Endothelial Functions

Shigeo Godo, Hiroaki Shimokawa

***Abstract*—**The endothelium plays important roles in modulating vascular tone by synthesizing and releasing a variety of endothelium-derived relaxing factors, including vasodilator prostaglandins, NO, and endothelium-dependent hyperpolarization factors, as well as endothelium-derived contracting factors. Endothelial dysfunction is mainly caused by reduced production or action of these relaxing mediators. Accumulating evidence has demonstrated that endothelial functions are essential to ensure proper maintenance of vascular homeostasis and that endothelial dysfunction is the hallmark of a wide range of cardiovascular diseases associated with pathological conditions toward vasoconstriction, thrombosis, and inflammatory state. In the clinical settings, evaluation of endothelial functions has gained increasing attention in view of its emerging relevance for cardiovascular disease. Recent experimental and clinical studies in the vascular biology field have demonstrated a close relationship between endothelial functions and cardiovascular disease and the highlighted emerging modulators of endothelial functions, new insight into cardiovascular disease associated with endothelial dysfunction, and potential therapeutic and diagnostic targets with major clinical implications. We herein will summarize the current knowledge on endothelial functions from bench to bedside with particular focus on recent publications in *Arteriosclerosis, Thrombosis, and Vascular Biology*. **(*Arterioscler Thromb Vasc Biol*. 2017;37: e108-e114. DOI: 10.1161/ATVBAHA.117.309813.)**

Key Words: cardiovascular diseases ■ endothelial function ■ endothelium ■ endothelium-dependent hyperpolarization ■ nitric oxide

The endothelium plays important roles in modulating vascular tone by synthesizing and releasing an array of endothelium-derived relaxing factors, including vasodilator prostaglandins, NO, and endothelium-dependent hyperpolarization (EDH) factors, as well as endothelium-derived contracting factors.[1,2] Such redundant mechanisms, like endogenous hyperglycemic hormones, are advantageous for ensuring proper maintenance of vascular tone under pathological conditions, where one of the vasoactive factor-mediated responses is compromised favoring a vasoconstrictor, prothrombotic, and proinflammatory state. Endothelial dysfunction is mainly caused by reduced production or action of endothelium-derived relaxing factors and could be an initial step toward cardiovascular disease.[1] Indeed, evaluation of endothelial functions in humans has attracted much attention in the clinical settings because it serves as an excellent surrogate marker of cardiovascular events. For instance, endothelial dysfunction, as evaluated by impaired flow-mediated dilation of the brachial artery or digital reactive hyperemia index in peripheral arterial tonometry, is associated with future cardiovascular events in patients with coronary artery disease,[3–5] and 1-SD decrease in flow-mediated dilation or reactive hyperemia index is associated with doubling of cardiovascular event risk.[6] These observations suggest that endothelial function in peripheral vascular beds could predict future cardiovascular events.

In this review, we will sum up the current advances and trends in the research on endothelial functions from bench to bedside with particular focus on recent publications in *Arteriosclerosis, Thrombosis, and Vascular Biology*. Earlier highlights of the journal on endothelial biology are extensively summarized in some review articles.[7–9]

Emerging Modulators of Endothelial Functions

Shear Stress

As Lüscher and Corti[10] described in an editorial, flow is the signal of life. This physical force is sensed by the endothelium lining internal surface of the whole cardiovascular system to be translated into numerous downstream signaling pathways in a moment to moment manner in response to diverse physiological demands in the body. Indeed, shear stress is one of the important physiological cues that make endothelial cells synthesize and release endothelium-derived relaxing factors to cause relaxation of underlying vascular smooth muscle and vasodilatation. In this context, novel mechanisms of endothelial mechanotransduction in health and disease have been unveiled and summarized in a review series published recently in *Arteriosclerosis, Thrombosis, and Vascular Biology*.[11,12] Briefly, Zhou et al[12] emphasized the distinct roles of atheroprotective laminar or pulsatile shear stress versus atheroprone oscillatory shear stress or disturbed flow and discussed in detail the underlying molecular mechanisms that are dependent on these patterns of flow. Abe and Berk[11] further reviewed the current knowledge on the dual roles of shear stress with emphasis placed on the 2 distinct types of flow; steady laminar flow provides atheroprotective effects on the vascular wall by enhancing endothelial production of prostacyclin and NO, whereas disturbed flow stimulates proinflammatory signaling

From the Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan.

Correspondence to Hiroaki Shimokawa, MD, PhD, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan. E-mail shimo@cardio.med.tohoku.ac.jp

***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org**
DOI: 10.1161/ATVBAHA.117.309813

with resultant endothelial dysfunction and subsequent development of atherosclerotic lesions. In addition, Warboys et al[13] demonstrated that such disturbed flow resembling that observed at atheroprone sites in vivo, such as arterial branches, bifurcations, and bends, accelerated endothelial senescence through a p53-p21-dependent pathway. Under their experimental conditions, activation of sirtuin-1 by using resveratrol or SRT1720 exerted a protective role against disturbed flow-induced endothelial senescence.[13] We also have recently demonstrated that endothelial mechanotransduction mechanisms play important roles in the therapeutic angiogenic effects of pulsed ultrasound.[14]

Reactive Oxygen Species

Reactive oxygen species (ROS) have been considered primarily detrimental because of their highly damaging entity to cells and tissues and pathological implications in a wide range of cardiovascular diseases and endothelial dysfunction.[15,16] In line with this concept, using adipocyte-specific NADPH (nicotinamide adenine dinucleotide phosphate) oxidase 4–deficient mice, Den Hartigh et al[17] demonstrated that adipocyte NADPH oxidase 4–derived ROS contributed to the development of obesity-related insulin resistance by triggering adipocyte inflammation. La Favor et al[18] developed a novel microdialysis technique that enables simultaneous measurement of ROS levels and microvascular endothelial functions in vivo. With this method, they showed that NADPH oxidase–derived ROS levels were elevated in obese subjects, associated with microvascular endothelial dysfunction as evidenced by impaired acetylcholine-induced blood flow increases.[18] Notably, an 8-week aerobic exercise training normalized both the elevated ROS levels and the microvascular endothelial dysfunction in this study.[18]

