Review

Caveolae and caveolin-1 as targets of dietary polyphenols for protection against vascular endothelial dysfunction

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(Received 21 February, 2024; Accepted 4 March, 2024; Released online in J-STAGE as advance publication 7 March, 2024)

Caveolae, consisting of caveolin-1 proteins, are ubiquitously present in endothelial cells and contribute to normal cardiovascular functions by acting as a platform for cellular signaling pathways as well as transcytosis and endocytosis. However, caveolin-1 is thought to have a proatherogenic role by inhibiting endothelial nitric oxide synthase activity and Nrf2 activation, or by promoting inflammation through NF-κB activation. Dietary polyphenols were suggested to exert anti-atherosclerotic effects by a mechanism involving the inhibition of endothelial dysfunction, by which they can regulate redox-sensitive signaling pathways in relation to NF-κB and Nrf2 activation. Some monomeric polyphenols and microbiota-derived catabolites from monomeric polyphenols or polymeric tannins might be responsible for the inhibition, because they can be transferred into the circulation from the digestive tract. Several polyphenols were reported to modulate caveolin-1 expression or its localization in caveolae. Therefore, we hypothesized that circulating polyphenols affect caveolae functions by altering its structure leading to the release of caveolin-1 from caveolae, and attenuating redox-sensitive signaling pathway-dependent caveolin-1 overexpression. Further studies using circulating polyphenols at a physiologically relevant level are necessary to clarify the mechanism of action of dietary polyphenols targeting caveolae and caveolin-1.

Key Words: **polyphenol, endothelial dysfunction, caveolae, caveolin-1, atherosclerosis**

Vascular endothelial cells cover the surface of the vascular lumen to form the intima layer together with connective tissue. These flat squamous cells have multiple functions in the vascular system, including a barrier function between the blood and vessel wall. Atherosclerosis is a potentially serious condition with fatty streaks in the vascular artery leading to cardiovascular disease (CVD).[1–3] Endothelial dysfunction is the initial hallmark related to the induction of atherosclerosis. Endothelial cells receive incessant and various stimuli from the blood including wall shear stress, resulting in the activation of signaling pathways responsible for the expression of proinflammatory cytokines and monocyte adhesion molecules, and for the modulation of vasodilative nitric oxide (NO) production. Stimulated endothelial cells promote the infiltration of monocytes into the intima. Macrophages, the differentiated monocytes within the intima, induce endothelial dysfunction by releasing proinflammatory mediators such as interleukin (IL)-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α). In addition, morphological changes of endothelial cells related to wall shear stress promote the subendothelial infiltration of plasma low-density lipoprotein (LDL) via endothelial transcytosis. Infiltrating LDL particles are subject to oxidative modification leading to the formation of oxidized LDL (oxLDL). Vascular endothelial cells can incorporate oxLDL via lectin-like oxidized LDL receptor-1 (LOX-1). This event accelerates endothelial dysfunction by inducing the production of proinflammatory cytokines. The continual repetition of this process gradually leads to the development of atheroma and finally atherosclerosis. Therefore, endothelial dysfunction has a key role in the progression of atherosclerosis.[4] Distinct plasma membrane microdomains called caveolae are universally present in vascular endothelial cells. Caveolae and caveolin proteins present in caveolae function as a platform for endothelial signaling pathways and endocytosis/transcytosis for xenobiotics as well as nutrients.[5,6] Rodent studies using *ApoE*$^{-/-}$ mice indicated that caveolin-1 (Cav-1) deficiency was protective against the development of aortic atheroma.[7,8] *ApoE*$^{-/-}$ mice with the endothelium-specific overexpression of Cav-1 demonstrated the contribution of this protein to the progression of atherosclerosis.[9]

A wide variety of mechanisms of action, including the prevention of endothelial dysfunction, have been proposed as anti-atherosclerotic effects of dietary polyphenols.[3,10–12] Endothelial cells in the arterial wall are critical targets for the anti-atherosclerotic effects of polyphenols because they make first contact with the surface of endothelial cells in vascular arteries.[13,14] Therefore, the relationship between polyphenols and caveolae/caveolin proteins in endothelial cells has attracted much attention regarding their anti-atherosclerotic effects.[15,16] This review article focuses on recent studies on the behavior of dietary polyphenols in endothelial cells and discusses the importance of caveolae and Cav-1 as potential targets for polyphenols in the prevention and therapy of atherosclerosis.

