can lead to ECs functional changes in terms of eNOS activation, NO production, proliferation, and migration. For example, heparan sulfate degradation leads to decreased sensitivity to shear stress and alterations in ECs alignment in the direction of flow and their suppressed proliferation in response to flow, which could influence the adhesion of leukocytes to the endothelium.[51]

Sphingosine-1-phosphate (S1P) plays a fundamental role in the endothelial permeability regulation as it binds to albumin, in this way inhibiting the shedding of the glycocalyx syndecan-1, therefore regulating the filtration through the endothelium.[52] All the above described basic science features are quite easily translated into clinical practice as we sum up the glycocalyx role in endothelial protection, permeability regulation, contractility modulator and coagulation balance. Recent data demonstrated the presence of a glycocalyx-like structure in the lymphatics, therefore widening the same glycocalyx involvement in fluid shifts and paving the way for its potential involvement in venous and lymphatic edema management.[53] Clearly, Starling law is not the only mechanism involved in edema formation and a sieve of various porosity as the glycocalyx could play a fundamental parts in vein-lymphatic filtration and therefore in both venous and lymphatic edema pathophysiology.[54] Interestingly, macrophages activity on the glycocalyx degradation mechanism were recently highlighted, paving the way as well for future clinical work on interstitial edema inhibition.[55] In light of the several scientific reports showing how the glycocalyx deterioration is associated with cardiovascular risk factors like hypertension, diabetes, obesity, and thrombo-inflammation, the glycocalyx is now considered a significant treatment target for related cardiovascular disorders.[56] Such systemic role has been highlighted during the COVID-19 pandemic, in which COVID infection represented a pan-endotheliopathy with potential damage to the glycocalyx integrity.[57]

Thus, glycocalyx is a pivotal translator of hemodynamic forces turning into biochemical signaling, while also acting as direct protector of the endothelial integrity. A vicious circle is established once the glycocalyx action is lost, for which the endothelial inflammation can negatively impact the same flow of hemodynamics and coagulation balance, leading to a pro-thrombotic state.[58] Summing up, glycocalyx represents a fundamental treatment target in CVD as it allows to counteract pathological shear stress, the related endothelial possible thrombo-inflammation, while also acting on the vessel tone. This is clinically supported by the evidence of glycocalyx restoration positive effect on venous inflammation signs and symptoms control, including edema, pigmentation, and skin ulceration.[59, 60]

Clinical manifestations and risk factors of CVD

Clinical manifestations and pathological features of CVD

CVD is a common venous disorder of the lower limb veins, affecting ~25 million of the adult population in the United States.[61] Based on the clinical-etiology-anatomy-pathophysiology (CEAP) categorization, CVD is classified into clinical stages C0-6. C0 indicates no visible signs of CVD, C1 shows telangiectasias (spider veins), C2 is manifested as VVs. and C3 shows tissue edema. C4a presents as eczema or skin pigmentation, C4b shows atrophie blanche or lipodermatosclerosis, C5 indicates healed VLU, and C6 presents as active VLU. The advanced CVD stages C4-6 are often designated as chronic venous insufficiency (CVI).[62] Other CEAP classifications use C2r for recurrent

VVs, C4c for corona phlebectatica with increased risk for VLU, and C6r for recurrent active VLU.[63]

VVs have a major socioeconomic impact, and their unsightly appearance exacerbates psychological distress. VVs are manifested as large, distended, engorged, and tortuous lower limb superficial veins. VVs also show incompetent venous valves and venous reflux that is maintained for more than half a second.[2] If untreated, VVs can progress to CVI with VLU and may increase the risk for thrombophlebitis and DVT.[62] Although VVs are thought as a localized dysfunction in the lower limb veins, increased distensibility and pathological changes are often observed in distant arm veins,[64] suggesting that VVs is one component of a systemic venous disorder, and it manifests in the lower limb because of the high venous hydrostatic pressure.

Tissue histology of VVs shows both hypertrophic regions with abnormal smooth muscle cells (SMCs) shape/orientation and extensive ECM deposition, as well as atrophic regions with ECM degradation and inflammatory cell infiltration.[65] VVs lack distinct vascular layers or clear boundaries between the intima, media, and adventitia. VVs show focal intima thickening and increased media thickness. In VVs, SMCs are disorganized in the media and in the vicinity of the intima, with undefined poorly-structured materials. The collagen fibers are disorganized, making it difficult to distinguish between the media and adventitia, while the elastin fibers are thick and fragmented in the intima and adventitia.[66]

Predisposing demographic and environmental factors in CVD

Several demographic, behavioral, and environmental factors increase the risk for VVs, including old age, female sex, use of contraceptive pills, estrogen therapy, pregnancy, obesity, history of leg injury, and venous inflammation and phlebitis. Some studies suggest that CVD is more prevalent in females than males. For instance, the Framingham Study showed a greater annual incidence of VVs in women (2.6%) than in men (1.9%).[67] Also, the Edinburgh Vein Study screened for CVD in 1456 subjects, and while the incidence rates appeared similar in men and women, the 13-year age-adjusted incidence of VVs was 17.4% (13.1-21.7%) in women and 15.2% (10.4-20.0%) in men.[68] Experimental studies also showed reduced contractile response in inferior vena cava (IVC) isolated from female *vs.* male rats. The sex differences in venous function are likely due to greater estrogen and estrogen receptor levels leading to increased venous relaxation and wall distention in females.[69] Hemodynamic and uterine changes during pregnancy could also promote venous dilation and VVs formation. Pregnancy is associated with elevated circulating levels of estrogen (E2) and progesterone, salt and water retention, and plasma volume expansion.[70] Also, the progressive fetal growth during pregnancy increase intra-abdominal pressure and venous hydrostatic pressure,[71] leading to venous dilation, valve incompetence and VVs.

Among women, obesity increases the risk for VVs.[72] A study found that overweight women (BMI 25.0-29.9 kg/m^2) were more likely, and obese women (BMI≥30 kg/m^2) were 3 times more likely to present with VVs than lean women. This may be partly related to the greater plasma estrogen levels in overweight *versus* lean women, particularly after menopause.[73] A positive relation between BMI and CVD was not found in men.[74]

Behavioral factors, including a sedentary lifestyle and prolonged sitting or standing, increase the risk for CVD.[75] Physical activity and ergonomics in a workplace also influence VVs epidemiology. A study in Jerusalem showed higher VVs prevalence among individuals spending most of their workday in a standing position. Also, the rate of reporting occupations involving prolonged standing was higher in women (31.4%) than in men (13.6%), although the ratio of standing *vs.* sitting workplace posture was higher in men (1.88) than in women (1.53).[76]

Predisposing hereditary and genetic factors in CVD

Family history and hereditary and genetic factors influence the risk for CVD.[77] The lower extremity venous hemodynamics and wall elasticity are reduced in children of patients with VVs.[78] The heritability of about 17% of cases suggests genetic risk factors for CVD.[79] Microarray analysis of 3,063 cDNAs from patients with VVs showed upregulation of 82 genes, especially those involved in the regulation of myofibroblasts, cytoskeletal proteins and ECM.[80] Also, GWAS in ~half a million VVs and control subjects found 30 genome loci associated with VVs, including genes encoding for blood pressure, mechanosensing channels, vascular development, maturation, and integrity, and genes near the hemochromatosis gene that is strongly associated with VLU and DVT.[1] Ehlers-Danlos syndrome is a connective tissue disorder involving defective *COL3A1* gene and abnormal collagen synthesis, and manifested as distensible skin, hypermobile joints, fragile and deformed bones, ocular disease, weak prone-to-rupture blood vessels and visceral walls, and VVs.[81]

Primary lymphedema-distichiasis syndrome involves mutation in the forkhead box C2 (*FOXC2)* gene on chromosome 16q24 and is associated with VVs in early age.[82]

Patients with Klippel-Trenaunay Syndrome present with congenital venous anomalies, atresia and agenesis of the deep veins, valve incompetence, venous aneurysms, embryonic veins, impaired venous muscle pump function, VVs, limb hypertrophy, dermal capillary hemangiomas or port wine stain, and abnormal lymphatics.[83] Also, *Notch3* gene mutation has been detected in the CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) pedigree with VVs.[84]

Single nucleotide polymorphism (SNP) -1562C/T in the promoter region of *MMP-9* gene has been linked to increased plasma MMP-9 levels and VVs in the Chinese population.[85] Also, genes for MMP-12, fibroblast growth factor receptor-2, hemochromatosis, coagulation factor XIII, and ferroportin have been associated with advanced CVI and VLU.[86]

The hemodynamics and molecular mechanisms in CVD

The pathophysiology of CVD and VLU is complex, and several mechanisms have been proposed. Increased venous hydrostatic pressure causes glycocalyx disruption and EC dysfunction and initiates a cascade of events, including venous hypoxia, SMC switch from contractile to synthetic phenotype, MMP imbalance and changes in ECM structural proteins, venous inflammation, release of cytokines, tissue metabolites, reactive oxygen species (ROS) and reactive nitrogen species (RNS) and iron deposition.

Venous hydrostatic pressure and CVD

Greater saphenous vein (GSV) valve incompetence and wall dilation result in venous retrograde flow in the lower extremities, increased distal venous pressure, and distended veins. A study also examined microvenous valves using retrograde resin injection and vein casting in amputated lower limbs of patients with CVD *versus* control subjects.[87] The network of tributaries was divided into six sequential generations before reaching the small venous networks. The valves in GSV and major tributaries were designated generation 0, and the valves in each subsequent tributary were consecutively designated generation 1-5. In control limbs and despite a competent GSV, retrograde venous filling was demonstrated, and microvalves were detected down to the 6th generation tributaries. Only in regions with incompetent microvalves out to the 3rd generation tributary ("the boundary"), the injected resin penetrated deeper into the microvenous networks of the dermis. In limbs with VVs and VLUs, reflux into the small venous networks and capillary loops was extensive, with more dense networks and greater tortuosity. Thus, in addition to superficial axial GVS insufficiency, microvenous valve insufficiency also exists, and compromised 3rd generation microvalves are associated with a greater risk for dermal VLUs. This may explain why some patients with long-standing VVs do not develop VLUs, likely because their 3rd generation microvenous valves are intact and prevent clinical deterioration.[87] On the other hand, CVD-related skin changes, hyperpigmentation, and small ulceration may be seen in patients with normal duplex ultrasound of the superficial, deep, and perforator venous systems, likely because of compromised 3rd generation microvalves.

The mechanisms linking the increased venous hydrostatic pressure to CVD and VLU could involve initial glycocalyx injury, EC dysfunction, and a cascade of other intermediary biological processes, including the early release of hypoxia-inducible factors (HIFs) and MMPs, and subsequent inflammation and late release of cytokines, metabolites, ROS, RNS, and iron deposition.[2, 88]

Glycocalyx injury, endothelial dysfunction, and inflammatory cell adherence in CVD

VVs show multiple structural and functional abnormalities. Histological examination of VVs show damaged endothelium areas,[89] and weak immunostaining of the EC marker CD31/PECAM-1 suggesting initial stages of EC-mesenchymal transition.[90] ECs are key regulators of vascular tone, blood flow, and coagulation. In CVD, elevated ambulatory venous pressure and altered shear stress in the venous microcirculation trigger ECs to release vasoactive factors, selectins, cytokines and chemokines, and prothrombotic factors.[91]

ECs can sense changes in blood flow and shear stress and respond by modulating NO production. L-arginine is metabolized by NOS, to produce NO, or by arginase to produce ornithine. Studies have shown increased levels/activity of both NOS and arginase in VLU, with ECs and inflammatory cells as the key sources. Excess NO interacts with hydroxyl free radicals to form peroxynitrite ($ONOO^-$), which promotes vascular inflammation and tissue destruction and delays VLU healing. Arginase enhances matrix deposition and the formation of matrix cuff around blood vessels, thus contributing to CVI, lipodermatosclerosis, and the development of VLU.[92]

VEGF is an EC mitogen that binds VEGFR1 (Flt-1) and VEGFR2 (Flk-1, KDR) to promote EC growth, proliferation, migration, survival, and differentiation. In concert with NO, VEGF maintains vascular integrity, reactivity and dilatory response. Aberrant VEGF release/activity per-

turbs vascular homeostasis and integrity, leading to VVs. A study suggested that among Russians polymorphism rs2010963 located in the 5' untranslated region of the VEGFA gene influences genetic susceptibility to VVs.[93] Another study showed increased mRNA expression of VEGF isoforms VEGF121/VEGF165 and their receptors Flt-1 and KDR in VVs overall and in VVs with incompetent sapheno-femoral junction *vs.* normal GSV.[94]

Prostaglandin E2 (PGE2) is an arachidonic acid derivative that regulates vascular tone and could influence inducible NOS (iNOS) and transforming growth factor-β1 (TGF-β1) signaling and MMP expression during the development/progression of VVs. Studies found an upregulation of PGE2 and TGF-β1 in the tunica intima and media of VVs. PGE2 promoted the migration and tube formation ability and upregulated iNOS, TGF-β1, and MMP-1 (collagenase-1) in human umbilical vascular endothelial cells (HUVECs). The PGE2-induced iNOS expression can promote venous relaxation and aggravate venous blood stasis. TGFβ-1 regulates cell growth, proliferation, differentiation, migration, and apoptosis, while MMP1 degrades collagen I and III. Thus, the PGE2-induced upregulation of TGF-β1 and MMP-1 can affect ECM remodeling and reduce the vein wall elasticity in VVs.[95]

GWAS have identified genes encoding for mechanosensing channels and proteins (calcium channels, glycocalyx) in association with VVs.[1] ECs sense changes in vein wall stretch via mechanosensitive transient receptor potential vanilloid channels (TRPVs).[96] Also, the glycocalyx functions as a mechanical sensor on EC surface, where it senses changes in shear stress, mediates mechanotransduction, acts as a selective permeability and electrostatic barrier to cells and proteins, and an anti-coagulation, anti-inflammatory, and anti-adhesive barrier, and counteracts EC injury induced by hemodynamics forces.[9] Thus, healthy glycocalyx prevents leukocyte adhesion, inflammation, and thrombosis.

Prolonged increases in venous hydrostatic pressure, altered shear stress and mechanical forces on the lower extremity vein wall cause EC injury, glycocalyx disruption, increased EC permeability, activation of adhesion molecules, and leukocyte adhesion to the vein wall.[65] Structural damage of the glycocalyx could be an initiating event leading to increased venous inflammation in CVD and VVs.[97] In support, VVs show disruption in endothelial glycocalyx and increased circulating levels of degraded sulfated glycosaminoglycans, likely due to glycocalyx degradation by heparanase and MMPs.[88]

Studies have examined the effect of shear stress on the expression of the glycocalyx (heparan sulfate proteoglycan, syndecan family, and glypican-1) in ECs, and their role in vascular dysfunction.[98] In rat model of acute mesenteric venous occlusion, the increased venous pressure and reduced shear stress were associated with leukocyte activation/adhesion, release of inflammatory markers, and MMPs expression.[99] Human leukocytes also show similar behavior in response to low shear stress.[100] Rat models of increased hind limb venous pressure induced by a femoral arterio-venous fistula also show increased saphenous vein venous pressure, upregulation of P-selectin and ICAM-1, and leukocyte infiltration and inflammation of the vein wall.[101] Changes in shear stress also promote the expression of ICAM-1, (CD54), vascular cell adhesion molecule-1 (VCAM-1, CD-106), endothelial leukocyte adhesion molecule-1 (ELAM-1, CD-62, E-selectin), L-selectin, and macrophage chemoattractant protein-1 (MCP-1), leading to adherence of leukocytes and their transmigration into the vein wall and valve, and initiation of an inflammatory cascade with increased release of chemokines (IL-8), cytokines (TNF-α, IL-1, TGF-β1), and MMPs.[2, 91, 102]

Leukocytes are a major source of MMPs, and the activation of adhesion molecules and subsequent leukocyte adhesion/infiltration in the vein wall augment the production of MMPs. MMPs in turn degrade different ECM proteins, leading to weakening of the vein wall, venous dilation, valve incompetence, further increases in the lower extremity venous hydrostatic pressure, and progression of CVD (Figure 1).[2] The relationship between increased lower limb venous hydrostatic pressure, inflammation of the vein wall, increased release of MMPs, and degradation of ECM proteins is typically observed in the VVs atrophic regions.

