

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 Corti¹⁰ 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 al¹² 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 Berk¹¹ 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 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan. E-mail shimo@cardio.med.tohoku.ac.jp

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with resultant endothelial dysfunction and subsequent development of atherosclerotic lesions. In addition, Warboys et al¹³ 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 al¹⁷ demonstrated that adipocyte NADPH oxidase 4-derived ROS contributed to the development of obesity-related insulin resistance by triggering adipocyte inflammation. La Favor et al¹⁸ 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 al¹⁹ 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 thio-redoxin reductase 2 has been shown to play a key role in the maintenance of healthy endothelial functions,²⁵ and peroxisome proliferator receptor- γ coactivator 1 α 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 al²⁷ 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 al²⁹ 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 PKG1 α (cyclic GMP-dependent protein kinase G1 α) 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 PKG1 α activation is a shared vasodilating mechanism of H_2O_2 as an EDH factor in resistance arteries as well.^{30,31} Moreover, Noblet et al³² have added another layer of complexity of PVAT-mediated responses. They showed that lean coronary PVAT inhibited K_{Ca} and K_{v7} channel-mediated vasodilations, 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 al³³ 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 $\alpha 1$ -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 vasodilations 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 al³⁸ 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 antisenesence effects in endothelial cells.⁴⁰ Shentu et al⁴¹ 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-mediated 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 al⁴³ 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 vasodilation, especially in those of EDH-mediated vascular smooth muscle relaxation and vasodilation. Stott et al⁴⁶ 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 al⁴⁷ showed that blocking $K_{Ca3.1}$ 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 $K_{Ca3.1}$ 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

P2Y₂ Receptor

Chen et al⁵² demonstrated that endothelium-specific deletion of P2Y₂ receptor exerted protective effects on plaque stabilization by promoting fibrous cap formation in an atherosclerotic mouse model. Considering that the endothelial cell-specific P2Y₂ receptor-deficient mice showed decreased nucleotide-mediated but preserved acetylcholine-induced endothelium-dependent relaxation of the aorta without causing systemic hypertension, endothelial P2Y₂ 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-1 β (interleukin-1 β) is one of the key proinflammatory cytokines. Honda et al⁵⁴ demonstrated a close relationship between endothelial dysfunction evaluated by flow-mediated dilation and vascular inflammation detected by (¹⁸F)-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 al⁵⁵ 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-1 β 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 al⁶¹ showed that wingless-type family member 5a/c-jun N terminal kinase pathway contributed to endothelial dysfunction associated with diabetic patients. Walther et al⁶² 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 al⁶³ 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 al⁶⁴ 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 al⁶⁵ 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 al⁶⁹ 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 al⁷² 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 al⁷³ 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 al⁷⁴ 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 al⁷⁵ 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 al⁷⁷ developed for the first time simple assays that can evaluate glycocalyx function by measuring its antithrombotic 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 angiotensin-like protein 2—a proinflammatory mediator that promotes endothelial dysfunction—were positively correlated with the risk for future cardiovascular disease.⁷⁸ Saita et al⁷⁹ 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.⁸⁵ 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.

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