In striking contrast, the physiological roles of ROS in the regulation of vascular homeostasis have been brought to light.[16] Gray et al[19] demonstrated the atheroprotective role of NADPH oxidase 4–derived hydrogen peroxide (H_2O_2) in a diabetic atherosclerosis mouse model. Moreover, we have recently demonstrated that excessive endothelial NO production by either deficiency of a negative regulator of endothelial NO synthase (eNOS) caveolin-1 or overexpression of eNOS disrupted the physiological balance between NO and H_2O_2 as an EDH factor in microcirculations, resulting in impaired cardiovascular homeostasis in mice.[20] A novel mechanism of microvascular dysfunction in human coronary artery disease has been proposed from the Gutterman laboratory.[21] Briefly, healthy human coronary circulation is regulated by NO and low physiological levels of H_2O_2 as an EDH factor. However, various atherosclerotic conditions and metabolic disorders cause a switch from NO to H_2O_2 in the mediator of endothelium-dependent relaxations, and resultant pathological levels of H_2O_2, like a double-edge sword, lead to microvascular dysfunction and the development of coronary artery disease.[21] Mechanistically, ceramide-induced reduction in telomerase activity in mitochondria has been shown to cause this switch.[22,23] Although the sources and regulatory mechanisms of physiological ROS are inconclusive, local subcellular concentrations at microdomains rather than net intracellular concentrations may be critical to determine whether the effects of ROS can be gainful or harmful to cellular processes, and colocalization of the source and target of ROS may help prevent nonspecific injurious oxidations.[24] Among redox regulating proteins, endothelial thioredoxin reductase 2 has been shown to play a key role in the maintenance of healthy endothelial functions,[25] and peroxisome proliferator receptor-γ coactivator 1α has emerged as a master regulator of endothelial functions, including protection against oxidative stress, inflammation, and atherosclerosis.[26]

These apparent dual roles of ROS again teach us a renewed recognition of physiologically relevant ROS as a significant endogenous signaling molecule, providing a clue for the development of better therapeutic strategies aiming at reducing pathological ROS (eg, isoform- or site-specific inhibitors of NADPH oxidase). See a review by Nowak et al[27] for further discussion on cell-specific roles of ROS.

Perivascular Adipose Tissue

Accumulating evidence has demonstrated the vasoprotective roles of perivascular adipose tissue (PVAT) in vascular health and disease.[28] PVAT is classified as white, brown, and beige with different pathophysiological roles, depending on its location in the body, and modulates vascular tone in a paracrine/autocrine manner by releasing an array of vasoactive substances, including adiponectin, NO, hydrogen sulfide, and others yet to be identified.[28] Friederich-Persson et al[29] have demonstrated that similar to PVAT surrounding the aorta or mesenteric artery, interscapular brown adipose tissue exerts an anticontractile effect via H_2O_2-induced PKG1α (cyclic GMP-dependent protein kinase G1α) activation and subsequent vasodilatation of small resistance arteries in mice, providing a therapeutic potential of targeting brown adipose tissue for cardiovascular disorders. Interestingly, this oxidant-mediated PKG1α activation is a shared vasodilating mechanism of H_2O_2 as an EDH factor in resistance arteries as well.[30,31] Moreover, Noblet et al[32] have added another layer of complexity of PVAT-mediated responses. They showed that lean coronary PVAT inhibited K_{Ca} and K_V7 channel-mediated vasodilatations, whereas obese coronary PVAT impaired a K_{ATP} channel-mediated vasodilatation in pigs ex vivo, implying potential roles of PVAT-derived factors in the pathogenesis of obesity-related coronary artery disease. Furthermore, Dou et al[33] revealed a novel mechanism by which human coronary microvascular dysfunction may develop; ADAM17 (aging and obesity increased a disintegrin and metalloprotease) activity and soluble tumor necrosis factor release in adipose tissue, leading to impaired bradykinin-induced endothelium-dependent vasodilatation of human coronary arterioles.

Obesity also impairs PVAT-mediated vascular function through mechanisms involving endothelium-derived relaxing factors. First, obesity promoted recruitment of proinflammatory macrophages to PVAT and impaired vasodilator property of PVAT by reducing endothelial and vascular smooth muscle production of hydrogen sulfide—a potent gaseous relaxing factor.[34] Second, diet-induced obesity caused eNOS uncoupling in PVAT by arginase-induced L-arginine deficiency.[35] Of note, obesity-induced loss of anticontractile effect of PVAT was reversed by calorie restriction.[36] Taken together, these new lines of evidence represent the therapeutic potential of targeting PVAT in the treatment of cardiovascular disease associated with vascular dysfunction.

AMP-Activated Protein Kinase

A growing number of studies have uncovered the diverse beneficial roles of AMPK (AMP-activated protein kinase) in the treatment of metabolic disorders, including diabetes mellitus and obesity, where vascular endothelial dysfunction is substantially involved. In this context, novel mechanistic insight into AMPK-mediated responses has emerged in recent publications in *Arteriosclerosis, Thrombosis, and Vascular Biology*. Using endothelium-specific AMPK knockout mice, we have demonstrated that α1-subunit of endothelial AMPK plays an important role in the regulation of blood pressure and coronary flow responses through EDH-mediated relaxations without affecting NO-mediated vasodilatations in mice in vivo.[37] Metformin is a drug of choice for the treatment of type 2 diabetes mellitus in the clinical settings and serves as an activator of AMPK as well. Cheang et al[38] showed that metformin reversed endothelial dysfunction of diabetic mouse aorta by inhibiting endoplasmic reticulum stress through activation of AMPK/peroxisome proliferator–activated receptor δ pathway. We also have recently demonstrated that endothelial AMPK plays an important protective role against the development of pulmonary hypertension in mice and that metformin could be a useful drug for the treatment of the disorder.[39] Similar to AMPK, sirtuin-1 is a senescence-associated protein that exhibits antisenescence effects in endothelial cells.[40] Shentu et al[41] reported a novel mechanism by which AMPK and sirtuin-1 collaborate in the process of vascular protection against atherosclerosis. Briefly, an F-actin-binding protein cortactin coregulated by AMPK-induced phosphorylation and sirtuin-1-medicated deacetylation in response to shear stress promoted compartmentalization and subsequent activation of eNOS, leading to atheroprotective effects in mice in vivo.[41,42] Moreover, Li and Kim et al[43] demonstrated that endothelial microRNA-34a was upregulated by oxidative stress in a diabetic mouse model and promoted endothelial dysfunction through inhibition of sirtuin-1. In addition, in a hyperlipidemic mouse model, inhibition of caspase-1 activation during early atherogenesis facilitated the accumulation of sirtuin-1 in endothelial cells with resultant anti-inflammatory effects.[44] Collectively, endothelial AMPK and sirtuin-1 may be promising therapeutic targets for the treatment of cardiovascular and metabolic disorders. Further information on the contribution of sirtuin-1 and AMPK to endothelial functions with a focus on EDH-mediated responses in aging, hypertension, and sex difference is available in a concise review published recently.[45]