Smooth muscle phenotypic switch and altered proliferation/apoptosis in CVD

Phenotypic transition of venous SMCs from contractile to synthetic/proliferative phenotype could contribute to the development of VVs. Longitudinal sections of VVs wall show considerable heterogeneity, with a succession of hypertrophic and atrophic segments, severe disorganization of the medial layer and numerous areas of intimal thickening.[89] In the hypertrophic regions, medial SMCs show altered shape, and the number of vasa vasorum is increased. In the atrophic regions, both cellular and ECM components are decreased. TGFβ1 and bFGF are increased in VVs. These observations suggest phenotypic transition of SMCs, altered ECM metabolism, and angiogenesis as major mechanisms contributing to the morphological and

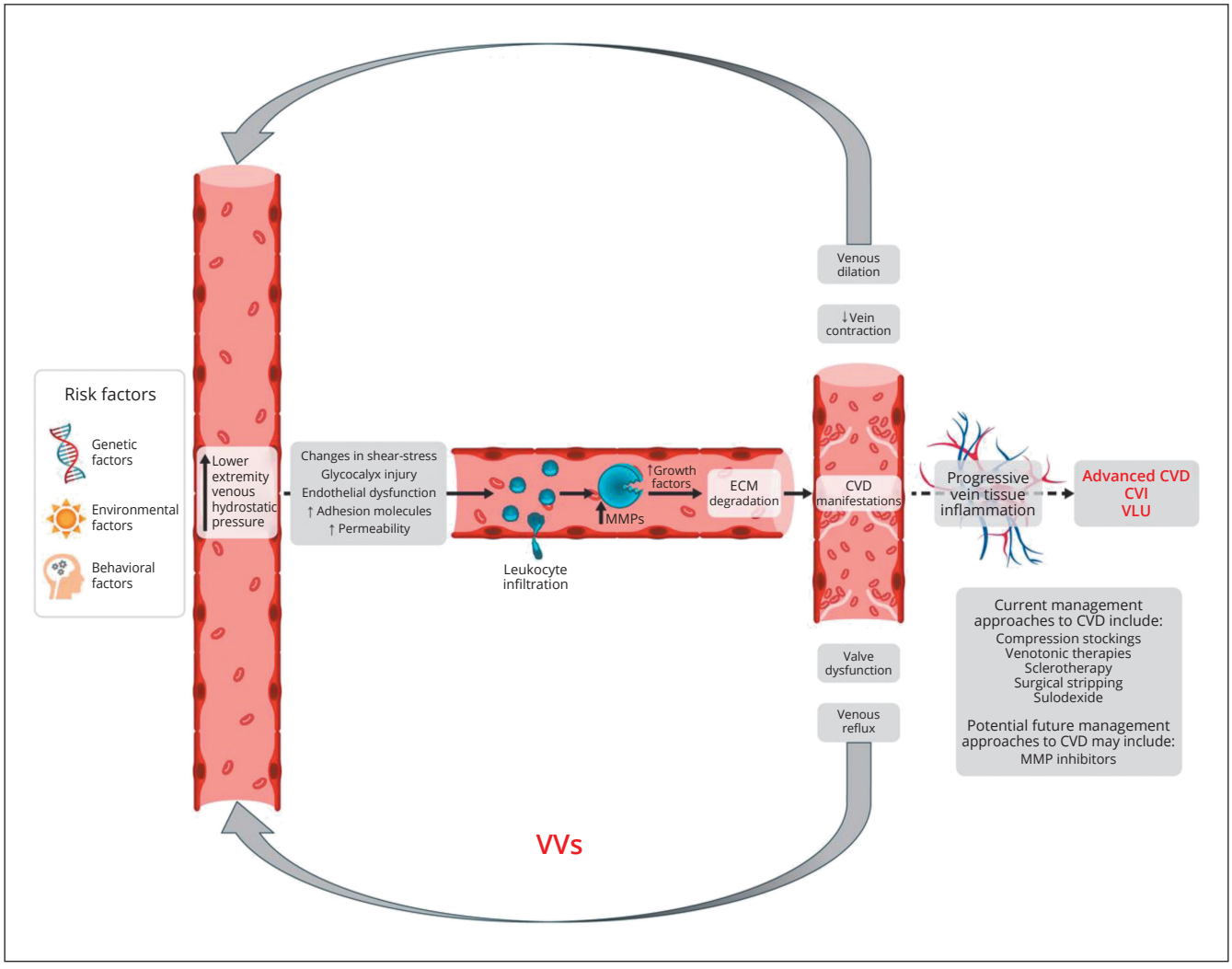

Figure 1.—Pathophysiology and management of CVD. Genetic, environmental and behavioral risk factors cause increases in venous hydrostatic pressure with subsequent changes in shear-stress, glycocalyx injury, endothelial dysfunction, augmented adhesion molecules, increased permeability, leukocyte infiltration, and the release of inflammatory cytokines and MMPs. MMPs increase the release of growth factors and cause ECM degradation, decreased vein contraction, venous dilation, valve dysfunction, venous reflux, various manifestations of CVD, and further increases in venous hydrostatic pressure (vicious cycle). Progressive vein tissue inflammation eventually leads to advanced CVD, CVI, and VLU. Current management of CVD includes elastic compression stockings, venotonic drugs, sclerotherapy or surgical removal of the affected veins. Treatment with sulodexide, which exhibits venotonic properties, has shown benefits in improving endothelial function, and inhibits MMPs and venous remodeling in CVD and VVs. MMP inhibitors may also offer a new approach to the management of CVD/CVI.
CVD: chronic venous disease; CVI: chronic venous insufficiency; ECM: extracellular matrix; MMP: matrix metalloproteinase; VLU: venous leg ulcer; VVs: varicose veins.

functional changes in VVs. MMPs-induced ECM degradation could be involved in the atrophic regions of VVs. Increased TGFβ1 and bFGF may promote SMC phenotypic transition and angiogenesis particularly in the hypertrophic regions of VVs.[103]

GWAS have identified changes in genes encoding for vascular cell differentiation, and vascular development and integrity in association with VVs.[1] Studies also examined the role of microRNA (miR)-199a-5p in VVs and in the phenotypic transition of SMCs. Bioinformatics analysis confirmed that miR-199a-5p had target sites on the FOXC2 3'-untranslated region. RT-qPCR revealed that miR-199a-5p was downregulated while FOXC2 was upregulated in VVs. Biomarkers of SMC contractile phenotype, including smooth muscle 22α (SM22α), calponin, α-SMA and myosin heavy chain 11 were downregulated

in VVs. Cell counting and transwell migration assays revealed that overexpression of miR-199a-5p by mimics suppressed, whereas depletion of miR-199a-5p enhanced SMC proliferation and migration. Notably, the effects of miR-199a-5p could be reversed by FOXC2 overexpression. Dual luciferase reporter analysis confirmed that FOXC2 was a target of miR-199a-5p. These observations suggest that miR-199a-5p regulates phenotypic switching in VSMCs by targeting FOXC2 during VVs formation.[104]

Desmuslin and desmin are intermediate filament proteins in the cytoskeletal network that help to mechanically reinforce protein connections and maintain the structural integrity of tissues. Desmuslin may also regulate SMC function. The desmulin gene is located on chromosome 15q26.3, and gene variants could be associated with VVs. Studies examining gene expression profiles of incompetent and normal GSV at the saphenofemoral junctions showed >30 differentially expressed cDNA. Sequence analysis revealed that a cDNA fragment downregulated in incompetent GSV was a portion of mRNA encoding desmuslin. RT-PCR revealed a similar mRNA expression profile of the desmuslin gene. The desmuslin protein was also markedly decreased in venous SMCs of incompetent veins, which could affect SMC integrity and cause vein wall deformity.[105] Also, in normal human GSV SMCs, desmuslin gene knockdown using siRNA increased collagen synthesis and MMP-2 expression, decreased the differentiation markers α-smooth muscle actin (α-SMA), SM-myosin heavy chain, and smoothelin, and caused disassembly of actin stress fibers consistent with a lower degree of differentiation. These observations suggest that desmuslin expression is necessary for maintaining SMC contractile phenotype, and decreased desmuslin levels could cause SMC switch from contractile to synthetic phenotype, leading to weakening of the vein wall and the development of VVs.[106]

Studies also examined the phenotypic and functional differences between SMCs derived from VVs and control veins. The microfilament structure of the framework and the proliferation, migration, adhesion, and the aging cell count were greater in SMCs from VVs. Bas and caspase-3 mRNA expression and protein content were decreased, whereas Bcl-2 mRNA expression and protein content were increased in VVs. MMP-2, MMP-9, TIMP-1, and TIMP-2 mRNA expression and protein levels were increased in VVs *vs.* control veins. Thus SMCs derived from VVs are more dedifferentiated and demonstrate increased proliferative and synthetic capacity, which may contribute to vein wall weakness and VVs.[107]

NELIN is an F-actin-binding protein involved in the organization of SMC cytoskeleton, NELIN and SM22α combine with actin simultaneously, and in turn alter the form/function of the cytoskeleton. NELIN and SM22α are also involved in SMC phenotypic transition. RT-PCR analysis revealed decreases in mRNA expression of NELIN and SM22α in VVs. Immunohistochemistry localized NELIN and SM22α in SMC cytoplasm, with marked decrease in VVs, suggesting the transformation of SMCs from contractile to synthetic secretory phenotype with subsequent release of MMPs, extensive vein remodeling, and VVs.[108]

C-fos is an immediate early gene involved in the regulation of cell proliferation, growth, migration, and possibly SMC phenotypic transition. VVs showed dilated vein cavities, disordered venous cells, and thicker media partly due to the proliferation of SMCs. Cell counting assay showed more numerous SMCs, and scratch-wound assay demonstrated greater migration speed in SMCs derived from VVs. VVs showed elevated c-fos and osteopontin mRNA expression/protein levels and decreased α-SMA levels n VVs, supporting a role of c-fos upregulation in SMC phenotypic switching and the pathogenesis of VVs.[109]

As variations in E2 levels with age and during pregnancy could contribute to the pathogenesis of varicosity in females, studies examined the role of E2 and estrogen receptors (ERs) in SMC migration in VVs *vs.* control veins. ERα expression was upregulated in VVs. In co-culture of HUVECs and human umbilical vein SMCs, the number of migrating SMCs and the migration rate were increased in E2-treated cells under hypoxia *vs.* E2-treated cells under normal oxygen or E2 non-treated cells under hypoxia and were decreased along with reduced expression of MMP-2 and MMP-9 in cells treated with E2+tamoxifen under hypoxia, suggesting that estrogen promotes SMC migration and VVs formation likely through activation of ER-α and increased expression of MMP-2 and MMP-9.[110] Other studies showed elevated plasma E2 levels in VVs patients and increased mRNA expression of G-protein-coupled receptor 30 (GPR30, GPER) in VVs *vs.* control veins. Upregulation of GPR30 was associated with maintained synthetic SMC phenotype, overexpression of osteopontin, MMP-1, and MMP-9, and poor expression of α-SMA, suggesting a role of GPER in the pathogenesis of VVs through maintaining a synthetic SMC phenotype.[111]

Osteopontin is highly expressed in synthetic SMCs where it regulates SMC proliferation, migration, and adhesion through integrinβ3 cell surface receptor. The methylation levels in the promoter regions of osteopontin and integrinβ3 genes are reduced, and the protein levels of os-

teopontin and integrinβ3 are increased in SMCs of VVs *vs.* control veins. Also, SMCs in the neointima of VVs were transformed into the synthetic phenotype. The hypomethylation of the promoter regions for ostepontin and integrinβ3 genes may increase their expression in VVs, where they promote SMC phenotypic switching.[112]

Foxc1 and Foxc2 are two closely related Fox transcription factors essential for arterial cell specification during development by inducing the transcription of Delta-like ligand 4 (Dll4), a ligand for Notch receptors. Studies have shown a positive association between c.-512C>T FoxC2 gene polymorphism and upregulated FoxC2 expression in VVs. Also, FoxC2 overexpression in venous ECs resulted in overexpression of arterial EC markers such as Dll4 and Hairy/enhancer-of-split related with YRPW motif protein 2 (Hey2). VVs tissue sections also showed elevated levels of Notch pathway components, including Dll4, Hey2, and EphrinB2, and the SMC markers α-SMA and vimentin. These observations suggest that molecular alterations in Dll4-Hey2 signaling are associated with SMC hypertrophy/hyperplasia in VVs, and further substantiate a role for altered FoxC2-Dll4 signaling in SMCs in VVs.[90]

An important factor in vessel wall remodeling is programmed cell death or apoptosis. Some studies have shown a decrease in apoptosis and marked differences in the expression and localization of the cell cycle regulatory protein cyclin D1 in VVs *vs.* healthy veins. Because cell cycle checkpoint controls are linked to apoptotic signaling, studies examined the expression of bcl-2 family members BAX and BCL-x, known mediators of apoptosis, and that of poly (ADP-ribose) polymerase (PARP), a downstream substrate of apoptosis and DNA cleavage, in VVs *vs.* control veins. Pro-apoptotic BAX was decreased in VVs *vs.* controls. PARP expression was diminished in VVs intima and media. Neither BAX nor PARP was observed in the VVs adventitia, but they were detected in the adventitia of control veins. The presence of proapoptotic protein bax in healthy veins supports its role in regulating SMC apoptosis, and the downregulation of both bax and PARP may reduce SMC apoptosis in VVs.[113]

However, other studies suggest increased apoptotic cells in VVs. Proximal GSV segments of VVs showed reduced SMC population and a marked increase of apoptotic markers p53, p21, and BCL-2 mRNA expression. The distal segments of VVs showed greater mRNA expression of BAX and BCL-2. Taking into account the patient's age, elevated p53 mRNA expression was observed in proximal GSV segments suggest that cell cycle disturbances may lead to weakening of the proximal GSV wall, and that valve injury is not the only factor causing VVs formation.[114] Another study showed increased BAX, Caspase 3, BCL-xl and BCL-xs and Ki-67 in GSV specimens from patients with CVD *vs.* healthy controls. In the CVD group, distal GSV showed increased BAX, Caspase 3, and BCL-xs compared with the proximal GSV. These observations suggest that VVs exhibit increased apoptotic activity through increased BAX, Caspase 3, BCL-xl, and BCL-xs. The increased apoptosis in the distal *vs.* proximal saphenous trunk among CVD patients suggests an association between chronic venous hypertension and apoptosis.[115]

Alterations in structural proteins and ECM remodeling in CVD and VLU

VVs histology shows disorganized SMCs, high density of vasa vasorum in the tunica media and adventitia, sclerotic blood vessels, and neoangiogenesis, suggesting marked remodeling of the vein wall. Immunohistochemistry shows positive protein gene product 9.5 (PGP 9.5)-containing innervation and laminin positive structures and a decrease in collagen IV in subendothelial layer of VVs.[89] VVs also demonstrate an imbalance in the main components of ECM proteins with marked changes in tissue content of collagen and elastin.[116]

VVs show increased collagen-I and decreased collagen-III compared with control veins.[116, 117] Studies examined the abnormal distensibility of VVs and measured hydroxyproline in VVs *vs.* control veins and the synthesis of collagen-I, -III, and -V in cultured venous SMCs and dermal fibroblasts from VVs patients and control subjects. Hydroxyproline was increased in VVs, suggesting increased collagen content. Collagen-I mRNA was overexpressed in VVs, whereas collagen-III mRNA was not altered. Cultured SMCs and dermal fibroblasts from VVs patients showed elevated protein levels of collagen-I and reduced collagen-III compared to cells from control subjects.[118] Collagen-III deficiency appears to be generalized in different tissues, suggesting genetic alteration of remodeling and a systemic condition influenced by underlying genetic factors in VVs patients.[119] Interestingly, the collagen-III gene transcription is normal in SMCs from VVs, but MMP-3 activity is increased, suggesting post-translational modification and degradation of collagen-III. In support, collagen-III production was partially restored in SMCs from VVs in the presence of the MMP inhibitor marimastat.[120] Collagen-I mainly confers rigidity while collagen-III determines the blood vessel elasticity, and alterations in collagen synthesis or proteolysis would alter the ratio between collagen-I and -III and negatively affect the vein structural integrity

and lead to weakening of the vein wall, and collagen-III deficiency would contribute to the decreased elasticity and increased distensibility of VVs.[66]

CVI is associated with lipodermatosclerosis (LDS) characterized by hardening and hyperpigmentation of lower limb skin. Studies examined whether elevated procollagen-I gene expression and increased cell proliferation are responsible for the fibrotic changes associated with LDS. Skin biopsies were obtained from the legs of patients with varying degrees of CVD and assessed for procollagen gene expression by *in-situ* hybridization and for cell proliferation by immunolocalization of proliferating cell nuclear antigen. The number of cells expressing procollagen-I mRNA was higher in the dermis of LDS-affected skin compared with samples from the patient other dermal areas. The number of dermal fibroblasts undergoing proliferation was markedly increased in both LDS samples and skin samples prior to LDS changes compared with control samples. There was no apparent difference in the level of inflammation in tissue specimens between patient classes. The results suggest the involvement of enhanced procollagen gene expression and cell proliferation in LDS development in the absence of significant inflammatory response, indicating that during CVI the release of profibrotic factors in the skin may play a role in LDS formation.[121]