Potassium Channels

A variety of potassium channels play pivotal roles in the mechanisms of vasodilatation, especially in those of EDH-mediated vascular smooth muscle relaxation and vasodilatation. Stott et al[46] showed that K_V7 channel-mediated relaxations in response to isoproterenol were dependent on exchange protein directly activated by cAMP in mesenteric artery but not in renal artery in rats, indicating that intermediate signaling steps from β-adrenoceptors to K_V7 channels vary depending on vascular beds. Xu et al[47] showed that blocking $K_{Ca}3.1$ channels reduced atherosclerotic burden and enhanced plaque stability in a mouse model of atherosclerosis by inhibiting macrophage differentiation toward proinflammatory M1 phenotype. However, nonspecific inhibition of $K_{Ca}3.1$ should require caution because it could lead to microvascular endothelial dysfunction by inhibiting EDH-mediated responses.[48,49]

Bone Morphogenic Protein 4

BMP4 (bone morphogenic protein 4) has been implicated in the development of cardiovascular disease and endothelial dysfunction in humans.[50,51] Recent studies from the Huang laboratory demonstrated a functional link between BMP4 and platelet-derived growth factors in the molecular mechanisms of diabetic endothelial dysfunction in mice,[50] and therapeutic potential of inhibiting BMP4 cascade for diabetic endothelial dysfunction.[51]

$P2Y_2$ Receptor

Chen et al[52] demonstrated that endothelium-specific deletion of $P2Y_2$ receptor exerted protective effects on plaque stabilization by promoting fibrous cap formation in an atherosclerotic mouse model. Considering that the endothelial cell-specific $P2Y_2$ receptor-deficient mice showed decreased nucleotide-mediated but preserved acetylcholine-induced endothelium-dependent relaxation of the aorta without causing systemic hypertension, endothelial $P2Y_2$ receptor may be a promising therapeutic target for atherosclerotic cardiovascular diseases.

Hallmark of Disease

Inflammation

Inflammatory conditions are substantially involved in the development of endothelial dysfunction,[53] where IL-1β (interleukin-1β) is one of the key proinflammatory cytokines. Honda et al[54] demonstrated a close relationship between endothelial dysfunction evaluated by flow-mediated dilation and vascular inflammation detected by (^{18}F)-fluorodeoxyglucose-positron emission tomography/computed tomography in subjects with mild cardiovascular risks, both of which were improved after a 6-month antihypertensive treatment. In an observational cohort study, Hertle et al[55] also showed that a number of molecules involved in the lectin-component pathway may contribute to endothelial dysfunction in subjects with mild metabolic risk factors. Furthermore, a recent meta-analysis has shown a positive association between higher serum levels of IL-1 receptor antagonist and increased incidence of cardiovascular disease in the general population.[56] Based on the inflammatory hypothesis of atherosclerotic cardiovascular diseases, a large-scale randomized clinical trial is currently ongoing to elucidate whether targeting IL-1β can reduce the risk of recurrent cardiovascular events (the CANTOS trial [canakinumab anti-inflammatory thrombosis outcomes study]).[57] A novel link between inflammation and coronary endothelial dysfunction has been reported from the Lerman laboratory; human coronary endothelial dysfunction was more severe in coronary artery segments with macrophage infiltration and vasa vasorum proliferation in an additive manner than in those without them, indicating an important role of inflammation and vasa vasorum proliferation in the pathogenesis of coronary artery disease.[58]

Diabetes Mellitus

Loader et al[59,60] revealed that acute hyperglycemia after sugar-sweetened beverage consumption in healthy subjects caused both microvascular and macrovascular endothelial dysfunction partly through oxidative stress-induced impairment of NO bioavailability. Their findings indicate potential burden of commercial sugar-sweetened beverage consumption on public health in general and the downside of hyperglycemia in patients with diabetes mellitus that are closely related with cardiovascular disease in particular. Bretón-Romero et al[61] showed that wingless-type family member 5a/c-jun N terminal kinase pathway contributed to endothelial dysfunction associated with diabetic patients. Walther et al[62] demonstrated that not only endothelium-dependent but also endothelium-independent vasodilatations in both micro- and macrocirculation were impaired in association with systemic inflammation in patients with metabolic syndrome with diabetes mellitus.

Pulmonary Hypertension

Pulmonary arterial hypertension (PAH) still remains a life-threatening disorder, for which better understanding of the underlying mechanisms is still required. To this end, several experiments have been conducted. Xue et al[63] demonstrated that endothelium-specific overexpression of cyclophilin A, which has been shown to induce vascular injury through multiple mechanisms, including endothelial dysfunction and vascular smooth muscle proliferation, caused spontaneous PAH in mice in vivo. Mechanistically, extracellular cyclophilin A induced endothelial cell dysfunction via endothelial apoptosis, inflammation, and oxidative stress production.[63] Meloche et al[64] proposed a mechanism by which coronary artery disease develops in PAH patients; expression of bromodomain-containing protein 4, which promotes atherogenic processes through inflammatory responses in endothelial cells, was increased not only in the lungs of PAH patients but also in their coronary arteries, promoting vascular remodeling through enhanced proliferation and suppressed apoptosis in vascular smooth muscle cells. These findings provide a clue for understanding why PAH patients are likely to be complicated by coronary artery disease even in the absence of metabolic disorders. Johns et al[65] showed that hypoxia-inducible factor-1 was an important downstream mediator of hypoxia-induced mitogenic factors that contributed to the development of pulmonary hypertension, in part, through pulmonary microvascular endothelial cell activation, apoptosis, and inflammation.

Chronic thromboembolic pulmonary hypertension is a distinct type of pulmonary hypertension, associated with organized thrombi of unknown origin in the pulmonary arteries leading to their mechanical obstruction. Although the endothelium plays crucial roles in the regulation of thrombosis, hemostasis, and fibrinolysis,[66] the pathophysiology of chronic thromboembolic pulmonary hypertension remains poorly understood. Based on the observation that the clot from patients with chronic thromboembolic pulmonary hypertension was resistant to fibrinolysis in vitro, Yaoita et al[67,68] provided new evidence that elevated plasma levels of thrombin-activatable fibrinolysis inhibitor from chronic thromboembolic pulmonary hypertension patients may confer a prothrombotic or hypercoagulable state during the development of this disorder.

Fabry Disease

Choi et al[69] showed that globotriaosylceramide—a pathogenic glycosphingolipid accumulating in a variety of cells (eg, endothelial cells) in Fabry disease—induced $K_{Ca}3.1$ degradation with resultant endothelial dysfunction, which may, in part, explain the mechanism of reduced myocardial perfusion reserve in patients with the disorder.

Novel Therapeutic and Diagnostic Targets

Agents for Metabolic Disorders

Ezetimibe—a potent cholesterol absorption inhibitor—has gained increasing attention in view of its potential role of reducing the residual risk for patients on statin therapy. The CuVIC trial (Effect of Cholesterol Absorption Inhibitor Usage on Target Vessel Dysfunction After Coronary Stenting) is a multicenter, randomized, controlled clinical trial, demonstrating that combination therapy with ezetimibe plus statins, as compared with statin monotherapy, improved coronary endothelial dysfunction in patients with coronary artery disease undergoing coronary stenting.[70] These findings are consistent with those of the IMPROVE-IT trial (Improved Reduction of Outcomes: Vytorin Efficacy International Trial) that demonstrated that ezetimibe provided an additional beneficial prognostic effect to statin therapy.[71] Lin et al[72] demonstrated that a 6-week treatment of ezetimibe in a standard dose not only reduced intestinal cholesterol absorption but also promoted reverse cholesterol transport from endogenous cholesterol pools into the stool. Ezetimibe did not affect the plasma concentration of high-density lipoprotein—a beneficial cholesterol with vasoprotective property of enhancing endothelial NO production through activation of eNOS.[72] Denimal et al[73] showed that this beneficial effect of high-density lipoprotein was impaired by the sphingosine-1-phosphate depletion of high-density lipoprotein in patients with metabolic syndrome.

On the basis of the premise that targeting glucagon-like peptide (GLP)-1 could provide better treatment of diabetes mellitus via pleiotropic cardiovascular protective effects beyond glycemic control, many clinical trials of GLP-1-based therapies have been conducted with variable effects on cardiovascular outcomes, and others are ongoing. For example, Smits et al[74] showed that in patients with type 2 diabetes mellitus, a 12-week treatment with a GLP-1 receptor agonist, liraglutide, or a GLP-1-degrading enzyme dipeptidyl peptidase-4 inhibitor, sitagliptin, had neutral effects on microvascular functions as assessed by nail fold skin capillary microscopy and laser Doppler flowmetry, implying that antihypertensive effects of GLP-1-based therapies are mediated by mechanisms other than improving microvascular functions.

Endothelial Function Tests

Bretón-Romero et al[75] showed that more than one third of a population-based cohort consisting of 5708 participants in the Framingham heart study exhibited brachial artery flow reversal, which was associated with endothelial dysfunction as evaluated by flow-mediated dilation or reactive hyperemic

flow and higher aortic stiffness. These results indicate that flow reversal may affect endothelial function in a flow pattern-dependent manner and may serve as an indicator of endothelial dysfunction. From another cross-sectional analysis in the Framingham heart study, the same group provided further insight into the relationship between nonalcoholic fatty liver disease and endothelial dysfunction independent of well-established cardiovascular risks, explaining, at least in part, why patients with this chronic liver condition are commonly affected by metabolic and cardiovascular disorders.[76]

Endothelial glycocalyx plays important roles in preserving healthy endothelial functions, including anticoagulation, mechanotransduction, and shear stress-mediated NO production. Dimitrievska et al[77] developed for the first time simple assays that can evaluate glycocalyx function by measuring its antithrombogenic capacity in vitro. This assay will pave the way for the development of a new diagnostic tool of endothelial function and a novel therapeutic approach targeting glycocalyx.

Biomarkers

Several new biomarkers that are associated with endothelial dysfunction and correlate with severity or clinical outcomes of cardiovascular disease have been identified. In the Hisayama cohort study including 3005 Japanese general population aged ≥40 from 1988, serum levels of angiopoietin-like protein 2—a proinflammatory mediator that promotes endothelial dysfunction—were positively correlated with the risk for future cardiovascular disease.[78] Saita et al[79] showed that plasma levels of soluble endoglin—a transforming growth factor-β receptor highly expressed on proliferating endothelial cells—were inversely associated with the severity of coronary artery disease. Given that conflicting results have been reported earlier in diabetic patients,[80] whether endoglin can serve as a biomarker for coronary artery disease awaits further investigation.[81] Hyperhomocysteinemia has been shown to cause endothelial dysfunction by inhibiting NO-mediated relaxations in conduit artery and EDH-mediated relaxations in resistant artery and has emerged as an independent predictor of cardiovascular events.[82,83] One of the mechanisms by which homocysteine causes endothelial dysfunction was acceleration of endothelial senescence via epigenetic regulation of human telomerase reverse transcriptase.[84] Indeed, elevated plasma levels of homocysteine decreased the blood pressure-lowering effect of an angiotensin-converting enzyme inhibitor, enalapril.[83] Finally, increased plasma levels of cyclophilin A, especially when combined with brain natriuretic peptide or high-sensitive C-reactive protein, can predict cardiovascular events in patients with coronary artery disease.[85]

Summary

Recent experimental and clinical studies in the vascular biology field have demonstrated a close relationship between endothelial dysfunctions and cardiovascular disease. Although it remains an open question how to modulate endothelial functions to improve clinical outcomes, recent publications in *Arteriosclerosis, Thrombosis, and Vascular Biology* highlighted the emerging modulators of endothelial functions, new insight into cardiovascular disease associated with endothelial dysfunction, and potential therapeutic and diagnostic targets with major clinical implications, making a significant contribution toward this end. In conclusion, further characterization and better understanding of endothelial functions is certainly required to develop novel therapeutic strategies in cardiovascular medicine.

Sources of Funding

This work was supported, in part, by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan (16K19383).

Disclosures

None.