Elastin expression is high in blood vessels, where elastic fibers are essential for maintaining vascular function. Among the possible mechanisms of VVs formation are changes in the vein elastin content. Some studies suggest that decreased elastin could reduce the vein wall elasticity and lead to venous dilation and VVs.[122] Studies have examined the expression of the elastin precursor tropoelastin, and lysyl oxidase-like 1 (LOXL1), a cross-linking enzyme responsible for elastin polymer deposition, in VVs *vs.* control veins. LOXL1 was markedly decreased in VVs *vs.* controls. The LOXL/tropoelastin ratio in the vein wall diminished with age. In the younger VVs patients an inverse relationship (LOXL decreased, tropoelastin increased) was observed. Thus the decreased elastin in VVs may be partly related to decreased LOXL1 levels.[123] VVs may also show a decrease in the elastin content due to increased elastolytic degradation by MMPs, other proteases or elastases produced by fibroblasts, platelets, macrophages and monocytes.[122] The net amount of collagen and elastin in VVs is influenced by the dynamic interaction between different biological processes at different stages of CVD. For example, increases in the vein collagen content could compensate for the decrease in elastin levels during the early stages of CVD. Conversely, the vein collagen content may decrease in the later stages of VVs. This may provide an explanation of the divergent reports of the collagen levels in VVs, showing a decrease, no change or even an increase *vs.* control veins.[2] However, other reports suggest an increase in the elastin network in VVs.[66] Studies have shown higher elastin mRNA expression and statistically insignificant upregulation of functionally related fibulin 5, MMP-2 and MMP-9 mRNA expression in VVs *vs.* control veins. Whether upregulation of elastin expression plays a role in the pathogenesis of VVs needs to be further examined.[124]

VVs also show changes in other ECM proteins including increases in tenascin and decreases in laminin levels. Cultured SMCs from VVs also show decreased fibronectin levels.[2]

MMPs Imbalance in CVD and VLU

MMPs are a large family of zinc-dependent metalloendopeptidases that are secreted in their latent pro-form by different cells in the vein wall including SMCs, fibroblasts, and leukocytes. MMPs by virtue of their proteolytic activities participate in cellular homeostasis, adaptation, tissue remodeling, and ECM organization/degradation. MMPs are also involved in SMC functional transformation, phenotypic transition, proliferation, and migration. Tissue homeostasis is achieved by a tight balance of MMPs and the expression/activity of endogenous tissue inhibitors of metalloproteinases (TIMPs), which prevent excessive ECM degradation. MMPs participate in the different stages of CVD; playing a role in the early events by affecting EC function, glycocalyx integrity, the EC-SMC interactions and venodilation, as well as the late events involving ECM degradation, changes in structural proteins, venous tissue remodeling and fibrosis, interstitial tissue proteolysis, and surrounding tissue damage including the dermal and subcutaneous structures leading to skin changes and VLU.[2, 88]

Several MMPs have been detected in vein specimens from CVD patients. In VVs the expression of MMP-1, -2, -3, -7, -9 and -13, and TIMP-1 and -3 is increased. The increased MMP-2 mRNA expression is strongly correlated with hyperlipidemia in patients with VVs. TGF-β1 is a multifunctional growth factor that is widely expressed in multiple tissues, with a critical role in maintaining venous tissue homeostasis. TGFβ1 activity is increased in CVI and dermal skin pathology. Studies examined whether increased TGFβ1 activity is associated with changes in MMPs and TIMP-1 in biopsies of the lower calf and lower thigh from patients with CVI *vs.* control. MMP-1 mRNA was increased in C-4 and C-6 patients, while TIMP-1 was

increased in C-6 patients only. Active MMP-2 was relatively increased compared with active MMP-1 and TIMP-1 in C-4 and C-5 patients. Gelatin zymography revealed both latent and active forms of MMP-2, but only the latent form of MMP-9. Immunohistochemistry revealed MMP-1 and MMP-2 in dermal fibroblasts and perivascular leukocytes. TIMP-1 was observed only in basal-layer keratinocytes of the epidermis. These findings suggest both transcriptional and post-transcriptional regulation of MMPs in CVI, and that dermal fibroblasts and migrating leukocytes are possible sources of MMPs. Alterations in MMP-2 activity, in conjunction with TGFβ1-mediated events, could disrupt the tissue homeostasis/remodeling balance and promote VLU formation.[125]

Several MMPs are also overexpressed in VLU and in the wound fluid, and increased proteinase activity is correlated with poor VLU healing. Multiplexed protein assay showed elevated MMP-1, -2, -3, -8, -9, -12, and -13 protein levels in VLU *vs.* healthy tissue. MMP-3, -8, and -9 markedly decreased after 4 weeks of high-strength compression therapy. Reduction in the levels of MMP-1, -2, and -3 was associated with higher rates of VLU healing at 4 weeks. These findings suggest that compression therapy reduces the pro-inflammatory environment in VLU partly through reduction in the expression/levels of specific MMPs.[126]

MMP release/activity is regulated by multiple factors including hypoxia-inducible factor (HIF), cytokines, urokinase-type plasminogen activator (uPA), extracellular MMP inducer (EMMPRIN, CD147), platelet-derived growth factor (PDGF) isoform AA, and mitogen-activated protein kinase (MAPK). Cytokines, chemokines, growth factors, MMPs, EMMPRIN, and TIMPs have been identified in VLU tissue and wound fluid. Changes in membrane type-MMPs (MT-MMP), and a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) have also been observed in CVD and VLU.[2, 127]

Some reports suggest that certain MMPs are decreased in VVs.[128] The discrepancy in MMP measurements may be related to the structural and pathological differences in the atrophic *vs.* hypertrophic regions of VVs. Increases in MMP activity is expected to cause degradation of ECM proteins in the atrophic regions,[65] while decreased MMP activity would preserve ECM proteins in the hypertrophic regions of VVs.[129] In support, studies on GSV samples from control subjects and patients with early and late stages of CVD showed decreased gene expression of MMP-12, TIMP-2, TIMP-3, TIMP-4, and TGFβR2 in VVs C2-C3 *vs.* control specimens from the ankle region. In CVD samples, findings were uneven across anatomical regions with decreased gene expression of MMP-9 and TGFβR3 and increased gene expression of MMP-2 and TIMP-3 in advanced clinical stages C4-C6 *vs.* control specimen from the ankle region.[130]

Alterations in MMP activity are expected to affect ECM composition and contribute to the abnormalities in vein structure and function associated with CVD. Beside the MMP-induced changes in ECM proteins, MMPs could participate in the pathophysiology of CVD by influencing SMC migration, growth, apoptosis and contractile function. MMP-mediated ECM proteolysis modulates cell-ECM adhesion and facilitates SMC migration. MMP-1 and -9 promote human aortic SMC migration.[131, 132] In rat aortic SMCs, upregulation of MMP-1 increases flow-mediated cell motility through phosphorylation of $ERK_{1/2}$ and increases in c-Jun and c-Fos transcription factors.[131] In cultured human SMCs, MMP-2 affects chemokine-induced chemotaxis.[133] Also, MMP-2 or MMP-9 knock-out reduces SMC migration and neointima formation in mouse models of filament loop injury and carotid artery occlusion.[134-136]

MMPs disrupt the basement membrane, facilitate the interaction between ECM components and integrins, and promote SMC migration. MMPs cause fragmentation of basement membrane proteins such as collagen-I, thus uncovering new integrin-binding sites. MMPs also cleave E-cadherin in epithelial cells, VE-cadherin in ECs and N-cadherin in SMCs, thus dissolving adherence junctions and allowing the cells to migrate.[137] In addition, MMP-1 binds to and cleaves protease-activated receptor-1 (PAR-1) and in turn uncovers tethered ligands that stimulate cell signaling and migration.[138] By sensing a proteolytic environment, the cells then actively and gradually move to the area where ECM is degraded.

SMC reorganization and migration into the vein intima may occur in CVD.[139, 140] SMCs in VVs appear disorganized, and show vacuolization and phagocytosis.[139, 140] Compared to the SMC contractile phenotype in healthy veins, SMCs isolated from VVs are largely dedifferentiated and show increased MMP-2 secretory potential and tendency for migration.[141] MMP-mediated SMC dedifferentiation, migration and phenotypic switch from contractile to synthetic phenotype cause decreases in the vein contractile response and venous dilation.

MMPs facilitate the release of growth factors from their binding proteins, which could promote SMC growth in the hypertrophic regions of VVs. MMPs allow a growth-permissive environment for SMCs in ECM through integrin-mediated signaling.[142] ECM-integrin interactions activates

FAK and regulates p53 signaling and cell survival.[143] Normal MMP levels are required for FAK activation and cell survival, but excessive MMP production increases degradation of ECM proteins and integrins and leads to anoikis.[144] Also, MMP-7 cleaves N-cadherin and regulates SMC apoptosis. MMPs could also regulate apoptosis by cleaving the death ligands TNF-α and Fas and their receptors.[2] While MMPs regulate cell apoptosis, the contribution of SMC apoptosis to VVs pathology needs to be further examined.

MMPs could have additional venous dilation effects.[2] MMPs are released in the vein wall in response to mechanical stretch and in turn affect different components of the vein wall.[44] Besides the tunica adventitia and ECM, the localization of MMPs in the tunica intima and media suggests additional effects on ECs and SMCs.[145] *In-vitro* studies have shown that mechanical stretch increases MMP expression in cultured ECs, SMCs and fibroblasts. MMPs regulate EC integrity and vascular permeability. MMP-2 and MMP-9 also disrupt membrane barrier integrity and the MMP inhibitor GM6001 prevents degradation of the tight junction protein occludin and reduces the intercellular gaps and vascular permeability in porcine cerebral microcapillary ECs.[146]

ECs regulate vascular tone by releasing NO, PGI_2 and endothelium-derived hyperpolarizing factor (EDHF). PARs 1-4 are G-protein coupled receptors (GPCRs). PAR-1 is expressed in ECs, VSMCs,[147] and platelets,[148] and is coupled to increased prostaglandin production.[149] Also, MMP-1 activates PAR-1,[138] which could contribute to venous dilation and CVD. EDHF causes vascular relaxation through the opening of small and intermediate conductance Ca^{2+}-activated K^+ channels and EC hyperpolarization. The hyperpolarization of ECs then spreads through myoendothelial gap junctions and causes SMC relaxation. EDHF-mediated vascular relaxation may involve epoxyeicosatrienoic acids, which are produced from the metabolism of arachidonic acid by cytochrome P450 epoxygenases. Other EDHFs include K^+ ion and hydrogen peroxide (H_2O_2). EDHF could then open large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) and cause hyperpolarization of SMCs.[150] *Ex-vivo* studies have shown that prolonged stretch of rat IVC increases the expression of MMP-2 and MMP-9 in the vein tunica intima and MMP-9 expression in the vein media. Also, prolonged vein stretch is associated with a decrease in contraction to the α-adrenergic receptor agonist phenylephrine or prostaglandin F2α, and the decrease was reversed by MMP inhibitors. Also, MMP-2 and MMP-9 inhibit Ca^{2+} influx in rat aorta,[151] and MMP-2 inhibits extracellular Ca^{2+}-dependent contraction in rat IVC.[152] Interestingly, the MMP-2 induced relaxation of rat IVC is prevented in veins incubated in a high KCl depolarizing solution, which blocks outward movement of K^+ ion via K^+ channels. Also, iberiotoxin, a blocker of BK_{Ca}, inhibited MMP-2 induced relaxation of rat IVC, suggesting that MMP-2 actions involve membrane hyperpolarization, activation of BK_{Ca}, and inhibition of Ca^{2+} entry through Ca^{2+} channels.[153] Long-term, the maintained MMP-induced hyperpolarization and inhibition of Ca^{2+} entry and vein contraction could lead to progressive venous dilation and VVs.[44] On the other hand, MMP-3 may impair EC function and endothelium-dependent vasodilation,[154] and therefore it is important to further study the effects of MMPs on ECs and vascular relaxation pathways.

Venous hypoxia, HIFs and CVD

Some studies suggest an association between venous hypoxia, vein cells apoptosis and CVD.[155] GWAS have shown associations between genes related to hypoxia and VLU.[1] Also, the average minimum O_2 tension is markedly lower in VVs *versus* non-VVs. Hypoxia-inducible factors (HIFs) are transcriptional factors that regulate the expression of genes involved in O_2 homeostasis. HIF-1α and HIF-2α are overexpressed in VVs *versus* control.[156] GSV specimens from patients with valve incompetence and venous reflux show marked increase in the levels of HIF-1α/HIF-2α and phosphoinositide 3-kinase (PI_3K)/mTOR, and the number of mast cells CD4+, CD8+, and CD19+ cells and mastocytes.[157] HIF-1α may also regulate MMP-2 and MMP-9 expression in arterio-venous fistulas and hemodialysis polytetrafluoroethylene grafts.[158] Also, HIF target genes and protein levels of glucose transporter-1 (GLUT-1), carbonic anhydrase-9, VEGF, and BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP-3) are elevated in VVs *versus* control. Additionally, the HIF regulatory enzymes prolyl-hydroxylase domain (PHD)-2 and PHD-3 are elevated in VVs *vs.* control. Furthermore, exposure of VVs and non-VVs cultures to hypoxia (O_2 tension 1%), or to the PHD inhibitor dimethyloxallyl glycine (DMOG) in normoxia is associated with increases in HIF-1α and HIF-2α and HIF target genes compared with normoxia alone. These observations support that hypoxia, HIF, and target genes contribute to the pathogenesis of CVD.[156]

In addition to hypoxia, mechanical stretch could affect HIF expression. Prolonged stretch of rat skeletal muscle increases HIF-1α and HIF-2α in the muscle capillaries ECs.[159] Also, mechanical stretch of the rat ventricular wall increases HIF-1α expression.[160] Prolonged mechanical

stretch of the rat IVC also causes increases in HIF-1α, HIF -2α, MMP-2 and MMP-9 accompanied with a decrease in vein contraction to phenylephrine. Pretreatment of rat IVC with DMOG, which inhibits PHD and prevents HIF inactivation, caused further reduction in vein contraction. On the other hand, the HIF inhibitors echinomycin and U0126 prevented the decrease in contraction in IVC under prolonged stretch, suggesting that HIF is an intermediary mechanism between the increase in venous hydrostatic pressure, increased MMP-2 and MMP-9 and the reduction in vein contraction.[161] It is likely that mechanical stretch activates Ca^{2+} entry via transient receptor potential vanilloid TRPV4 channel, leading to activation of PI_3K and induction of HIFs.[162] Mechanical stretch may also affect membrane integrins and trigger a signaling cascade that causes activation of mitogen-activated protein kinase (MAPK) and induction of HIF mRNA expression. Also, mechanical stretch may stimulate GPCRs or tyrosine kinases or increase ROS, leading to MAPK activation and increased HIF expression. Interestingly, MAPK inhibitors reverse the increases in HIFs and the decreases in contraction in IVC under prolonged mechanical stretch, supporting a role of MAPK as a linking factor between mechanical stretch, HIF expression and reduced vein contraction.[161] Beside hypoxia and mechanical stretch, low pH, metallic ions, heat exposure and hormones could affect the expression of HIFs and MMPs in lower extremity veins.

Metabolic abnormalities in CVD and VLU

Metabolic products could play a role in venous dysfunction and CVD. Creatine, lactate, and myoinositol metabolites are increased in VVs *versus* control veins.[163] Also, studies have shown higher levels of glutamate, taurine, myo-inositol, creatine and inosine in aqueous extracts, and phosphatidylcholine, phosphatidylethanolamine and sphingomyelin in lipid extracts of specimens from VVs *versus* control veins. Out of 7 differentially expressed miRNAs, hsa-miR-642a-3p, hsa-miR-4459 and hsa-miR-135a-3p expression was correlated with inosine in the vein tissue, while miR-216a-5p, conversely, was correlated with phosphatidylcholine and phosphatidylethanolamine. Phosphatidylcholine and sphingomyelin were correlated with inflammation and myo-inositol was correlated with cell proliferation, thus implicating major metabolic pathways in the pathogenesis of CVD.[164] Other studies have shown upregulated metabolites in vein specimens from patients with CVD including lipids, branched-chain amino acids, glutamate, taurine, lactate, and myo-inositol, as well as in VLU wound fluid and ulcer biopsies including lactate, branched-chain amino acids, lysine, 3-hydroxybutyrate, and glutamate.[165] These observations are consistent with the pathology observed in VVs and CVD, and provide insight into the underlying metabolic pathways.

Increased levels of valine, choline and triglyceride moieties were also detected in association with decreased contraction observed in rat IVC under prolonged stretch *vs.* non-stretched vein. The increased branched-chain amino acid valine and cell membrane constituent choline suggest increased muscle breakdown. The increased triglyceride moieties in stretched IVC suggest an inflammatory response to prolonged IVC stretch and high venous tension. These observations support that increased vein wall tension/venous pressure could alter the tissue metabolic profile in the setting of VVs.[166] Whether the increases in tissue metabolites affect vein contraction through changes in the expression of HIFs and MMPs remains to be examined.