References

1. Vanhoutte PM, Shimokawa H, Feletou M, Tang EH. Endothelial dysfunction and vascular disease - a 30th anniversary update. *Acta Physiol (Oxf)*. 2017;219:22–96. doi: 10.1111/apha.12646.
2. Shimokawa H. 2014 Williams Harvey lecture: importance of coronary vasomotion abnormalities-from bench to bedside. *Eur Heart J*. 2014;35:3180–3193. doi: 10.1093/eurheartj/ehu427.
3. Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol*. 2004;44:2137–2141. doi: 10.1016/j.jacc.2004.08.062.
4. Kitta Y, Obata JE, Nakamura T, Hirano M, Kodama Y, Fujioka D, Saito Y, Kawabata K, Sano K, Kobayashi T, Yano T, Nakamura K, Kugiyama K. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. *J Am Coll Cardiol*. 2009;53:323–330. doi: 10.1016/j.jacc.2008.08.074.
5. Matsuzawa Y, Sugiyama S, Sugamura K, et al. Digital assessment of endothelial function and ischemic heart disease in women. *J Am Coll Cardiol*. 2010;55:1688–1696. doi: 10.1016/j.jacc.2009.10.073.
6. Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A. Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. *J Am Heart Assoc*. 2015;4:e002270.
7. Shimokawa H, Satoh K. Vascular function. *Arterioscler Thromb Vasc Biol*. 2014;34:2359–2362. doi: 10.1161/ATVBAHA.114.304119.
8. Vallerie SN, Bornfeldt KE. Metabolic flexibility and dysfunction in cardiovascular cells. *Arterioscler Thromb Vasc Biol*. 2015;35:e37–e42. doi: 10.1161/ATVBAHA.115.306226.
9. Boulanger CM. Endothelium. *Arterioscler Thromb Vasc Biol*. 2016;36:e26–e31. doi: 10.1161/ATVBAHA.116.306940.
10. Lüscher TF, Corti R. Flow: the signal of life. *Circ Res*. 2004;95:749–751. doi: 10.1161/01.RES.0000146513.73748.78.
11. Abe J, Berk BC. Novel mechanisms of endothelial mechanotransduction. *Arterioscler Thromb Vasc Biol*. 2014;34:2378–2386. doi: 10.1161/ATVBAHA.114.303428.
12. Zhou J, Li YS, Chien S. Shear stress-initiated signaling and its regulation of endothelial function. *Arterioscler Thromb Vasc Biol*. 2014;34:2191–2198. doi: 10.1161/ATVBAHA.114.303422.
13. Warboys CM, de Luca A, Amini N, et al. Disturbed flow promotes endothelial senescence via a p53-dependent pathway. *Arterioscler Thromb Vasc Biol*. 2014;34:985–995. doi: 10.1161/ATVBAHA.114.303415.
14. Shindo T, Ito K, Ogata T, et al. Low-intensity pulsed ultrasound enhances angiogenesis and ameliorates left ventricular dysfunction in a mouse model of acute myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2016;36:1220–1229. doi: 10.1161/ATVBAHA.115.306477.
15. Lee MY, Griendling KK. Redox signaling, vascular function, and hypertension. *Antioxid Redox Signal*. 2008;10:1045–1059. doi: 10.1089/ars.2007.1986.
16. Godo S, Shimokawa H. Divergent roles of endothelial nitric oxide synthases system in maintaining cardiovascular homeostasis. *Free Radic Biol Med*. 2017;109:4–10. doi: 10.1016/j.freeradbiomed.2016.12.019.
17. Den Hartigh LJ, Omer M, Goodspeed L, Wang S, Wietecha T, O'Brien KD, Han CY. Adipocyte-specific deficiency of NADPH oxidase 4 delays the onset of insulin resistance and attenuates adipose tissue inflammation in obesity. *Arterioscler Thromb Vasc Biol*. 2017;37:466–475. doi: 10.1161/ATVBAHA.116.308749.

18. La Favor JD, Dubis GS, Yan H, White JD, Nelson MA, Anderson EJ, Hickner RC. Microvascular endothelial dysfunction in sedentary, obese humans is mediated by NADPH oxidase: influence of exercise training. *Arterioscler Thromb Vasc Biol*. 2016;36:2412–2420. doi: 10.1161/ATVBAHA.116.308339.
19. Gray SP, Di Marco E, Kennedy K, Chew P, Okabe J, El-Osta A, Calkin AC, Biessen EA, Touyz RM, Cooper ME, Schmidt HH, Jandeleit-Dahm KA. Reactive oxygen species can provide atheroprotection via NOX4-dependent inhibition of inflammation and vascular remodeling. *Arterioscler Thromb Vasc Biol*. 2016;36:295–307. doi: 10.1161/ATVBAHA.115.307012.
20. Godo S, Sawada A, Saito H, Ikeda S, Enkhjargal B, Suzuki K, Tanaka S, Shimokawa H. Disruption of physiological balance between nitric oxide and endothelium-dependent hyperpolarization impairs cardiovascular homeostasis in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:97–107. doi: 10.1161/ATVBAHA.115.306499.
21. Gutterman DD, Chabowski DS, Kadlec AO, Durand MJ, Freed JK, Ait-Aissa K, Beyer AM. The human microcirculation: regulation of flow and beyond. *Circ Res*. 2016;118:157–172. doi: 10.1161/CIRCRESAHA.115.305364.
22. Freed JK, Beyer AM, LoGiudice JA, Hockenberry JC, Gutterman DD. Ceramide changes the mediator of flow-induced vasodilation from nitric oxide to hydrogen peroxide in the human microcirculation. *Circ Res*. 2014;115:525–532. doi: 10.1161/CIRCRESAHA.115.303881.
23. Durand MJ, Zinkevich NS, Riedel M, Gutterman DD, Nasci VL, Salato VK, Hijjawi JB, Reuben CF, North PE, Beyer AM. Vascular actions of angiotensin 1-7 in the human microcirculation: novel role for telomerase. *Arterioscler Thromb Vasc Biol*. 2016;36:1254–1262. doi: 10.1161/ATVBAHA.116.307518.
24. Holmström KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat Rev Mol Cell Biol*. 2014;15:411–421. doi: 10.1038/nrm3801.
25. Kirsch J, Schneider H, Pagel JI, et al. Endothelial dysfunction, and a prothrombotic, proinflammatory phenotype is caused by loss of mitochondrial thioredoxin reductase in endothelium. *Arterioscler Thromb Vasc Biol*. 2016;36:1891–1899. doi: 10.1161/ATVBAHA.116.307843.
26. Kadlec AO, Chabowski DS, Ait-Aissa K, Gutterman DD. Role of PGC-1α in vascular regulation: implications for atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2016;36:1467–1474. doi: 10.1161/ATVBAHA.116.307123.
27. Nowak WN, Deng J, Ruan XZ, Xu Q. Reactive oxygen species generation and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2017;37:e41–e52. doi: 10.1161/ATVBAHA.117.309228.
28. Brown NK, Zhou Z, Zhang J, Zeng R, Wu J, Eitzman DT, Chen YE, Chang L. Perivascular adipose tissue in vascular function and disease: a review of current research and animal models. *Arterioscler Thromb Vasc Biol*. 2014;34:1621–1630. doi: 10.1161/ATVBAHA.114.303029.
29. Friederich-Persson M, Nguyen Dinh Cat A, Persson P, Montezano AC, Touyz RM. Brown adipose tissue regulates small artery function through NADPH oxidase 4-derived hydrogen peroxide and redox-sensitive protein kinase G-1α. *Arterioscler Thromb Vasc Biol*. 2017;37:455–465. doi: 10.1161/ATVBAHA.116.308659.
30. Burgoyne JR, Madhani M, Cuello F, Charles RL, Brennan JP, Schröder E, Browning DD, Eaton P. Cysteine redox sensor in PKGIa enables oxidant-induced activation. *Science*. 2007;317:1393–1397. doi: 10.1126/science.1144318.
31. Prysyazhna O, Rudyk O, Eaton P. Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension. *Nat Med*. 2012;18:286–290. doi: 10.1038/nm.2603.
32. Noblet JN, Owen MK, Goodwill AG, Sassoon DJ, Tune JD. Lean and obese coronary perivascular adipose tissue impairs vasodilation via differential inhibition of vascular smooth muscle K+ channels. *Arterioscler Thromb Vasc Biol*. 2015;35:1393–1400. doi: 10.1161/ATVBAHA.115.305500.
33. Dou H, Feher A, Davila AC, Romero MJ, Patel VS, Kamath VM, Gooz MB, Rudic RD, Lucas R, Fulton DJ, Weintraub NL, Bagi Z. Role of adipose tissue endothelial ADAM17 in age-related coronary microvascular dysfunction. *Arterioscler Thromb Vasc Biol*. 2017;37:1180–1193. doi: 10.1161/ATVBAHA.117.309430.
34. Candela J, Wang R, White C. Microvascular endothelial dysfunction in obesity is driven by macrophage-dependent hydrogen sulfide depletion. *Arterioscler Thromb Vasc Biol*. 2017;37:889–899. doi: 10.1161/ATVBAHA.117.309138.
35. Xia N, Horke S, Habermeier A, Closs EI, Reifenberg G, Gericke A, Mikhed Y, Münzel T, Daiber A, Förstermann U, Li H. Uncoupling of endothelial nitric oxide synthase in perivascular adipose tissue of diet-induced obese mice. *Arterioscler Thromb Vasc Biol*. 2016;36:78–85. doi: 10.1161/ATVBAHA.115.306263.