Inflammation in CVD and VLU

GWAS have shown associations of genes related to inflammation with VLU.[1] Also, GSV specimens from VVs show increased monocyte/macrophage infiltration in the vein wall and valves,[167, 168] and increased expression of ICAM-1 and VCAM-1 in ECs.[169] VVs patients also show increases in plasma levels of ICAM-1, VCAM-1, angiotensin converting enzyme, L-selectin and proMMP-9, supporting a relationship between postural blood stasis, increased lower limb venous hydrostatic pressure, infiltration of polymorphonuclear leukocytes in the vein wall, and increased MMP release in VVs.[170] Pro-inflammatory cytokines play an important role in different stages of CVD by contributing to the inflammatory process and its propagation in the interstitial space and the VLU bed.[2, 88] A study examined the expression of cytokines and chemokines in surgically-retrieved primary VVs *vs.* control veins, and the effect of treating VVs patients with acetylsalicylic acid (Aspirin) 15 days prior to removal of varices on cytokine expression. Control veins expressed low levels of MCP-1 and IL-8 mRNA. In contrast, VVs showed marked upregulation of MCP-1 and IL-8 and increased expression of interferon-γ (INFγ)-induced protein 10 (IP-10), RANTES, macrophage inflammatory protein-1α (MIP-1α) and MIP-1β mRNA. On the other hand, VVs obtained from patients treated with acetylsalicylic acid showed a decrease in chemokine expression.[171] Also, among CVD patients, untreated VLU displayed high levels of TNF-α, interleukins and IFN-γ, but after 4 weeks of compression therapy, the cytokines levels markedly decreased, transforming growth factor (TGF-β1) increased and the VLU began to heal. In-

terestingly, healing was much better in VLUs with higher levels of IL-1 and IFN-γ before compression therapy.[172]

Inflammatory cytokines promote the release of MMPs. Cytokines could also increase ROS which in turn affect the expression/activity of MMPs. TNF-α, IL-17 and IL-18 increase MMP-9 gene promoter activity and MMP-9 mRNA expression through activation of specificity protein-1 (Sp-1), activator protein-1 (AP-1), and nuclear factor κ light chain enhancer of activated B cells (NF-κB).[173] Also, the levels of TNF-α, IL-1, IL-6, and IL-8, MMP-1 and MMP-8, and VEGF are greater in infected *vs.* non-infected VLU, supporting an association between inflammation, cytokine secretion, and MMP activation in CVI.[174] Another study showed marked increases in the levels of MMP-1, 2, 3, 8, 9, 12, and 13 in VLUs compared to healthy tissue, and 4 weeks compression therapy was associated with reduced MMP-3, 8, and 9 levels. The study also showed a correlation between reduced MMP-1, 2, and 3 levels and higher VLU healing rates.[126] Also, studies on inflammatory *vs.* granulating VLUs showed marked differences in the levels of cytokines, chemokines, granulocyte-monocyte colony-stimulating factor, and growth factors in the wound fluid depending on the wound environment. Notably, marked differences in the levels of MMPs and TIMPs are also observed depending on the stage of the VLU wound; inflammatory *versus* granulating.[175] In addition to venous hypertension, inflammation, vein wall remodeling, and increased expression of cytokines and MMPs, a fibrin cuff containing collagen I and III, fibronectin, vitronectin, laminin, tenascin, fibrin, TGF-β1, and α2-macroglobulin is often identified in the post-capillary venules of VLU and represent a major abnormality in the dermal microcirculation.[176] Interestingly, macrophages and mast cells have been detected in the fibrin cuff, and may represent a major source of the elevated cytokines and MMPs and the consequent pathological changes in CVD, skin changes and VLU.

Urokinase plasminogen activator (uPA) contributes to the inflammatory process by increasing TNF-α expression in injured vessels. Binding of uPA to the uPA receptor also potentiates the activity of MMP -2 and aggravate VLU pathology. Studies found elevated uPA and uPA receptor levels in VLU *vs.* healthy skin. Also, fibrin zymography showed greater endogenous uPA fibrinolytic activity in VLU *vs.* healthy skin. These observations suggest that elevated plasminogen activation is crucial in maintaining proteolytic activity in VLU.[177] Hyaluronan synthases 2 gene (HAS2) code for ECM proteins and scaffolding vascular structure and plays a role in angiogenesis, cell adhesion and vascular injury. HAS2 mRNA expression is decreased in specimens of VVs. Also, HAS2 knockdown in zebrafish resulted in dilated venous structures with static venous flow, supporting that downregulation of HAS2 contributes to the pathogenesis of VVs.[178]

Gap junctions participate in the different processes involved in chronic wounds including inflammation, edema and fibrosis. Connexins are the channel forming components of gap junctions, facilitating electrical propagation between excitable cells and the passage of small molecules between the cytoplasm of adjacent cells. Connexins may facilitate the spread of inflammation in CVD and VLU, and connexin43 is elevated in the wound margin of VLU.[165] Interestingly, ACT1, a peptide inhibitor of connexin43, may accelerate fibroblast proliferation and wound epithelialization. In support, VLU treated with compression plus ACT1 gel showed greater mean percent re-epithelialization at 12 weeks and reduced median time to 100% ulcer healing *vs.* ulcers treated with compression alone.[179]

Reactive oxygen/nitrogen species in CVD/VLU

Early indications of oxidative stress are observed in VVs. Studies measured the levels of the antioxidant defense enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase, and the oxidative stress indicator malondialdehyde in GSV specimens of VVs *versus* control veins. Measurements revealed marked increase in catalase and malondialdehyde levels but no differences in superoxide dismutase, glutathione peroxidase and glutathione S-transferase enzyme activity in VVs *versus* control veins. An increase in the levels of the antioxidant enzyme catalase may represent a compensatory response to the increase in the levels of the oxidative molecule malondialdehyde in VVs.[180]

Oxidative stress is also elevated in patients with VLU and can lead to slow-healing or non-healing.[181] The highly oxidative state within VLU results from activation of different oxidative and nitrating pathways. A study of VLU tissue showed elevated oxidative stress markers (increased lipid peroxidation, gluthathione activity, and radical scavenging activity), and tissue injury (as indicated by elevated lactate dehydrogenase). Generation of ROS, RNS, protein carbonylation, lipid peroxidation, and DNA oxidation and nitration result in DNA damage and single-strand breaks.[182] Peroxynitrite ($ONOO^-$) is a potent oxidizing and nitrating agent that causes irreparable damage to the mitochondria and DNA, as well as lipid peroxidation, and protein oxidation and nitration, leading to disruption of different cellular functions.[2] $ONOO^-$ causes DNA single-strand breaks, which activates poly-ADP ribose poly-

merase (PARP) and builds at the DNA nicks a branched poly-ADP-ribose (bPAR) polymer to initiate the repair process A study found elevation of both bPAR (indicating PARP activity, DNA damage/repair) and nitrotyrosine (an $ONOO^-$ metabolite) in tissue biopsies of VLU *vs.* control tissue.[182] $ONOO^-$ and its damaging effect on DNA could lead to gene mutations and carcinogenic transformation of VLU as is seen with squamous cell carcinoma.[2]

In fibroblasts, MMP expression is regulated by NADPH oxidase-1 (Nox-1).[183] Also, uPA affects the expression of MMP-9 in part through increasing ROS production.[184] ROS could activate MMPs through oxidation of the MMP prodomain thiol and its autolytic cleavage. ROS may also modify the critical amino acids required for MMP proteolytic activity and lead to MMP inactivation, and thereby provide a feedback-mechanism that controls any undesirable bursts in MMP activity.[185] $ONOO^-$ inhibits superoxide dismutase, causing further increases in ROS generation and activation of MMPs. Future work should examine if inhibition of $ONOO^-$ formation can alter the progression of VLU and improve healing.

Iron deposition in CVD/VLU

Changes in blood levels of iron have been associated with multiple clinical disorders. Genetically predicted higher blood levels of iron (serum iron, ferritin, and transferrin saturation) are associated with lower risks of iron deficiency anemia, but an enhanced risk of VVs. While higher iron status exerts a detrimental effect on VVs in both genders, sex-stratified Mendelian randomization analysis revealed some sex differences in their clinical effects.[186, 187] GWAS have shown an association between genes related to hemochromatosis and VLU.[1] Local iron overload and deposits have been implicated in the pathogenesis of CVD, the development of skin changes and the progression to VLU.[188] The number of patients affected by primary CVD is ~20-40% of the general population and ~10% of these patients would develop VLU. Detection of venous tissue iron overload could help in predicting in advance who will develop VLU. Mutations in iron metabolism genes could be involved in VVs pathology. Also, prolonged venous reflux could cause iron overload and dermal hemosiderin deposition which is directly correlated with manifestations of CVI such as lipodermatosclerosis and skin changes.[189] Iron deposition promotes free radical formation, thus aggravating tissue injury, and causing further progression to CVI and VLU.[190] Also, coagulation factor XIII is a cross-linking protein that is critical for VLU healing.[191] and mutations in hemochromatosis *HFE* gene C282Y and *Factor XIII* gene V34L have been associated with severe CVI, skin changes and the size of VLU.[192-194] A cohort study examined patients with severe CVD (CEAP C4-C6) subdivided into patients with VLU (C5-C6, primary and post-thrombotic), and cases with no skin lesions (C4) *vs.* healthy controls, and all subjects were PCR genotyped for HFE mutations (C282Y and H63D). C282Y mutation increased the risk of VLU in primary CVD ~7-fold. Application of the HFE test in primary CVD demonstrated increased specificity and positive predictive values. The high specificity of C282Y blood genetic test in predicting VLU development suggests that applying this test starting from the C2 class VVs could be useful in predicting and strengthening the priorities for surgical correction of CVI.[192]

Studies have examined the role of HFE-C282Y and FXIII V34L and P564L gene variants in VLU healing time in patients with primary VLU (CEAP C6) who underwent superficial venous surgery. Generally, VLU cases had a postoperative mean healing time of 8.5 +/- 5.7 weeks. For the subset of cases above and below the median value (8.0 weeks), FXIII-V34L genotype distribution markedly differed. Kaplan-Meier analysis yielded specific healing time profiles for the different FXIII-V34L classes of genotype, with an increased risk of delayed healing for the FXIII-VV genotype. FXIII-P54L genotype distributions did not differ, but homozygous 564LL cases and double carriers for both FXIII variants had a markedly reduced healing time *vs.* control types. No differences in healing time were observed between carriers and noncarriers of the HFE-C282Y variant, but when these cases were stratified by FXIII-V34L genotypes, the L34 carriers had a markedly shorter healing time, irrespective of the HFE genotype. Thus, the FXIII-34L variant is associated with shorter VLU healing time after surgery, suggesting a role in the healing and tissue regeneration phases. While HFE-C282Y is involved in VLU development, it did not affect the postoperative healing time.[194]

Other studies used DNA-array technology to evaluate SNPs in candidate genes for hemochromatosis HFE (C282Y, H63D), exporter of non-heme iron ferroportin 1 (FPN1) (-8CG), MMP-12 (-82AG) and FXIII (V34L) as potential prognostic markers in VLU healing in 638 subjects, 221 with VLU (171 primary and 50 post-thrombosis), 112 with severe CVD (C3-C4), and 305 healthy controls. The HFE and FXIII SNPs were previously genotyped by conventional PCR in the same cohort,[192-194] and re-genotyping by DNA-array resulted in a 100% matching. In the risk computation, the FPN1 -8GG genotype had

an overall CVD risk of 4.3 and a VLU risk of 5.2 virtually the same among primary VLU (4.98). The MMP-12-82AA genotype had a VLU risk of 1.96 only in primary VLU. In the genotype-ulcer size association studies, a smaller mean ulcer size was observed in the MMP-12 GG-genotype compared with the other genotypes.[86]

Erythrodiapedesis is a process in which red blood cells exit the capillaries and pericapillary network and enter into the interstitial tissue space, leading to erythrocyte disruption, hemoglobin degradation, and storing of ferric iron as hemosiderin. Erythrodiapedesis has been detected in CVD patients with skin changes, lipodermatosclerosis and VLU.[195] Also, the extremely toxic ferric ion (Fe^{3+}) is detected in tissue biopsies of advanced CVI, lipodermatosclerosis and VLU, but not in VVs or only edema and hyperpigmentation CVD patients.[195] Free ferrous ion (Fe^{2+}) also stimulates macrophages, and promotes cytokine and chemokine release, leading to tissue inflammation, oxidative stress, hemolysis of red cells, and perpetuation of skin changes and VLU development.[196]

Management of CVD

Conservative and surgical approaches

Treatment of CVD includes conservative approaches in the early stages, and interventional surgical approaches in advanced stages. VVs is first managed using graduated elastic compression stockings to promote venous emptying, decrease pain and edema, and slow VVs progression to CVI.[197] Compression elastic stockings could also reduce venous thromboembolism after VVs surgical procedures, and improve the hemodynamics in post-thrombotic syndrome.[198]

In more advanced CVD, several approaches are used to obliterate the engorged VVs. Sclerotherapy is performed under the guidance of Duplex ultrasound to infuse hypertonic saline solution or the sclerosing compounds ethanolamine oleate and sodium morrhuate in the dilated VVs. Other sclerosing agents such as the liquid detergent sodium tetradecyl sulfate (STS) and polidocanol produce foam that displace blood and cause vasoconstriction and eventually thrombosis and occlusion of VVs.[199, 200]

Endovenous ablation utilizes radiofrequency or infrared laser at wavelengths ranging between 810 to 1550 nm to produce high heat that denatures venous proteins and occludes VVs.[201] Ablation therapy produces acceptable vein occlusion rates, good clinical outcomes, and ~2% vein recanalization rate 4 years following radiofrequency therapy,[202] and only 3 to 7% VVs recurrence rate 2 to 3 years following infrared laser therapy.[203] Other endovenous approaches including cyanoacrylate glue and related mechanochemical techniques show promising initial clinical outcomes,[204, 205] but further studies are needed to evaluate their benefits *vs.* the thermal ablation procedures. Stripping of GSV and high ligation of the saphenofemoral junction are common surgical approaches with low VVs recurrence rate.[206] Ambulatory micro-phlebectomy involves avulsion of clusters of large VVs and incompetent saphenous vein. Transilluminated powered phlebectomy is also used to remove clusters of VVs through fewer incisions and a shorter surgical procedure time.[207]

Venotonics in management of CVD

Venotonic drugs enhance venous tone, improve capillary permeability, and decrease leukocyte infiltration in the vein wall. Venotonics include α-benzopyrones (coumarins), γ-benzopyrones (flavonoids), plant extracts (blueberry and grape seed, ergots, Ginkgo biloba), saponosides (Centella asiatica, escin, horse chestnut seed extract, ruscus extract), catechin (Green tea), escletin, hesperitin, hesperidin, oxerutin, quercetin, rutosides, troxerutin, umbelliferone, calcium dobesilate, and Daflon (an oral micronized purified phlebotonic flavonoid fraction containing 90% diosmin and 10% hesperidin).[208, 209]

Flavonoids affect EC permeability and leukocyte infiltration and decrease edema and inflammation, while saponosides reduce vein wall distensibility and morphologic changes. Flavonoids such as diosmin and saponosides such as Aesculus hippocastanum, Aescin, and Escin have been used in the management of VVs and VLU.[209, 210] Escin reduces leg edema, pain, fatigue/heaviness, calf itching and cramps.[211] Escin exerts its venotonic action through several mechanisms including improved EC permeability, release of endothelium-derived vasoconstrictors such as prostaglandin-F2α, and vein sensitization to the contractile actions of histamine and serotonin.[212, 213] Escin also forms small pores in the plasma membrane and permit Ca^{2+} and other biologically-relevant factors (>3000 dalton) including calmodulin and heparin to diffuse into the cell without damaging membrane receptors or coupling mechanisms.[214] Diosmin, could improve venous tone, microcirculatory flow, microvascular permeability and lymphatic drainage and decrease vein inflammation.[209, 215] Diosmin may also inhibit venous cathecol-O-methyltransferase (COMT) thus decreasing norepinephrine metabolism and prolonging its venoconstrictive effects.[216] Diosmin may also enhance the venotonic effects of escin.[217] Studies compared the effects of escin and diosmin on isolated veins.[218] In rat IVC seg-

ments pretreated with escin in a 0 Ca^{2+} solution, gradual increases in extracellular $CaCl_2$ caused stepwise increases in vein contraction. In escin-pretreated rat IVC, the contraction to phenylephrine, angiotensin II (Ang II) and high KCl was reduced. In comparison, in rat IVC pretreated with diosmin in a 0 Ca^{2+} solution, gradual increases in extracellular $CaCl_2$ caused negligible contraction. Diosmin did not augment the IVC contractile response to phenylephrine, Ang II or escin, but increased the vein contraction in response to high KCl solution. These observations suggest that escin promotes extracellular Ca^{2+}-dependent venous contraction, but disrupts α-adrenergic receptor- and angiotensin receptor-mediated contraction mechanisms, which would limit its long-term usefulness as a venotonic in VVs. Also, diosmin does not appear to promote vein contraction on its own or enhance the effects of escin or endogenous venoconstricting factors, and therefore its benefits as a venotonic agent need further examination.[218]

In patients with advanced CVI, rutosides have shown some improvement of EC function.[219] Also, pentoxifylline, a xanthine derivative with anti-inflammatory and hemorheologic effects including TNF-α inhibition, decreased leukotrienes synthesis, and reduced red blood cells deformability, may have benefits in advanced CVI.[2] PGE1 and red vine leaves (AS 195) may also improve microcirculatory blood flow and transcutaneous oxygen tension and reduce leg edema in CVI.[2] Also, calcium dobesilate may have venoactive properties by reducing VEGF-induced cell proliferation, vascular permeability, venous inflammation, oxidative stress, platelet aggregation and blood viscosity associated with VVs and VLU.[220-223]

Sulodexide mechanism of action in VLU and CVD

Sulodexide is a highly-purified blend of two glycosaminoglycans; a fast-moving heparin fraction extracted from porcine intestinal mucosa and a dermatan sulfate component. Central to sulodexide's vascular beneficial effects are protection and repair of the endothelium, and preservation of the thickness of the glycocalyx.[97]

Sulodexide is biologically active when administered parenterally or orally, producing antithrombotic and profibrinolytic effects similar to heparin, but with fewer changes in blood clotting mechanisms and less bleeding risks. The sulodexide-induced decrease in blood viscosity together with its fibrinolytic and lipolytic properties have shown cardiovascular benefits including prevention of cardiovascular events in survivors after acute myocardial infarction, improvement of intermittent claudication in patients with peripheral arterial disease and chronic leg ischemia, and improved venous circulation in post-thrombotic venous syndrome and venous thromboembolism.[58, 224-226] Sulodexide has also shown some benefits in stabilizing renal function and reducing proteinuria in patients with diabetic microangiopathy and nephropathy.[227-230] Clinical and preclinical observations have also supported beneficial effects of sulodexide not only in advanced CVI and VLU, but also in the initial stages of CVD though multiple mechanisms on ECs, SMCs, MMPs and the inflammatory processes in the veins and surrounding tissue.[225, 227]

Sulodexide undergoes substantial uptake by ECs, where it induces protective effects.[65, 231-233] When given orally, sulodexide maintains EC structural integrity and function, by reducing metabolites- and toxins-induced EC injury, stabilizing EC-blood cell interactions, and inhibiting the microvascular inflammatory response.[227] Experimental data also suggest that sulodexide promotes arterial vasodilation through endothelium-dependent mechanisms. Phenylephrine-induced contraction is reduced in rat aortic and mesenteric artery segments pretreated with 1 mg/ml sulodexide. Also, in rat aortic and mesenteric artery rings precontracted with phenylephrine, sulodexide (0.001 to 1 mg/ml) caused concentration-dependent relaxation, that was reduced by endothelium removal or treatment with the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). Sulodexide also enhances acetylcholine-induced arterial relaxation and nitrate/nitrite production. These observations suggest that sulodexide enhances arterial relaxation through endothelium-mediated NO release, a beneficial effect that could improve vasodilation and reduce arterial constriction in occlusive arterial disease.[4] In support, meta-analyses of data from clinical trials have shown that treatment with sulodexide for at least one month may reduce blood pressure in patients with hypertension.[229, 234]

Interestingly, sulodexide shows different effects in veins *vs.* arteries. In contrast with the reduced arterial contraction and enhanced endothelium-dependent arterial relaxation, sulodexide promotes contraction of venous SMCs. In rat IVC segments under normal basal tension, Sulodexide (0.001–1mg/ml) caused concentration-dependent contraction. In IVC segments under prolonged stretch, high KCl and phenylephrine-induced contraction was reduced, but was markedly enhanced in veins pretreated with sulodexide. Also, MMP-2 and MMP-9 levels were increased in IVC segments under prolonged stretch, and reversed to control levels in veins pretreated with sulodexide. These observations suggest that sulodexide enhances contraction in veins under protracted stretch likely through decreases in MMP-2 and MMP-9 activity.[235]

Prolonged MMP-2 and MMP-9 activation could change venous SMCs from the contractile to the synthetic proliferative phenotype likely due to inducing venous SMC hyperpolarization[153] and the release of growth factors in the veins and surrounding tissue. The sulodexide-induced decrease in MMP-2 and MMP-9 would reverse their inhibitory effects on venous SMC contraction, restore venous SMC contractile phenotype and promotes venotonic effects in early stages of CVD.

Sulodexide could also maintain the structure and integrity of ECM and preserve venous structural proteins such as collagen and elastin through inhibition of MMPs and other proteases.[227] In CVD patients at CEAP stage C5, sulodexide treatment for 2 months caused reduction in MMP-9 serum level.[236] Also, treatment of leukemia white blood cells in culture with sulodexide reduced MMP-9 in a concentration-dependent manner.[65] Thus sulodexide could provide additional vascular benefits that could help alleviate CVI symptoms and promote VLU healing,[225, 227] likely through decreasing MMP expression, modulation of growth factors, promoting anti-angiogenic effects, reducing inflammatory cell infiltration in the veins and surrounding tissue and decreasing the release of inflammation products including cytokines, chemokines, metabolites, ROS and RNS.

MMPs modulators as potential tools in management of CVD

The growing evidence of a role of MMPs in the pathogenesis of VVs has generated interest in MMP inhibitors (MMPIs) to reduce the development/recurrence of CVD. MMPIs are either endogenous such as TIMPs and α2-macroglobulin, or synthetic Zn^{2+}-dependent and Zn^{2+}-independent compounds. TIMPs bind to MMPs in a 1:1 stoichiometry.[237] TIMPs have 4 homologous subtypes, TIMP-1, -2, -3 and -4, with different efficacies in inhibiting various MMPs. MMP/TIMP imbalance could contribute to the pathogenesis of CVD, and serve as a biomarker for the progress of CVI and VLU healing.

Divalent ions such as Cu^{2+}, Mg^{2+}, and Mn^{2+} inhibit MMPs by interfering with Zn^{2+} binding in the MMP catalytic domain.[238] Several MMPIs have a Zn^{2+} binding side-chain such as carboxylic acid, hydroxamic acid, or a sulfhydryl group.[239] Zn^{2+} binding globulins (ZBGs) inhibit MMPs by displacing the Zn^{2+}-bound water molecule in the MMP catalytic domain.[240] Hydroxamic acid-based MMPIs include phosphinamide, succinyl, and sulfonamide hydroxamates.[241, 242] Succinyl hydroxamates such as batimastat (BB-94), marimastat (BB-2516), and ilomastat (GM6001) have a structure similar to collagen, and act as broad spectrum MMPIs through bidentate chelation of Zn^{2+}.[243] Tetracyclines such as doxycycline also inhibit MMPs by chelating Zn^{2+} from the MMP active site.[243] Mechanism-based MMPIs such as SB-3CT not only coordinates with the MMP Zn^{2+} but also form a strong covalent bond with the MMP molecule thus minimizing its dissociation and reducing the concentration required to saturate the MMP active site.[240, 244]

Other MMPIs such as compound-37 do not have ZBGs, and do not bind to the Zn^{2+} binding site, but rather interact non-covalently with the MMP molecule in a manner similar to that of the substrate[243, 245] MMP-specific siRNA inhibits the transcription of specific MMPs.[246] Statins such as atorvastatin have pleiotropic properties that decrease MMP-1, -2, and -9 expression in human retinal pigment epithelial cells,[247] and inhibit MMP-1, -2, -3, and -9 release from human GSV SMCs, rabbit macrophages and rabbit aortic SMCs.[248] Also, treatment of rat models of heart failure with pravastatin suppresses the increases in activity of MMP-2 and -9.[249]

Although several MMPIs have been developed, doxycycline remains the only MMPI approved by the United States Food and Drug Administration (FDA). Patients with VLU who received compression therapy with or without VVs surgery in addition to oral doxycycline 20 mg b.i.d. for 3 months showed a higher rate of healed VLU than patients receiving compression therapy alone, suggesting that doxycycline therapy through its anti-inflammatory and MMPI effects could preserve ECM integrity and facilitate VLU healing.[250] However. MMPIs cause several musculoskeletal side-effects including joint pain, stiffness, inflammation, and tendonitis.[251] Improved specificity of MMPIs and their directed targeting locally to the dilated veins could enhance their therapeutic potential and minimize their systemic side-effects in the management of CVD.

Conclusions and future perspective

The glycocalyx is an integral structure in the vascular wall and an important functional component in ECs. Extensive changes in shear-stress cause damage to the glycocalyx and lead to endothelial dysfunction. Secretion of adhesion molecules, recruitment of inflammatory cells and release of inflammatory cytokines cause further damage to the endothelium and vascular integrity. Endothelial dysfunction has been observed in several vascular disorders and animal models of vascular disease. In clinical practice, decreased brachial artery flow-mediated vasodilation and increases

in biomarkers of vascular injury and endothelial dysfunction have been observed in hypertension, atherosclerosis and other arterial and venous disorders. Specifically, endothelial dysfunction could contribute to the pathogenesis and manifestations of CVD and VLU. Increased lower extremity venous hydrostatic pressure and changes in venous shear-stress cause injury to the glycocalyx, endothelial dysfunction, activation of different adhesion molecules, and leukocyte recruitment to the vein wall. Increased lower extremity venous pressure could also activate HIFs and increase MMP expression/activity. MMPs promote proteolytic degradation of ECM structural proteins including collagen and elastin, leading to weakening of the vein wall architecture, progressive dilation of the vein wall, valve incompetence and venous reflux. MMPs also promote SMC migration, apoptosis and phenotypic transition, and could modulate K^+ channel activity, Ca^{2+} signaling and SMC contraction. MMPs could also adversely affect the glycocalyx integrity, EC function and endothelium-derived relaxing factors. This conglomerate of events leads to leukocyte activation and transmigration into the vein wall, valves and the interstitium, with the secretion of different cytokines, chemokines, and growth factors, and the release of MMPs and other proteolytic enzymes causing further venous dysfunction and inflammation in the lower limb, and clinical manifestations of CVD. Accumulation of tissue metabolites, generation of ROS and RNS, erythrodiapedesis, red blood cell degradation, and iron deposition further escalate inflammation and tissue damage, eventually progressing to advanced CVD, CVI, and VLU.

The complexity of the pathophysiology and mechanisms of CVD have posed a challenge for effective management. Current management of VVs includes compression stockings, venotonic drugs, sclerotherapy or surgical removal of the affected veins. Sulodexide treatment have shown benefits in CVI and VLU healing largely through endothelium protection and restoration, reducing inflammatory cell infiltration, decreasing the release/accumulation of inflammatory products such as cytokines, chemokines, metabolites and oxidative stress and may have venotonic properties and MMP inhibitory effects that could improve venous function in patients with CVD. Further genetic mapping and research of the early events underlying glycocalyx injury, EC dysfunction, venous SMC phenotypic switch, ECM and proteases imbalance, and subsequent venous inflammation and tissue damage would provide a better understanding of the pathophysiologic mechanisms and disease process and help develop specific targeted therapies for CVD and VLU.

References

1. Fukaya E, Flores AM, Lindholm D, Gustafsson S, Zanetti D, Ingelsson E, *et al.* Clinical and Genetic Determinants of Varicose Veins. Circulation 2018;138:2869–80.

2. Raffetto JD, Khalil RA. Mechanisms of Lower Extremity Vein Dysfunction in Chronic Venous Disease and Implications in Management of Varicose Veins. Vessel Plus 2021;5.

3. Raffetto JD, Yu W, Wang X, Calanni F, Mattana P, Khalil RA. Sulodexide Improves Contraction and Decreases Matrix Metalloproteinase-2 and -9 in Veins Under Prolonged Stretch. J Cardiovasc Pharmacol 2020;75:211–21.

4. Raffetto JD, Calanni F, Mattana P, Khalil RA. Sulodexide promotes arterial relaxation via endothelium-dependent nitric oxide-mediated pathway. Biochem Pharmacol 2019;166:347–56.

5. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. Circ Res 2007;100:158–73.

6. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch 2007;454:345–59.

7. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. Pflugers Arch 2000;440:653–66.

8. Mulivor AW, Lipowsky HH. Role of glycocalyx in leukocyte-endothelial cell adhesion. Am J Physiol Heart Circ Physiol 2002;283:H1282–91.

9. Zeng Y. Endothelial glycocalyx as a critical signalling platform integrating the extracellular haemodynamic forces and chemical signalling. J Cell Mol Med 2017;21:1457–62.

10. Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. J Intern Med 2016;280:97–113.

11. Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 2007;446:1030–7.

12. Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mechanisms of action. Chem Rev 2006;106:818–39.

13. Varki A, Angata T. Siglecs—the major subfamily of I-type lectins. Glycobiology 2006;16:1R–27R.

14. Sonnino S, Prinetti A. Membrane domains and the "lipid raft" concept. Curr Med Chem 2013;20:4–21.

15. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. Annu Rev Biomed Eng 2007;9:121–67.

16. Pahakis MY, Kosky JR, Dull RO, Tarbell JM. The role of endothelial glycocalyx components in mechanotransduction of fluid shear stress. Biochem Biophys Res Commun 2007;355:228–33.

17. Aird WC. Endothelium as an organ system. Crit Care Med 2004;32(Suppl):S271–9.

18. Topper JN, Gimbrone MA Jr. Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. Mol Med Today 1999;5:40–6.

19. Clarke H, Smith SR, Vasdekis SN, Hobbs JT, Nicolaides AN. Role of venous elasticity in the development of varicose veins. Br J Surg 1989;76:577–80.

20. Hsiai TK, Cho SK, Wong PK, Ing M, Salazar A, Sevanian A, *et al.* Monocyte recruitment to endothelial cells in response to oscillatory shear stress. FASEB J 2003;17:1648–57.

21. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. Arterioscler Thromb Vasc Biol 1998;18:677–85.

22. Chiu JJ, Wang DL, Chien S, Skalak R, Usami S. Effects of disturbed flow on endothelial cells. J Biomech Eng 1998;120:2–8.

23. Dejana E. Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis. J Clin Invest 1996;98:1949–53.

24. Bardin N, Blot-Chabaud M, Despoix N, Kebir A, Harhouri K, Ar-

santo JP, *et al.* CD146 and its soluble form regulate monocyte transendothelial migration. Arterioscler Thromb Vasc Biol 2009;29:746–53.

25. Harhouri K, Kebir A, Guillet B, Foucault-Bertaud A, Voytenko S, Piercecchi-Marti MD, *et al.* Soluble CD146 displays angiogenic properties and promotes neovascularization in experimental hind-limb ischemia. Blood 2010;115:3843–51.

26. Lawson C, Wolf S. ICAM-1 signaling in endothelial cells. Pharmacol Rep 2009;61:22–32.

27. Tisato V, Zauli G, Voltan R, Gianesini S, di Iasio MG, Volpi I, *et al.* Endothelial cells obtained from patients affected by chronic venous disease exhibit a pro-inflammatory phenotype. PLoS One 2012;7:e39543.

28. Mironiuc A, Palcău L, Rogojan L, Micula S, Gherman C. Is there a correlation between the CEAP score and the histopathological findings in varicose disease? Rom J Morphol Embryol 2011;52:117–21.

29. Venuraju SM, Yerramasu A, Corder R, Lahiri A. Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. J Am Coll Cardiol 2010;55:2049–61.

30. Prochaska JH, Arnold N, Falcke A, Kopp S, Schulz A, Buch G, *et al.* Chronic venous insufficiency, cardiovascular disease, and mortality: a population study. Eur Heart J 2021;42:4157–65.

31. Tisato V, Zauli G, Gianesini S, Menegatti E, Brunelli L, Manfredini R, *et al.* Modulation of circulating cytokine-chemokine profile in patients affected by chronic venous insufficiency undergoing surgical hemodynamic correction. J Immunol Res 2014;2014:473765.

32. Tisato V, Zamboni P, Menegatti E, Gianesini S, Volpi I, Zauli G, *et al.* Endothelial PDGF-BB produced ex vivo correlates with relevant hemodynamic parameters in patients affected by chronic venous disease. Cytokine 2013;63:92–6.

33. Garoffolo G, Pesce M. Mechanotransduction in the Cardiovascular System: From Developmental Origins to Homeostasis and Pathology. Cells 2019;8:1607.

34. Driscoll TP, Cosgrove BD, Heo SJ, Shurden ZE, Mauck RL. Cytoskeletal to Nuclear Strain Transfer Regulates YAP Signaling in Mesenchymal Stem Cells. Biophys J 2015;108:2783–93.