36. Bussey CE, Withers SB, Aldous RG, Edwards G, Heagerty AM. Obesity-related perivascular adipose tissue damage is reversed by sustained weight loss in the rat. *Arterioscler Thromb Vasc Biol*. 2016;36:1377–1385. doi: 10.1161/ATVBAHA.116.307210.
37. Enkhjargal B, Godo S, Sawada A, Suvd N, Saito H, Noda K, Satoh K, Shimokawa H. Endothelial AMP-activated protein kinase regulates blood pressure and coronary flow responses through hyperpolarization mechanism in mice. *Arterioscler Thromb Vasc Biol*. 2014;34:1505–1513. doi: 10.1161/ATVBAHA.114.303735.
38. Cheang WS, Tian XY, Wong WT, Lau CW, Lee SS, Chen ZY, Yao X, Wang N, Huang Y. Metformin protects endothelial function in diet-induced obese mice by inhibition of endoplasmic reticulum stress through 5' adenosine monophosphate-activated protein kinase-peroxisome proliferator-activated receptor δ pathway. *Arterioscler Thromb Vasc Biol*. 2014;34:830–836. doi: 10.1161/ATVBAHA.113.301938.
39. Omura J, Satoh K, Kikuchi N, et al. Protective roles of endothelial amp-activated protein kinase against hypoxia-induced pulmonary hypertension in mice. *Circ Res*. 2016;119:197–209. doi: 10.1161/CIRCRESAHA.115.308178.
40. Bai B, Vanhoutte PM, Wang Y. Loss-of-SIRT1 function during vascular ageing: hyperphosphorylation mediated by cyclin-dependent kinase 5. *Trends Cardiovasc Med*. 2014;24:81–84. doi: 10.1016/j.tcm.2013.07.001.
41. Shentu TP, He M, Sun X, Zhang J, Zhang F, Gongol B, Marin TL, Zhang J, Wen L, Wang Y, Geary GG, Zhu Y, Johnson DA, Shyy JY. AMP-activated protein kinase and sirtuin 1 coregulation of cortactin contributes to endothelial function. *Arterioscler Thromb Vasc Biol*. 2016;36:2358–2368. doi: 10.1161/ATVBAHA.116.307871.
42. Belvitch P, Rizzo AN, Dudek SM. Cortactin in atherosclerosis: just say NO. *Arterioscler Thromb Vasc Biol*. 2016;36:2278–2280. doi: 10.1161/ATVBAHA.116.308497.
43. Li Q, Kim YR, Vikram A, Kumar S, Kassan M, Gabani M, Lee SK, Jacobs JS, Irani K. P66Shc-induced MicroRNA-34a causes diabetic endothelial dysfunction by downregulating sirtuin1. *Arterioscler Thromb Vasc Biol*. 2016;36:2394–2403. doi: 10.1161/ATVBAHA.116.308321.
44. Yin Y, Li X, Sha X, et al. Early hyperlipidemia promotes endothelial activation via a caspase-1-sirtuin 1 pathway. *Arterioscler Thromb Vasc Biol*. 2015;35:804–816. doi: 10.1161/ATVBAHA.115.305282.
45. Leung SW, Vanhoutte PM. Endothelium-dependent hyperpolarization: age, gender and blood pressure, do they matter? *Acta Physiol (Oxf)*. 2017;219:108–123. doi: 10.1111/apha.12628.
46. Stott JB, Barrese V, Greenwood IA. Kv7 channel activation underpins EPAC-dependent relaxations of rat arteries. *Arterioscler Thromb Vasc Biol*. 2016;36:2404–2411. doi: 10.1161/ATVBAHA.116.308517.
47. Xu R, Li C, Wu Y, Shen L, Ma J, Qian J, Ge J. Role of KCa3.1 channels in macrophage polarization and its relevance in atherosclerotic plaque instability. *Arterioscler Thromb Vasc Biol*. 2017;37:226–236. doi: 10.1161/ATVBAHA.116.308461.
48. Köhler R, Ruth P. Endothelial dysfunction and blood pressure alterations in K+-channel transgenic mice. *Pflugers Arch*. 2010;459:969–976. doi: 10.1007/s00424-010-0819-z.
49. Wulff H, Köhler R. Endothelial small-conductance and intermediate-conductance KCa channels: an update on their pharmacology and usefulness as cardiovascular targets. *J Cardiovasc Pharmacol*. 2013;61:102–112. doi: 10.1097/FJC.0b013e318279ba20.
50. Hu W, Zhang Y, Wang L, Lau CW, Xu J, Luo JY, Gou L, Yao X, Chen ZY, Ma RC, Tian XY, Huang Y. Bone morphogenic protein 4-smad-induced upregulation of platelet-derived growth factor AA impairs endothelial function. *Arterioscler Thromb Vasc Biol*. 2016;36:553–560. doi: 10.1161/ATVBAHA.115.306302.
51. Zhang Y, Liu J, Tian XY, Wong WT, Chen Y, Wang L, Luo J, Cheang WS, Lau CW, Kwan KM, Wang N, Yao X, Huang Y. Inhibition of bone morphogenic protein 4 restores endothelial function in db/db diabetic mice. *Arterioscler Thromb Vasc Biol*. 2014;34:152–159. doi: 10.1161/ATVBAHA.113.302696.
52. Chen X, Qian S, Hoggatt A, Tang H, Hacker TA, Obukhov AG, Herring PB, Seye CI. Endothelial cell-specific deletion of P2Y2 receptor promotes plaque stability in atherosclerosis-susceptible ApoE-null mice. *Arterioscler Thromb Vasc Biol*. 2017;37:75–83. doi: 10.1161/ATVBAHA.116.308561.
53. Paneni F, Diaz Cañestro C, Libby P, Lüscher TF, Camici GG. The aging cardiovascular system: understanding it at the cellular and clinical levels. *J Am Coll Cardiol*. 2017;69:1952–1967. doi: 10.1016/j.jacc.2017.01.064.
54. Honda A, Tahara N, Nitta Y, et al. Vascular inflammation evaluated by [18F]-fluorodeoxyglucose-positron emission tomography/computed tomography is associated with endothelial dysfunction.