35. Tajik A, Zhang Y, Wei F, Sun J, Jia Q, Zhou W, *et al.* Transcription upregulation via force-induced direct stretching of chromatin. Nat Mater 2016;15:1287–96.

36. Ley K. Integration of inflammatory signals by rolling neutrophils. Immunol Rev 2002;186:8–18.

37. Pala R, Jamal M, Alshammari Q, Nauli SM. The Roles of Primary Cilia in Cardiovascular Diseases. Cells 2018;7:233.

38. Jiang XZ, Luo KH, Ventikos Y. Understanding the Role of Endothelial Glycocalyx in Mechanotransduction via Computational Simulation: A Mini Review. Front Cell Dev Biol 2021;9:732815.

39. Carroll BJ, Piazza G, Goldhaber SZ. Sulodexide in venous disease. J Thromb Haemost 2019;17:31–8.

40. Gonzalez-Ochoa AJ, Raffetto JD, Hernández AG, Zavala N, Gutiérrez O, Vargas A, *et al.* Sulodexide in the Treatment of Patients with Early Stages of COVID-19: A Randomized Controlled Trial. Thromb Haemost 2021;121:944–54.

41. Zha D, Fu M, Qian Y. Vascular Endothelial Glycocalyx Damage and Potential Targeted Therapy in COVID-19. Cells 2022;11:1972.

42. Gouverneur M, Berg B, Nieuwdorp M, Stroes E, Vink H. Vasculoprotective properties of the endothelial glycocalyx: effects of fluid shear stress. J Intern Med 2006;259:393–400.

43. Zeng Y, Waters M, Andrews A, Honarmandi P, Ebong EE, Rizzo V, *et al.* Fluid shear stress induces the clustering of heparan sulfate via mobility of glypican-1 in lipid rafts. Am J Physiol Heart Circ Physiol 2013;305:H811–20.

44. Raffetto JD, Qiao X, Koledova VV, Khalil RA. Prolonged increases in vein wall tension increase matrix metalloproteinases and decrease constriction in rat vena cava: potential implications in varicose veins. J Vasc Surg 2008;48:447–56.

45. Doganci S, Ince ME, Demeli M, Ors Yildirim N, Pehlivanoglu B, Yildirim AK, *et al.* Sulodexide Develops Contraction in Human Saphenous Vein via Endothelium-Dependent Nitric Oxide Pathway. J Clin Med 2023;12:1019.

46. Ebong EE, Lopez-Quintero SV, Rizzo V, Spray DC, Tarbell JM. Shear-induced endothelial NOS activation and remodeling via heparan sulfate, glypican-1, and syndecan-1. Integr Biol (Camb) 2014;6:338–47.

47. Lamorte S, Ferrero S, Aschero S, Monitillo L, Bussolati B, Omedè P, *et al.* Syndecan-1 promotes the angiogenic phenotype of multiple myeloma endothelial cells. Leukemia 2012;26:1081–90.

48. Baeyens N, Mulligan-Kehoe MJ, Corti F, Simon DD, Ross TD, Rhodes JM, *et al.* Syndecan 4 is required for endothelial alignment in flow and atheroprotective signaling. Proc Natl Acad Sci USA 2014;111:17308–13.

49. Mitra R, O'Neil GL, Harding IC, Cheng MJ, Mensah SA, Ebong EE. Glycocalyx in Atherosclerosis-Relevant Endothelium Function and as a Therapeutic Target. Curr Atheroscler Rep 2017;19:63.

50. Zeng Y, Liu J. Role of glypican-1 in endothelial NOS activation under various steady shear stress magnitudes. Exp Cell Res 2016;348:184–9.

51. Yao Y, Rabodzey A, Dewey CF Jr. Glycocalyx modulates the motility and proliferative response of vascular endothelium to fluid shear stress. Am J Physiol Heart Circ Physiol 2007;293:H1023–30.

52. Zhang L, Zeng M, Fan J, Tarbell JM, Curry FR, Fu BM. Sphingosine-1-phosphate Maintains Normal Vascular Permeability by Preserving Endothelial Surface Glycocalyx in Intact Microvessels. Microcirculation 2016;23:301–10.

53. Gianesini S, Rimondi E, Raffetto JD, Melloni E, Pellati A, Menegatti E, *et al.* Human collecting lymphatic glycocalyx identification by electron microscopy and immunohistochemistry. Sci Rep 2023;13:3022.

54. Michel CC, Woodcock TE, Curry FE. Understanding and extending the Starling principle. Acta Anaesthesiol Scand 2020;64:1032–7.

55. Nishida R, Suzuki D, Akimoto Y, Matsubara S, Hayakawa J, Ushiyama A, *et al.* Exploring the pathophysiological mechanism of interstitial edema focusing on the role of macrophages and their interaction with the glycocalyx. J Oral Biosci 2023;65:111–8.

56. Milusev A, Rieben R, Sorvillo N. The Endothelial Glycocalyx: A Possible Therapeutic Target in Cardiovascular Disorders. Front Cardiovasc Med 2022;9:897087.

57. Fraser DD, Patterson EK, Slessarev M, Gill SE, Martin C, Daley M, *et al.* Endothelial Injury and Glycocalyx Degradation in Critically Ill Coronavirus Disease 2019 Patients: Implications for Microvascular Platelet Aggregation. Crit Care Explor 2020;2:e0194.

58. Andreozzi GM, Bignamini AA, Davì G, Palareti G, Matuška J, Holý M, *et al.*; SURVET Study Investigators. Sulodexide for the Prevention of Recurrent Venous Thromboembolism: The Sulodexide in Secondary Prevention of Recurrent Deep Vein Thrombosis (SURVET) Study: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial. Circulation 2015;132:1891–7.

59. Bignamini AA, Matuška J. Sulodexide for the Symptoms and Signs of Chronic Venous Disease: A Systematic Review and Meta-analysis. Adv Ther 2020;37:1013–33.

60. Raffetto JD. Pathophysiology of wound healing and alterations in venous leg ulcers-review. Phlebology 2016;31(Suppl):56–62.

61. Beebe-Dimmer JL, Pfeifer JR, Engle JS, Schottenfeld D. The epidemiology of chronic venous insufficiency and varicose veins. Ann Epidemiol 2005;15:175–84.

62. Eklöf B, Rutherford RB, Bergan JJ, Carpentier PH, Gloviczki P, Kistner RL, *et al.*; American Venous Forum International Ad Hoc Committee for Revision of the CEAP Classification. Revision of the CEAP classification for chronic venous disorders: consensus statement. J Vasc Surg 2004;40:1248–52.

63. Lurie F, Passman M, Meisner M, Dalsing M, Masuda E, Welch H, *et al.* The 2020 update of the CEAP classification system and reporting standards. J Vasc Surg Venous Lymphat Disord 2020;8:342–52.

64. Zsotér T, Cronin RF. Venous distensibility in patients with varicose veins. Can Med Assoc J 1966;94:1293–7.

65. Mannello F, Medda V, Ligi D, Raffetto JD. Glycosaminoglycan sulodexide inhibition of MMP-9 gelatinase secretion and activity: possible pharmacological role against collagen degradation in vascular chronic diseases. Curr Vasc Pharmacol 2013;11:354–65.

66. Sansilvestri-Morel P, Fioretti F, Rupin A, Senni K, Fabiani JN, Godeau G, *et al.* Comparison of extracellular matrix in skin and saphenous veins from patients with varicose veins: does the skin reflect venous matrix changes? Clin Sci (Lond) 2007;112:229–39.

67. Naoum JJ, Hunter GC, Woodside KJ, Chen C. Current advances in the pathogenesis of varicose veins. J Surg Res 2007;141:311–6.

68. Robertson L, Lee AJ, Evans CJ, Boghossian S, Allan PL, Ruckley CV, *et al.* Incidence of chronic venous disease in the Edinburgh Vein Study. J Vasc Surg Venous Lymphat Disord 2013;1:59–67.

69. Raffetto JD, Qiao X, Beauregard KG, Khalil RA. Estrogen receptor-mediated enhancement of venous relaxation in female rat: implications in sex-related differences in varicose veins. J Vasc Surg 2010;51:972–81.

70. Bernstein IM, Ziegler W, Badger GJ. Plasma volume expansion in early pregnancy. Obstet Gynecol 2001;97:669–72.

71. Stansby G. Women, pregnancy, and varicose veins. Lancet 2000;355:1117–8.

72. Mekky S, Schilling RS, Walford J. Varicose veins in women cotton workers. An epidemiological study in England and Egypt. BMJ 1969;2:591–5.

73. Kaye SA, Folsom AR, Soler JT, Prineas RJ, Potter JD. Associations of body mass and fat distribution with sex hormone concentrations in postmenopausal women. Int J Epidemiol 1991;20:151–6.

74. Seidell JC, Bakx KC, Deurenberg P, van den Hoogen HJ, Hautvast JG, Stijnen T. Overweight and chronic illness—a retrospective cohort study, with a follow-up of 6-17 years, in men and women of initially 20-50 years of age. J Chronic Dis 1986;39:585–93.

75. Lacroix P, Aboyans V, Preux PM, Houlès MB, Laskar M. Epidemiology of venous insufficiency in an occupational population. Int Angiol 2003;22:172–6.

76. Abramson JH, Hopp C, Epstein LM. The epidemiology of varicose veins. A survey in western Jerusalem. J Epidemiol Community Health 1981;35:213–7.

77. Anwar MA, Georgiadis KA, Shalhoub J, Lim CS, Gohel MS, Davies AH. A review of familial, genetic, and congenital aspects of primary varicose vein disease. Circ Cardiovasc Genet 2012;5:460–6.

78. Reagan B, Folse R. Lower limb venous dynamics in normal persons and children of patients with varicose veins. Surg Gynecol Obstet 1971;132:15–8.

79. Ellinghaus E, Ellinghaus D, Krusche P, Greiner A, Schreiber C, Nikolaus S, *et al.* Genome-wide association analysis for chronic venous disease identifies EFEMP1 and KCNH8 as susceptibility loci. Sci Rep 2017;7:45652.

80. Lee S, Lee W, Choe Y, Kim D, Na G, Im S, *et al.* Gene expression profiles in varicose veins using complementary DNA microarray. Dermatol Surg 2005;31:391–5.

81. Badauy CM, Gomes SS, Sant'Ana Filho M, Chies JA. Ehlers-Danlos syndrome (EDS) type IV: review of the literature. Clin Oral Investig 2007;11:183–7.

82. Ng MY, Andrew T, Spector TD, Jeffery S; Lymphoedema Consortium. Linkage to the FOXC2 region of chromosome 16 for varicose veins in otherwise healthy, unselected sibling pairs. J Med Genet 2005;42:235–9.

83. Delis KT, Gloviczki P, Wennberg PW, Rooke TW, Driscoll DJ. Hemodynamic impairment, venous segmental disease, and clinical severity scoring in limbs with Klippel-Trenaunay syndrome. J Vasc Surg 2007;45:561–7.

84. Saiki S, Sakai K, Saiki M, Kitagawa Y, Umemori T, Murata K, *et al.* Varicose veins associated with CADASIL result from a novel mutation in the Notch3 gene. Neurology 2006;67:337–9.

85. Xu HM, Zhao Y, Zhang XM, Zhu T, Fu WG. Polymorphisms in MMP-9 and TIMP-2 in Chinese patients with varicose veins. J Surg Res 2011;168:e143–8.

86. Gemmati D, Federici F, Catozzi L, Gianesini S, Tacconi G, Scapoli GL, *et al.* DNA-array of gene variants in venous leg ulcers: detection of prognostic indicators. J Vasc Surg 2009;50:1444–51.

87. Vincent JR, Jones GT, Hill GB, van Rij AM. Failure of microvenous valves in small superficial veins is a key to the skin changes of venous insufficiency. J Vasc Surg 2011;54(6 Suppl):62S–69S.

88. Raffetto JD, Ligi D, Maniscalco R, Khalil RA, Mannello F. Why Venous Leg Ulcers Have Difficulty Healing: Overview on Pathophysiology, Clinical Consequences, and Treatment. J Clin Med 2020;10:29.

89. Birdina J, Pilmane M, Ligers A. The Morphofunctional Changes in the Wall of Varicose Veins. Ann Vasc Surg 2017;42:274–84.

90. Surendran S, S Ramegowda K, Suresh A, Binil Raj SS, Lakkappa RK, Kamalapurkar G, *et al.* Arterialization and anomalous vein wall remodeling in varicose veins is associated with upregulated FoxC2-Dll4 pathway. Lab Invest 2016;96:399–408.

91. Bergan JJ, Schmid-Schönbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. N Engl J Med 2006;355:488–98.

92. Abd-El-Aleem SA, Ferguson MW, Appleton I, Kairsingh S, Jude EB, Jones K, *et al.* Expression of nitric oxide synthase isoforms and arginase in normal human skin and chronic venous leg ulcers. J Pathol 2000;191:434–42.

93. Shadrina AS, Smetanina MA, Sokolova EA, Shamovskaya DV, Sevost'ianova KS, Shevela AI, *et al.* Allele rs2010963 C of the VEGFA gene is associated with the decreased risk of primary varicose veins in ethnic Russians. Phlebology 2018;33:27–35.

94. Hollingsworth SJ, Powell G, Barker SG, Cooper DG. Primary varicose veins: altered transcription of VEGF and its receptors (KDR, flt-1, soluble flt-1) with sapheno-femoral junction incompetence. Eur J Vasc Endovasc Surg 2004;27:259–68.

95. Wang JC, Gu J, Li Y, Ma Q, Feng J, Lu S. Transforming growth factor-β1 and inducible nitric oxide synthase signaling were involved in effects of prostaglandin E2 on progression of lower limb varicose veins. J Vasc Surg Venous Lymphat Disord 2021;9:1535–44.

96. Chen YS, Lu MJ, Huang HS, Ma MC. Mechanosensitive transient receptor potential vanilloid type 1 channels contribute to vascular remodeling of rat fistula veins. J Vasc Surg 2010;52:1310–20.

97. Zieliński A, Jasińska-Sumińska K, Bręborowicz A, Kowalska K, Zabel M, Wysocka T, *et al.* Changes of the serum properties and its effect on the endothelial cells restoration in patients with chronic venous disease treated with sulodexide. J Vasc Surg Venous Lymphat Disord 2024;12:101941.

98. Liu JX, Yan ZP, Zhang YY, Wu J, Liu XH, Zeng Y. Hemodynamic shear stress regulates the transcriptional expression of heparan sulfate proteoglycans in human umbilical vein endothelial cell. Cell Mol Biol (Noisy-le-grand) 2016;62:28–34.

99. Alsaigh T, Pocock ES, Bergan JJ, Schmid-Schönbein GW. Acute venous occlusion enhances matrix metalloprotease activity: implications on endothelial dysfunction. Microvasc Res 2011;81:108–16.

100. Moazzam F, DeLano FA, Zweifach BW, Schmid-Schönbein GW. The leukocyte response to fluid stress. Proc Natl Acad Sci USA 1997;94:5338–43.

101. Takase S, Pascarella L, Bergan JJ, Schmid-Schönbein GW. Hypertension-induced venous valve remodeling. J Vasc Surg 2004;39:1329–34.

102. Takase S, Bergan JJ, Schmid-Schönbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. Ann Vasc Surg 2000;14:427–35.

103. Badier-Commander C, Couvelard A, Henin D, Verbeuren T, Michel JB, Jacob MP. Smooth muscle cell modulation and cytokine overproduction in varicose veins. An in situ study. J Pathol 2001;193:398–407.

104. Cao Y, Cao Z, Wang W, Jie X, Li L. MicroRNA-199-5p regulates

FOXC2 to control human vascular smooth muscle cell phenotypic switch. Mol Med Rep 2021;24.

105. Yin H, Zhang X, Wang J, Yin W, Zhang G, Wang S, *et al.* Down-regulation of desmuslin in primary vein incompetence. J Vasc Surg 2006;43:372–8.

106. Xiao Y, Huang Z, Yin H, Zhang H, Wang S. Desmuslin gene knockdown causes altered expression of phenotype markers and differentiation of saphenous vein smooth muscle cells. J Vasc Surg 2010;52:684–90.

107. Xu Y, Bei Y, Li Y, Chu H. Phenotypic and functional transformation in smooth muscle cells derived from varicose veins. J Vasc Surg Venous Lymphat Disord 2017;5:723–33.

108. Chen S, Qin S, Wang M, Zhang S. Expression and significance of NELIN and SM22α in varicose vein tissue. Exp Ther Med 2015;9:845–9.

109. Guo Z, Luo C, Zhu T, Li L, Zhang W. Elevated c-fos expression is correlated with phenotypic switching of human vascular smooth muscle cells derived from lower limb venous varicosities. J Vasc Surg Venous Lymphat Disord 2021;9:242–51.

110. Zhao MY, Zhao T, Meng QY, Zhao L, Li XC. Estrogen and estrogen receptor affects MMP2 and MMP9 expression through classical ER pathway and promotes migration of lower venous vascular smooth muscle cells. Eur Rev Med Pharmacol Sci 2020;24:1460–7.

111. Zha B, Qiu P, Zhang C, Li X, Chen Z. GPR30 Promotes the Phenotypic Switching of Vascular Smooth Muscle Cells via Activating the AKT and ERK Pathways. OncoTargets Ther 2020;13:3801–8.

112. Jiang H, Lun Y, Wu X, Xia Q, Zhang X, Xin S, *et al.* Association between the hypomethylation of osteopontin and integrin β3 promoters and vascular smooth muscle cell phenotype switching in great saphenous varicose veins. Int J Mol Sci 2014;15:18747–61.

113. Ascher E, Jacob T, Hingorani A, Tsemekhin B, Gunduz Y. Expression of molecular mediators of apoptosis and their role in the pathogenesis of lower-extremity varicose veins. J Vasc Surg 2001;33:1080–6.

114. Urbanek T, Skop B, Wiaderkiewicz R, Wilczok T, Ziaja K, Lebda-Wyborny T, *et al.* Smooth muscle cell apoptosis in primary varicose veins. Eur J Vasc Endovasc Surg 2004;28:600–11.

115. Filis K, Kavantzas N, Isopoulos T, Antonakis P, Sigalas P, Vavouranakis E, *et al.* Increased vein wall apoptosis in varicose vein disease is related to venous hypertension. Eur J Vasc Endovasc Surg 2011;41:533–9.

116. Sansilvestri-Morel P, Rupin A, Badier-Commander C, Kern P, Fabiani JN, Verbeuren TJ, *et al.* Imbalance in the synthesis of collagen type I and collagen type III in smooth muscle cells derived from human varicose veins. J Vasc Res 2001;38:560–8.

117. Kirsch D, Dienes HP, Küchle R, Duschner H, Wahl W, Böttger T, *et al.* Changes in the extracellular matrix of the vein wall—the cause of primary varicosis? Vasa 2000;29:173–7.

118. Sansilvestri-Morel P, Rupin A, Jaisson S, Fabiani JN, Verbeuren TJ, Vanhoutte PM. Synthesis of collagen is dysregulated in cultured fibroblasts derived from skin of subjects with varicose veins as it is in venous smooth muscle cells. Circulation 2002;106:479–83.

119. Sansilvestri-Morel P, Rupin A, Badier-Commander C, Fabiani JN, Verbeuren TJ. Chronic venous insufficiency: dysregulation of collagen synthesis. Angiology 2003;54(Suppl 1):S13–8.

120. Sansilvestri-Morel P, Rupin A, Jullien ND, Lembrez N, Mestries-Dubois P, Fabiani JN, *et al.* Decreased production of collagen Type III in cultured smooth muscle cells from varicose vein patients is due to a degradation by MMPs: possible implication of MMP-3. J Vasc Res 2005;42:388–98.

121. Degiorgio-Miller AM, Treharne LJ, McAnulty RJ, Coleridge Smith PD, Laurent GJ, Herrick SE. Procollagen type I gene expression and cell proliferation are increased in lipodermatosclerosis. Br J Dermatol 2005;152:242–9.

122. Venturi M, Bonavina L, Annoni F, Colombo L, Butera C, Peracchia A, *et al.* Biochemical assay of collagen and elastin in the normal and varicose vein wall. J Surg Res 1996;60:245–8.

123. Pascual G, Mendieta C, Mecham RP, Sommer P, Bellón JM, Buján J. Down-regulation of lysyl oxydase-like in aging and venous insufficiency. Histol Histopathol 2008;23:179–86.

124. Görmüs U, Timirci-Kahraman O, Ergen A, Kunt AT, Isbir S, Dalan AB, *et al.* Expression levels of elastin and related genes in human varicose veins. Folia Biol (Praha) 2014;60:68–73.

125. Saito S, Trovato MJ, You R, Lal BK, Fasehun F, Padberg FT Jr, *et al.* Role of matrix metalloproteinases 1, 2, and 9 and tissue inhibitor of matrix metalloproteinase-1 in chronic venous insufficiency. J Vasc Surg 2001;34:930–8.

126. Beidler SK, Douillet CD, Berndt DF, Keagy BA, Rich PB, Marston WA. Multiplexed analysis of matrix metalloproteinases in leg ulcer tissue of patients with chronic venous insufficiency before and after compression therapy. Wound Repair Regen 2008;16:642–8.

127. Serra R, Gallelli L, Butrico L, Buffone G, Caliò FG, De Caridi G, *et al.* From varices to venous ulceration: the story of chronic venous disease described by metalloproteinases. Int Wound J 2017;14:233–40.

128. Gomez I, Benyahia C, Louedec L, Leséche G, Jacob MP, Longrois D, *et al.* Decreased PGE2 content reduces MMP-1 activity and consequently increases collagen density in human varicose vein. PLoS One 2014;9:e88021.

129. Badier-Commander C, Verbeuren T, Lebard C, Michel JB, Jacob MP. Increased TIMP/MMP ratio in varicose veins: a possible explanation for extracellular matrix accumulation. J Pathol 2000;192:105–12.

130. Serralheiro P, Novais A, Cairrão E, Maia C, Costa Almeida CM, Verde I. Variability of MMP/TIMP and TGF-β1 Receptors throughout the Clinical Progression of Chronic Venous Disease. Int J Mol Sci 2017;19:6.

131. Shi ZD, Ji XY, Berardi DE, Qazi H, Tarbell JM. Interstitial flow induces MMP-1 expression and vascular SMC migration in collagen I gels via an ERK1/2-dependent and c-Jun-mediated mechanism. Am J Physiol Heart Circ Physiol 2010;298:H127–35.

132. Jin UH, Suh SJ, Chang HW, Son JK, Lee SH, Son KH, *et al.* Tanshinone IIA from Salvia miltiorrhiza BUNGE inhibits human aortic smooth muscle cell migration and MMP-9 activity through AKT signaling pathway. J Cell Biochem 2008;104:15–26.

133. Haque NS, Fallon JT, Pan JJ, Taubman MB, Harpel PC. Chemokine receptor-8 (CCR8) mediates human vascular smooth muscle cell chemotaxis and metalloproteinase-2 secretion. Blood 2004;103:1296–304.

134. Johnson C, Galis ZS. Matrix metalloproteinase-2 and -9 differentially regulate smooth muscle cell migration and cell-mediated collagen organization. Arterioscler Thromb Vasc Biol 2004;24:54–60.

135. Cho A, Reidy MA. Matrix metalloproteinase-9 is necessary for the regulation of smooth muscle cell replication and migration after arterial injury. Circ Res 2002;91:845–51.

136. Galis ZS, Johnson C, Godin D, Magid R, Shipley JM, Senior RM, *et al.* Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. Circ Res 2002;91:852–9.

137. Uglow EB, Slater S, Sala-Newby GB, Aguilera-Garcia CM, Angelini GD, Newby AC, *et al.* Dismantling of cadherin-mediated cell-cell contacts modulates smooth muscle cell proliferation. Circ Res 2003;92:1314–21.

138. Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell 2005;120:303–13.

139. Somers P, Knaapen M. The histopathology of varicose vein disease. Angiology 2006;57:546–55.

140. Wali MA, Eid RA. Intimal changes in varicose veins: an ultrastructural study. J Smooth Muscle Res 2002;38:63–74.

141. Xiao Y, Huang Z, Yin H, Lin Y, Wang S. In vitro differences between smooth muscle cells derived from varicose veins and normal veins. J Vasc Surg 2009;50:1149–54.

142. Morla AO, Mogford JE. Control of smooth muscle cell proliferation and phenotype by integrin signaling through focal adhesion kinase. Biochem Biophys Res Commun 2000;272:298–302.

143. Ilić D, Almeida EA, Schlaepfer DD, Dazin P, Aizawa S, Damsky

CH. Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. J Cell Biol 1998;143:547–60.

144. Levkau B, Kenagy RD, Karsan A, Weitkamp B, Clowes AW, Ross R, *et al.* Activation of metalloproteinases and their association with integrins: an auxiliary apoptotic pathway in human endothelial cells. Cell Death Differ 2002;9:1360–7.

145. Woodside KJ, Hu M, Burke A, Murakami M, Pounds LL, Killewich LA, *et al.* Morphologic characteristics of varicose veins: possible role of metalloproteinases. J Vasc Surg 2003;38:162–9.

146. Lischper M, Beuck S, Thanabalasundaram G, Pieper C, Galla HJ. Metalloproteinase mediated occludin cleavage in the cerebral microcapillary endothelium under pathological conditions. Brain Res 2010;1326:114–27.

147. McNamara CA, Sarembock IJ, Gimple LW, Fenton JW 2nd, Coughlin SR, Owens GK. Thrombin stimulates proliferation of cultured rat aortic smooth muscle cells by a proteolytically activated receptor. J Clin Invest 1993;91:94–8.

148. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258–64.

149. Garcia JG, Patterson C, Bahler C, Aschner J, Hart CM, English D. Thrombin receptor activating peptides induce Ca2+ mobilization, barrier dysfunction, prostaglandin synthesis, and platelet-derived growth factor mRNA expression in cultured endothelium. J Cell Physiol 1993;156:541–9.

150. Félétou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? Arterioscler Thromb Vasc Biol 2006;26:1215–25.

151. Chew DK, Conte MS, Khalil RA. Matrix metalloproteinase-specific inhibition of Ca2+ entry mechanisms of vascular contraction. J Vasc Surg 2004;40:1001–10.

152. Raffetto JD, Barros YV, Wells AK, Khalil RA. MMP-2 induced vein relaxation via inhibition of [Ca2+]e-dependent mechanisms of venous smooth muscle contraction. Role of RGD peptides. J Surg Res 2010;159:755–64.

153. Raffetto JD, Ross RL, Khalil RA. Matrix metalloproteinase 2-induced venous dilation via hyperpolarization and activation of K+ channels: relevance to varicose vein formation. J Vasc Surg 2007;45:373–80.

154. Lee HY, You HJ, Won JY, Youn SW, Cho HJ, Park KW, *et al.* Forkhead factor, FOXO3a, induces apoptosis of endothelial cells through activation of matrix metalloproteinases. Arterioscler Thromb Vasc Biol 2008;28:302–8.

155. Lim CS, Gohel MS, Shepherd AC, Paleolog E, Davies AH. Venous hypoxia: a poorly studied etiological factor of varicose veins. J Vasc Res 2011;48:185–94.

156. Lim CS, Kiriakidis S, Paleolog EM, Davies AH. Increased activation of the hypoxia-inducible factor pathway in varicose veins. J Vasc Surg 2012;55:1427–39.

157. Ortega MA, Asúnsolo Á, Leal J, Romero B, Alvarez-Rocha MJ, Sainz F, *et al.* Implication of the PI3K/Akt/mTOR Pathway in the Process of Incompetent Valves in Patients with Chronic Venous Insufficiency and the Relationship with Aging. Oxid Med Cell Longev 2018;2018:1495170.

158. Misra S, Fu AA, Rajan DK, Juncos LA, McKusick MA, Bjarnason H, *et al.* Expression of hypoxia inducible factor-1 alpha, macrophage migration inhibition factor, matrix metalloproteinase-2 and -9, and their inhibitors in hemodialysis grafts and arteriovenous fistulas. J Vasc Interv Radiol 2008;19:252–9.

159. Milkiewicz M, Doyle JL, Fudalewski T, Ispanovic E, Aghasi M, Haas TL. HIF-1alpha and HIF-2alpha play a central role in stretch-induced but not shear-stress-induced angiogenesis in rat skeletal muscle. J Physiol 2007;583:753–66.

160. Kim CH, Cho YS, Chun YS, Park JW, Kim MS. Early expression of myocardial HIF-1alpha in response to mechanical stresses: regulation by stretch-activated channels and the phosphatidylinositol 3-kinase signaling pathway. Circ Res 2002;90:E25–33.

161. Lim CS, Qiao X, Reslan OM, Xia Y, Raffetto JD, Paleolog E, *et al.* Prolonged mechanical stretch is associated with upregulation of hypoxia-inducible factors and reduced contraction in rat inferior vena cava. J Vasc Surg 2011;53:764–73.

162. Thodeti CK, Matthews B, Ravi A, Mammoto A, Ghosh K, Bracha AL, *et al.* TRPV4 channels mediate cyclic strain-induced endothelial cell reorientation through integrin-to-integrin signaling. Circ Res 2009;104:1123–30.

163. Anwar MA, Shalhoub J, Vorkas PA, Lim CS, Want EJ, Nicholson JK, *et al.* In-vitro identification of distinctive metabolic signatures of intact varicose vein tissue via magic angle spinning nuclear magnetic resonance spectroscopy. Eur J Vasc Endovasc Surg 2012;44:442–50.

164. Anwar MA, Adesina-Georgiadis KN, Spagou K, Vorkas PA, Li JV, Shalhoub J, *et al.* A comprehensive characterisation of the metabolic profile of varicose veins; implications in elaborating plausible cellular pathways for disease pathogenesis. Sci Rep 2017;7:2989.

165. Onida S, Tan MK, Kafeza M, Bergner RT, Shalhoub J, Holmes E, *et al.* Metabolic Phenotyping in Venous Disease: The Need for Standardization. J Proteome Res 2019;18:3809–20.

166. Anwar MA, Vorkas PA, Li J, Adesina-Georgiadis KN, Reslan OM, Raffetto JD, *et al.* Prolonged Mechanical Circumferential Stretch Induces Metabolic Changes in Rat Inferior Vena Cava. Eur J Vasc Endovasc Surg 2016;52:544–52.

167. Sayer GL, Smith PD. Immunocytochemical characterisation of the inflammatory cell infiltrate of varicose veins. Eur J Vasc Endovasc Surg 2004;28:479–83.

168. Ono T, Bergan JJ, Schmid-Schönbein GW, Takase S. Monocyte infiltration into venous valves. J Vasc Surg 1998;27:158–66.

169. Aunapuu M, Arend A. Histopathological changes and expression of adhesion molecules and laminin in varicose veins. Vasa 2005;34:170–5.

170. Jacob MP, Cazaubon M, Scemama A, Prié D, Blanchet F, Guillin MC, *et al.* Plasma matrix metalloproteinase-9 as a marker of blood stasis in varicose veins. Circulation 2002;106:535–8.

171. Solá LR, Aceves M, Dueñas AI, González-Fajardo JA, Vaquero C, Crespo MS, *et al.* Varicose veins show enhanced chemokine expression. Eur J Vasc Endovasc Surg 2009;38:635–41.

172. Beidler SK, Douillet CD, Berndt DF, Keagy BA, Rich PB, Marston WA. Inflammatory cytokine levels in chronic venous insufficiency ulcer tissue before and after compression therapy. J Vasc Surg 2009;49:1013–20.

173. Reddy VS, Prabhu SD, Mummidi S, Valente AJ, Venkatesan B, Shanmugam P, *et al.* Interleukin-18 induces EMMPRIN expression in primary cardiomyocytes via JNK/Sp1 signaling and MMP-9 in part via EMMPRIN and through AP-1 and NF-kappaB activation. Am J Physiol Heart Circ Physiol 2010;299:H1242–54.

174. Serra R, Grande R, Buffone G, Molinari V, Perri P, Perri A, *et al.* Extracellular matrix assessment of infected chronic venous leg ulcers: role of metalloproteinases and inflammatory cytokines. Int Wound J 2016;13:53–8.

175. Ligi D, Mosti G, Croce L, Raffetto JD, Mannello F. Chronic venous disease - Part II: proteolytic biomarkers in wound healing. Biochim Biophys Acta 2016;1862:1900–8.

176. Pappas PJ, DeFouw DO, Venezio LM, Gorti R, Padberg FT Jr, Silva MB Jr, *et al.* Morphometric assessment of the dermal microcirculation in patients with chronic venous insufficiency. J Vasc Surg 1997;26:784–95.

177. Herouy Y, Trefzer D, Hellstern MO, Stark GB, Vanscheidt W, Schöpf E, *et al.* Plasminogen activation in venous leg ulcers. Br J Dermatol 2000;143:930–6.

178. Hsieh CS, Tsai CT, Chen YH, Chang SN, Hwang JJ, Chuang EY, *et al.* Global Expression Profiling Identifies a Novel Hyaluronan Synthases 2 Gene in the Pathogenesis of Lower Extremity Varicose Veins. J Clin Med 2018;7:537.

179. Ghatnekar GS, Grek CL, Armstrong DG, Desai SC, Gourdie RG. The effect of a connexin43-based Peptide on the healing of chronic venous leg ulcers: a multicenter, randomized trial. J Invest Dermatol 2015;135:289–98.