Arterioscler Thromb Vasc Biol. 2016;36:1980–1988. doi: 10.1161/ATVBAHA.116.307293.
55. Hertle E, Arts IC, van der Kallen CJ, Feskens EJ, Schalkwijk CG, Hoffmann-Petersen IT, Thiel S, Stehouwer CD, van Greevenbroek MM. Distinct longitudinal associations of MBL, MASP-1, MASP-2, MASP-3, and MAp44 with endothelial dysfunction and intima-media thickness: the cohort on diabetes and atherosclerosis maastricht (CODAM) study. *Arterioscler Thromb Vasc Biol*. 2016;36:1278–1285. doi: 10.1161/ATVBAHA.115.306552.
56. Herder C, de Las Heras Gala T, Carstensen-Kirberg M, et al. Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of six population-based cohorts. *Arterioscler Thromb Vasc Biol*. 2017;37:1222–1227. doi: 10.1161/ATVBAHA.117.309307.
57. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the canakinumab anti-inflammatory thrombosis outcomes study (CANTOS). *Am Heart J*. 2011;162:597–605. doi: 10.1016/j.ahj.2011.06.012.
58. Choi BJ, Matsuo Y, Aoki T, Kwon TG, Prasad A, Gulati R, Lennon RJ, Lerman LO, Lerman A. Coronary endothelial dysfunction is associated with inflammation and vasa vasorum proliferation in patients with early atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2014;34:2473–2477. doi: 10.1161/ATVBAHA.114.304445.
59. Loader J, Meziat C, Watts R, Lorenzen C, Sigaudo-Roussel D, Stewart S, Reboul C, Meyer G, Walther G. Effects of sugar-sweetened beverage consumption on microvascular and macrovascular function in a healthy population. *Arterioscler Thromb Vasc Biol*. 2017;37:1250–1260. doi: 10.1161/ATVBAHA.116.308010.
60. Loader J, Montero D, Lorenzen C, Watts R, Méziat C, Reboul C, Stewart S, Walther G. Acute hyperglycemia impairs vascular function in healthy and cardiometabolic diseased subjects: systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol*. 2015;35:2060–2072. doi: 10.1161/ATVBAHA.115.305530.
61. Bretón-Romero R, Feng B, Holbrook M, Farb MG, Fetterman JL, Linder EA, Berk BD, Masaki N, Weisbrod RM, Inagaki E, Gokce N, Fuster JJ, Walsh K, Hamburg NM. Endothelial dysfunction in human diabetes is mediated by Wnt5a-JNK signaling. *Arterioscler Thromb Vasc Biol*. 2016;36:561–569. doi: 10.1161/ATVBAHA.115.306578.
62. Walther G, Obert P, Dutheil F, Chapier R, Lesourd B, Naughton G, Courteix D, Vinet A. Metabolic syndrome individuals with and without type 2 diabetes mellitus present generalized vascular dysfunction: cross-sectional study. *Arterioscler Thromb Vasc Biol*. 2015;35:1022–1029. doi: 10.1161/ATVBAHA.114.304591.
63. Xue C, Sowden M, Berk BC. Extracellular cyclophilin A, especially acetylated, causes pulmonary hypertension by stimulating endothelial apoptosis, redox stress, and inflammation. *Arterioscler Thromb Vasc Biol*. 2017;37:1138–1146. doi: 10.1161/ATVBAHA.117.309212.
64. Meloche J, Lampron MC, Nadeau V, Maltais M, Potus F, Lambert C, Tremblay E, Vitry G, Breuils-Bonnet S, Boucherat O, Charbonneau E, Provencher S, Paulin R, Bonnet S. Implication of inflammation and epigenetic readers in coronary artery remodeling in patients with pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol*. 2017;37. DOI: 10.1161/ATVBAHA.117.309156.
65. Johns RA, Takimoto E, Meuchel LW, Elsaigh E, Zhang A, Heller NM, Semenza GL, Yamaji-Kegan K. Hypoxia-inducible factor 1α is a critical downstream mediator for hypoxia-induced mitogenic factor (FIZZ1/RELMα)-induced pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2016;36:134–144. doi: 10.1161/ATVBAHA.115.306710.
66. Feletou M. The Endothelium: Part 1: Multiple Functions of the Endothelial Cells-Focus on Endothelium-Derived Vasoactive Mediators. San Rafael, CA: Morgan & Claypool Life Sciences Publishers; 2011.
67. Yaoita N, Shirakawa R, Fukumoto Y, Sugimura K, Miyata S, Miura Y, Nochioka K, Miura M, Tatebe S, Aoki T, Yamamoto S, Satoh K, Kimura T, Shimokawa H, Horiuchi H. Platelets are highly activated in patients of chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2014;34:2486–2494. doi: 10.1161/ATVBAHA.114.304404.
68. Yaoita N, Satoh K, Satoh T, Sugimura K, Tatebe S, Yamamoto S, Aoki T, Miura M, Miyata S, Kawamura T, Horiuchi H, Fukumoto Y, Shimokawa H. Thrombin-activatable fibrinolysis inhibitor in chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2016;36:1293–1301. doi: 10.1161/ATVBAHA.115.306845.
69. Choi S, Kim JA, Na HY, Cho SE, Park S, Jung SC, Suh SH. Globotriaosylceramide induces lysosomal degradation of endothelial KCa3.1 in fabry disease. *Arterioscler Thromb Vasc Biol*. 2014;34:81–89. doi: 10.1161/ATVBAHA.113.302200.
70. Takase S, Matoba T, Nakashiro S, et al. Ezetimibe in combination with statins ameliorates endothelial dysfunction in coronary arteries after stenting: the CuVIC trial (effect of cholesterol absorption inhibitor usage on target vessel dysfunction after coronary stenting), a multicenter randomized controlled trial. *Arterioscler Thromb Vasc Biol*. 2017;37:350–358. doi: 10.1161/ATVBAHA.116.308388.
71. Cannon CP, Blazing MA, Giugliano RP, et al; IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med*. 2015;372:2387–2397. doi: 10.1056/NEJMoa1410489.
72. Lin X, Racette SB, Ma L, Wallendorf M, Ostlund RE Jr. Ezetimibe increases endogenous cholesterol excretion in humans. *Arterioscler Thromb Vasc Biol*. 2017;37:990–996. doi: 10.1161/ATVBAHA.117.309119.
73. Denimal D, Monier S, Brindisi MC, Petit JM, Bouillet B, Nguyen A, Demizieux L, Simoneau I, Pais de Barros JP, Vergès B, Duvillard L. Impairment of the ability of HDL from patients with metabolic syndrome but without diabetes mellitus to activate eNOS: correction by S1P enrichment. *Arterioscler Thromb Vasc Biol*. 2017;37:804–811. doi: 10.1161/ATVBAHA.117.309287.
74. Smits MM, Tonneijck L, Muskiet MH, Hoekstra T, Kramer MH, Diamant M, Serné EH, van Raalte DH. GLP-1-based therapies have no microvascular effects in type 2 diabetes mellitus: an acute and 12-week randomized, double-blind, placebo-controlled trial. *Arterioscler Thromb Vasc Biol*. 2016;36:2125–2132. doi: 10.1161/ATVBAHA.116.307930.
75. Bretón-Romero R, Wang N, Palmisano J, Larson MG, Vasan RS, Mitchell GF, Benjamin EJ, Vita JA, Hamburg NM. Cross-sectional associations of flow reversal, vascular function, and arterial stiffness in the Framingham heart study. *Arterioscler Thromb Vasc Biol*. 2016;36:2452–2459. doi: 10.1161/ATVBAHA.116.307948.
76. Long MT, Wang N, Larson MG, Mitchell GF, Palmisano J, Vasan RS, Hoffmann U, Speliotes EK, Vita JA, Benjamin EJ, Fox CS, Hamburg NM. Nonalcoholic fatty liver disease and vascular function: cross-sectional analysis in the Framingham heart study. *Arterioscler Thromb Vasc Biol*. 2015;35:1284–1291. doi: 10.1161/ATVBAHA.114.305200.
77. Dimitrievska S, Gui L, Weyers A, Lin T, Cai C, Wu W, Tuggle CT, Sundaram S, Balestrini JL, Slattery D, Tchouta L, Kyriakides TR, Tarbell JM, Linhardt RJ, Niklason LE. New functional tools for antithrombogenic activity assessment of live surface glycocalyx. *Arterioscler Thromb Vasc Biol*. 2016;36:1847–1853. doi: 10.1161/ATVBAHA.116.308023.
78. Hata J, Mukai N, Nagata M, Ohara T, Yoshida D, Kishimoto H, Shibata M, Hirakawa Y, Endo M, Ago T, Kitazono T, Oike Y, Kiyohara Y, Ninomiya T. Serum angiopoietin-like protein 2 is a novel risk factor for cardiovascular disease in the community: the Hisayama study. *Arterioscler Thromb Vasc Biol*. 2016;36:1686–1691. doi: 10.1161/ATVBAHA.116.307291.
79. Saita E, Miura K, Suzuki-Sugihara N, Miyata K, Ikemura N, Ohmori R, Ikegami Y, Kishimoto Y, Kondo K, Momiyama Y. Plasma soluble endoglin levels are inversely associated with the severity of coronary atherosclerosis-brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:49–52. doi: 10.1161/ATVBAHA.116.308494.
80. Blázquez-Medela AM, García-Ortiz L, Gómez-Marcos MA, Recio-Rodríguez JI, Sánchez-Rodríguez A, López-Novoa JM, Martínez-Salgado C. Increased plasma soluble endoglin levels as an indicator of cardiovascular alterations in hypertensive and diabetic patients. *BMC Med*. 2010;8:86. doi: 10.1186/1741-7015-8-86.
81. Pasterkamp G, Goumans MJ. The microvasculature: the next battlefield where transforming growth factor-β and endoglin draw their double-edged swords? *Arterioscler Thromb Vasc Biol*. 2017;37:10–12. doi: 10.1161/ATVBAHA.116.308610.
82. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ*. 2002;325:1202.
83. Qin X, Li Y, Sun N, et al. Elevated homocysteine concentrations decrease the antihypertensive effect of angiotensin-converting enzyme inhibitors in hypertensive patients. *Arterioscler Thromb Vasc Biol*. 2017;37:166–172. doi: 10.1161/ATVBAHA.116.308515.
84. Zhang D, Sun X, Liu J, Xie X, Cui W, Zhu Y. Homocysteine accelerates senescence of endothelial cells via DNA hypomethylation of human telomerase reverse transcriptase. *Arterioscler Thromb Vasc Biol*. 2015;35:71–78. doi: 10.1161/ATVBAHA.114.303899.
85. Ohtsuki T, Satoh K, Omura J, Kikuchi N, Satoh T, Kurosawa R, Nogi M, Sunamura S, Yaoita N, Aoki T, Tatebe S, Sugimura K, Takahashi J, Miyata S, Shimokawa H. prognostic impacts of plasma levels of cyclophilin A in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2017;37:685–693. doi: 10.1161/ATVBAHA.116.308986.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Endothelial Functions

Shigeo Godo and Hiroaki Shimokawa

Arterioscler Thromb Vasc Biol. 2017;37:e108-e114
doi: 10.1161/ATVBAHA.117.309813
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636