180. Saribal D, Kanber EM, Hocaoglu-Emre FS, Akyolcu MC. Effects of

the oxidative stress and genetic changes in varicose vein patients. Phlebology 2019;34:406–13.

181. Yeoh-Ellerton S, Stacey MC. Iron and 8-isoprostane levels in acute and chronic wounds. J Invest Dermatol 2003;121:918–25.

182. Bodnár E, Bakondi E, Kovács K, Hegedűs C, Lakatos P, Robaszkiewicz A, *et al.* Redox Profiling Reveals Clear Differences between Molecular Patterns of Wound Fluids from Acute and Chronic Wounds. Oxid Med Cell Longev 2018;2018:5286785.

183. Arbiser JL, Petros J, Klafter R, Govindajaran B, McLaughlin ER, Brown LF, *et al.* Reactive oxygen generated by Nox1 triggers the angiogenic switch. Proc Natl Acad Sci USA 2002;99:715–20.

184. Zubkova ES, Men'shikov MY, Plekhanova OS, Beloglazova IB, Ratner EI, Parfenova EV. Urokinase stimulates production of matrix metalloproteinase-9 in fibroblasts with involvement of reactive oxygen species. Bull Exp Biol Med 2014;157:18–21.

185. Fu X, Kao JL, Bergt C, Kassim SY, Huq NP, d'Avignon A, *et al.* Oxidative cross-linking of tryptophan to glycine restrains matrix metalloproteinase activity: specific structural motifs control protein oxidation. J Biol Chem 2004;279:6209–12.

186. Zhou J, Liu C, Francis M, Sun Y, Ryu MS, Grider A, *et al.* The Causal Effects of Blood Iron and Copper on Lipid Metabolism Diseases: Evidence from Phenome-Wide Mendelian Randomization Study. Nutrients 2020;12:3174.

187. Yang F, Bao Q, Wang Z, Ma M, Shen J, Ye F, *et al.* Sex-Specific Genetically Predicted Iron Status in relation to 12 Vascular Diseases: A Mendelian Randomization Study in the UK Biobank. BioMed Res Int 2020;2020:6246041.

188. Zamboni P. Is Leg Ulceration a Defending Mechanism against Toxic Iron Accumulation. Acta Haematol 2016;135:122–3.

189. Christopoulos D, Nicolaides AN, Szendro G. Venous reflux: quantification and correlation with the clinical severity of chronic venous disease. Br J Surg 1988;75:352–6.

190. Zamboni P, Izzo M, Tognazzo S, Carandina S, De Palma M, Catozzi L, *et al.* The overlapping of local iron overload and HFE mutation in venous leg ulcer pathogenesis. Free Radic Biol Med 2006;40:1869–73.

191. Zamboni P, De Mattei M, Ongaro A, Fogato L, Carandina S, De Palma M, *et al.* Factor XIII contrasts the effects of metalloproteinases in human dermal fibroblast cultured cells. Vasc Endovascular Surg 2004;38:431–8.

192. Zamboni P, Tognazzo S, Izzo M, Pancaldi F, Scapoli GL, Liboni A, *et al.* Hemochromatosis C282Y gene mutation increases the risk of venous leg ulceration. J Vasc Surg 2005;42:309–14.

193. Tognazzo S, Gemmati D, Palazzo A, Catozzi L, Carandina S, Legnaro A, *et al.* Prognostic role of factor XIII gene variants in nonhealing venous leg ulcers. J Vasc Surg 2006;44:815–9.

194. Gemmati D, Tognazzo S, Catozzi L, Federici F, De Palma M, Gianesini S, *et al.* Influence of gene polymorphisms in ulcer healing process after superficial venous surgery. J Vasc Surg 2006;44:554–62.

195. Caggiati A, Franceschini M, Heyn R, Rosi C. Skin erythrodiapedesis during chronic venous disorders. J Vasc Surg 2011;53:1649–53.

196. Wlaschek M, Singh K, Sindrilaru A, Crisan D, Scharffetter-Kochanek K. Iron and iron-dependent reactive oxygen species in the regulation of macrophages and fibroblasts in non-healing chronic wounds. Free Radic Biol Med 2019;133:262–75.

197. Lattimer CR, Kalodiki E, Kafeza M, Azzam M, Geroulakos G. Quantifying the degree graduated elastic compression stockings enhance venous emptying. Eur J Vasc Endovasc Surg 2014;47:75–80.

198. Lattimer CR, Azzam M, Kalodiki E, Makris GC, Geroulakos G. Compression stockings significantly improve hemodynamic performance in post-thrombotic syndrome irrespective of class or length. J Vasc Surg 2013;58:158–65.

199. Mann MW. Sclerotherapy: it is back and better. Clin Plast Surg 2011;38:475–87, vii. [vii.]

200. King JT, O'Byrne M, Vasquez M, Wright D; VANISH-1 Investigator Group. Treatment of Truncal Incompetence and Varicose Veins with a Single Administration of a New Polidocanol Endovenous Microfoam Preparation Improves Symptoms and Appearance. Eur J Vasc Endovasc Surg 2015;50:784–93.

201. Proebstle TM, Lehr HA, Kargl A, Espinola-Klein C, Rother W, Bethge S, *et al.* Endovenous treatment of the greater saphenous vein with a 940-nm diode laser: thrombotic occlusion after endoluminal thermal damage by laser-generated steam bubbles. J Vasc Surg 2002;35:729–36.

202. Merchant RF, Pichot O, Myers KA. Four-year follow-up on endovascular radiofrequency obliteration of great saphenous reflux. Dermatol Surg 2005;31:129–34.

203. Min RJ, Khilnani N, Zimmet SE. Endovenous laser treatment of saphenous vein reflux: long-term results. J Vasc Interv Radiol 2003;14:991–6.

204. Bootun R, Lane TR, Davies AH. The advent of non-thermal, non-tumescent techniques for treatment of varicose veins. Phlebology 2016;31:5–14.

205. Tekin AI, Tuncer ON, Memetoğlu ME, Arslan Ü, Öztekin A, Yağmur B, *et al.* Nonthermal, Nontumescent Endovenous Treatment of Varicose Veins. Ann Vasc Surg 2016;36:231–5.

206. Sarin S, Scurr JH, Coleridge Smith PD. Stripping of the long saphenous vein in the treatment of primary varicose veins. Br J Surg 1994;81:1455–8.

207. Aremu MA, Mahendran B, Butcher W, Khan Z, Colgan MP, Moore DJ, *et al.* Prospective randomized controlled trial: conventional versus powered phlebectomy. J Vasc Surg 2004;39:88–94.

208. Krajnović P. [Effect of a benzopyrone preparation in venous diseases during pregnancy]. Med Monatsschr 1977;31:86–8.

209. Frick RW. Three treatments for chronic venous insufficiency: escin, hydroxyethylrutoside, and Daflon. Angiology 2000;51:197–205.

210. Sirtori CR. Aescin: pharmacology, pharmacokinetics and therapeutic profile. Pharmacol Res 2001;44:183–93.

211. Siebert U, Brach M, Sroczynski G, Berla K. Efficacy, routine effectiveness, and safety of horsechestnut seed extract in the treatment of chronic venous insufficiency. A meta-analysis of randomized controlled trials and large observational studies. Int Angiol 2002;21:305–15.

212. Berti F, Omini C, Longiave D. The mode of action of aescin and the release of prostaglandins. Prostaglandins 1977;14:241–9.

213. Carrasco OF, Vidrio H. Endothelium protectant and contractile effects of the antivaricose principle escin in rat aorta. Vascul Pharmacol 2007;47:68–73.

214. Kobayashi S, Kitazawa T, Somlyo AV, Somlyo AP. Cytosolic heparin inhibits muscarinic and alpha-adrenergic Ca2+ release in smooth muscle. Physiological role of inositol 1,4,5-trisphosphate in pharmacomechanical coupling. J Biol Chem 1989;264:17997–8004.

215. Coleridge Smith PD. From skin disorders to venous leg ulcers: pathophysiology and efficacy of Daflon 500 mg in ulcer healing. Angiology 2003;54(Suppl 1):S45–50.

216. Araujo D, Viana F, Osswald W. Diosmin therapy alters the in vitro metabolism of noradrenaline by the varicose human saphenous vein. Pharmacol Res 1991;24:253–6.

217. Savineau JP, Marthan R. Diosmin-induced increase in sensitivity to Ca2+ of the smooth muscle contractile apparatus in the rat isolated femoral vein. Br J Pharmacol 1994;111:978–80.

218. Raffetto JD, Khalil RA. Ca(2+)-dependent contraction by the saponoside escin in rat vena cava: implications in venotonic treatment of varicose veins. J Vasc Surg 2011;54:489–96.

219. Cesarone MR, Belcaro G, Pellegrini L, Ledda A, Vinciguerra G, Ricci A, *et al.* Venoruton vs. Daflon: evaluation of effects on quality of life in chronic venous insufficiency. Angiology 2006;57:131–8.

220. Kaur C, Sarkar R, Kanwar AJ, Attri AK, Dabra AK, Kochhar S. An open trial of calcium dobesilate in patients with venous ulcers and stasis dermatitis. Int J Dermatol 2003;42:147–52.

221. Alda O, Valero MS, Pereboom D, Serrano P, Azcona JM, Garay RP.

In vitro effect of calcium dobesilate on oxidative/inflammatory stress in human varicose veins. Phlebology 2011;26:332–7.

222. Tejerina T, Ruiz E. Calcium dobesilate: pharmacology and future approaches. Gen Pharmacol 1998;31:357–60.

223. Angulo J, Peiró C, Romacho T, Fernández A, Cuevas B, González-Corrochano R, *et al.* Inhibition of vascular endothelial growth factor (VEGF)-induced endothelial proliferation, arterial relaxation, vascular permeability and angiogenesis by dobesilate. Eur J Pharmacol 2011;667:153–9.

224. Coccheri S, Scondotto G, Agnelli G, Aloisi D, Palazzini E, Zamboni V; Venous arm of the SUAVIS (Sulodexide Arterial Venous Italian Study) Group. Randomised, double blind, multicentre, placebo controlled study of sulodexide in the treatment of venous leg ulcers. Thromb Haemost 2002;87:947–52.

225. Scondotto G, Aloisi D, Ferrari P, Martini L. Treatment of venous leg ulcers with sulodexide. Angiology 1999;50:883–9.

226. Coccheri S. Biological and clinical effects of sulodexide in arterial disorders and diseases. Int Angiol 2014;33:263–74.

227. Coccheri S, Mannello F. Development and use of sulodexide in vascular diseases: implications for treatment. Drug Des Devel Ther 2013;8:49–65.

228. Andreozzi GM. Role of sulodexide in the treatment of CVD. Int Angiol 2014;33:255–62.

229. Olde Engberink RH, Heerspink HJ, de Zeeuw D, Vogt L. Blood pressure-lowering effects of sulodexide depend on albuminuria severity: post hoc analysis of the sulodexide microalbuminuria and macroalbuminuria studies. Br J Clin Pharmacol 2016;82:1351–7.

230. Liu YN, Zhou J, Li T, Wu J, Xie SH, Liu HF, *et al.* Sulodexide Protects Renal Tubular Epithelial Cells from Oxidative Stress-Induced Injury via Upregulating Klotho Expression at an Early Stage of Diabetic Kidney Disease. J Diabetes Res 2017;2017:4989847.

231. Ciszewicz M, Polubinska A, Antoniewicz A, Suminska-Jasinska K, Breborowicz A. Sulodexide suppresses inflammation in human endothelial cells and prevents glucose cytotoxicity. Transl Res 2009;153:118–23.

232. Suminska-Jasinska K, Polubinska A, Ciszewicz M, Mikstacki A, Antoniewicz A, Breborowicz A. Sulodexide reduces senescence-related changes in human endothelial cells. Med Sci Monit 2011;17:CR222–6.

233. Połubińska A, Staniszewski R, Baum E, Sumińska-Jasińska K, Bręborowicz A. Sulodexide modifies intravascular homeostasis what affects function of the endothelium. Adv Med Sci 2013;58:304–10.

234. Olde Engberink RH, Rorije NM, Lambers Heerspink HJ, De Zeeuw D, van den Born BJ, Vogt L. The blood pressure lowering potential of sulodexide—a systematic review and meta-analysis. Br J Clin Pharmacol 2015;80:1245–53.

235. Raffetto JD, Yu W, Wang X, Calanni F, Mattana P, Khalil RA. Sulodexide Improves Contraction and Decreases Matrix Metalloproteinase-2 and -9 in Veins Under Prolonged Stretch. J Cardiovasc Pharmacol 2020;75:211–21.

236. Urbanek T, Zbigniew K, Begier-Krasińska B, Baum E, Bręborowicz A. Sulodexide suppresses inflammation in patients with chronic venous insufficiency. Int Angiol 2015;34:589–96.

237. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 2006;69:562–73.

238. Li PC, Pan CH, Sheu MJ, Wu CC, Ma WF, Wu CH. Deep sea water prevents balloon angioplasty-induced hyperplasia through MMP-2: an in vitro and in vivo study. PLoS One 2014;9:e96927.

239. Benjamin MM, Khalil RA. Matrix metalloproteinase inhibitors as investigative tools in the pathogenesis and management of vascular disease. Experientia Suppl 2012;103:209–79.

240. Jacobsen JA, Major Jourden JL, Miller MT, Cohen SM. To bind zinc or not to bind zinc: an examination of innovative approaches to improved metalloproteinase inhibition. Biochim Biophys Acta 2010;1803:72–94.

241. Scozzafava A, Supuran CT. Carbonic anhydrase and matrix metalloproteinase inhibitors: sulfonylated amino acid hydroxamates with MMP inhibitory properties act as efficient inhibitors of CA isozymes I, II, and IV, and N-hydroxysulfonamides inhibit both these zinc enzymes. J Med Chem 2000;43:3677–87.

242. Pochetti G, Gavuzzo E, Campestre C, Agamennone M, Tortorella P, Consalvi V, *et al.* Structural insight into the stereoselective inhibition of MMP-8 by enantiomeric sulfonamide phosphonates. J Med Chem 2006;49:923–31.

243. Hu J, Van den Steen PE, Sang QX, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat Rev Drug Discov 2007;6:480–98.

244. Bernardo MM, Brown S, Li ZH, Fridman R, Mobashery S. Design, synthesis, and characterization of potent, slow-binding inhibitors that are selective for gelatinases. J Biol Chem 2002;277:11201–7.

245. Johnson AR, Pavlovsky AG, Ortwine DF, Prior F, Man CF, Bornemeier DA, *et al.* Discovery and characterization of a novel inhibitor of matrix metalloprotease-13 that reduces cartilage damage in vivo without joint fibroplasia side effects. J Biol Chem 2007;282:27781–91.

246. Chetty C, Bhoopathi P, Joseph P, Chittivelu S, Rao JS, Lakka S. Adenovirus-mediated small interfering RNA against matrix metalloproteinase-2 suppresses tumor growth and lung metastasis in mice. Mol Cancer Ther 2006;5:2289–99.

247. Dorecka M, Francuz T, Garczorz W, Siemianowicz K, Romaniuk W. The influence of elastin degradation products, glucose and atorvastatin on metalloproteinase-1, -2, -9 and tissue inhibitor of metalloproteinases-1, -2, -3 expression in human retinal pigment epithelial cells. Acta Biochim Pol 2014;61:265–70.

248. Luan Z, Chase AJ, Newby AC. Statins inhibit secretion of metalloproteinases-1, -2, -3, and -9 from vascular smooth muscle cells and macrophages. Arterioscler Thromb Vasc Biol 2003;23:769–75.

249. Ichihara S, Noda A, Nagata K, Obata K, Xu J, Ichihara G, *et al.* Pravastatin increases survival and suppresses an increase in myocardial matrix metalloproteinase activity in a rat model of heart failure. Cardiovasc Res 2006;69:726–35.

250. Serra R, Gallelli L, Buffone G, Molinari V, Stillitano DM, Palmieri C, *et al.* Doxycycline speeds up healing of chronic venous ulcers. Int Wound J 2015;12:179–84.

251. Renkiewicz R, Qiu L, Lesch C, Sun X, Devalaraja R, Cody T, *et al.* Broad-spectrum matrix metalloproteinase inhibitor marimastat-induced musculoskeletal side effects in rats. Arthritis Rheum 2003;48:1742–9.

Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Funding
Dr. R.A. Khalil was supported by grants from National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA (R56HL147889, and R01HL147889-A1) and by Alfasigma S.p.A., Bologna, Italy. Prof. Diaz and Prof. Gianesini were supported by grants from Alfasigma S.p.A. Alfasigma S.p.A. funded fee relating to the publication of the manuscript, including Open Access.

Authors' contributions
All authors read and approved the final version of the manuscript.

History
Manuscript accepted: December 17, 2024. - Manuscript revised: November 8, 2024. - Manuscript received: September 20, 2024.