Characteristics of Caveolae and Cav-1 in Vascular Endothelial Cells

Background of caveolae in cell membranes. In the 1950s, heterogeneous cell membranes containing a flask-shape 50–100 nm invagination were detected by electron microscopy.[17] These unique, partially invaginated vesicles are particularly rich in vascular endothelial cells and were termed caveolae by Yamada in 1955.[18] Caveolin was first discovered as a constitute protein of caveolae in 1992.[19] Thereafter, caveolin was found to possess cholesterol-binding properties,[20] which serve a specific function in caveolae. Furthermore, lipid membranes, which are part of caveolae structures, are rich in sphingolipids and cholesterol. In 1997, Simons and Ikonen[21] proposed this characteristic microdomain structure was a "lipid raft" in cell membranes. Gly-

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doi: 10.3164/jcbn.24-30

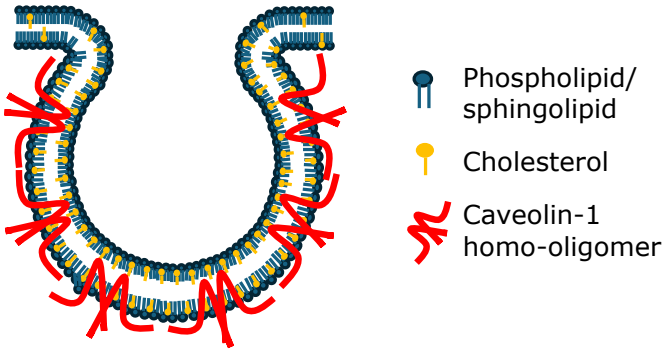

Fig. 1. Diagram of caveolae in endothelial cell membranes. Caveolin-1 (Cav-1) monomers assemble into discrete homo-oligomers of 14–16 molecules.[24] Binding of Cav-1 to cholesterol in lipid bilayers promotes this oligomerization.[20]

cosphingolipids and phosphatidylcholines with saturated acyl groups are the major lipid species in the outer membrane and the content of cholesterol is relatively high in the outer and inner membranes. This heterogeneous distribution in lipid bilayers yields specific liquid-ordered phases in cell membranes. Lipid rafts were suggested to function as a platform for the binding of receptors and ligands, and to allow access to signaling molecules. Lipid rafts without caveolin proteins, which are distinct from caveolae, are thought to be a functional platform in cell membranes, although the existence of such microdomains remains controversial. Lipid rafts without caveolin proteins were recently claimed to be unstable and formed by the on-demand binding of receptors with ligands in cell membranes.[22] In contrast, caveolae are regarded as a specific form of lipid rafts characterized by an invagination at the surface of cell membranes and the presence of caveolin proteins (Fig. 1).[23,24] Thus, caveolae and lipid rafts are two different concepts for the microdomains in cell membranes. Caveolin proteins in caveolae interact directly with signaling molecules through their scaffolding site. Thus, caveolae allow cell membranes to regulate signaling cascades.[25]

Role of caveolae and Cav-1 in vascular endothelial cells. Transcytosis, the transport of albumin, insulin, LDL and other biochemical substances from the vascular luminal site to the subendothelial space, was long assumed to be the sole function of caveolae in vascular endothelial cells.[6,26] However, the discovery of the caveolin protein expanded the role of caveolae in the regulation of a variety of endothelial functions. The mammalian caveolin gene family consists of Cav-1, caveolin-2, and caveolin-3. Cav-1 and Cav-2 are ubiquitous in many types of cells, whereas Cav-3 is exclusively present in muscle-specific cardiac and skeletal muscles, and smooth muscle cells.[24] The levels of Cav-1 proteins and cholesterol are determinants for the caveolae number in cell membranes.[27] Cav-1, a membrane-constituting protein with a molecular weight of 22–24 kDa, possesses a transmembrane hairpin loop, a juxtamembrane domain responsible for its oligomerization, and a regulatory amino terminal tail that contains a phosphorylatable site. The juxtamembrane domain acts as a scaffold protein for signaling molecules.[28] Phosphorylation of Trp^{14}-Cav-1 regulates the oligomerization of Cav-1 and induces the formation of caveolae and its internalization.[28] Cav-1 was recently found to be involved in the liquid-ordered phase in caveolae by acting as a spur to lipid bilayers.[29] A number of molecules that interact with Cav-1 have been discovered including G protein-coupled receptors, nonreceptor tyrosine kinases, and structural proteins.[30,31] In cultured endothelial cells, Cav-1 acts as a major scaffolding protein that helps to sequestrate signaling molecules such as TNF-α receptors and TNF-α-converting enzymes into caveolae.[5] Caveolae also function as mechanoreceptors for endothelial responses toward hemodynamic forces such as wall shear stress, together with transport across the endothelial barrier.[7]

At present, caveolae are recognized as essential sites for the initiation of cellular signaling pathways and as carriers of biological components by transcytosis/endocytosis. Therefore, caveolae and Cav-1 in cell membranes are required for the proper organization of functional proteins within vascular endothelial cells.[32]

Participation of Caveolae and Cav-1 in Endothelial Dysfunction. Figure 2 summarizes the potential involvement of caveolae and Cav-1 in endothelial dysfunction leading to atherosclerosis.

Uptake of plasma LDL and oxLDL into endothelial cells. Circulating LDLs are taken up by endothelial cells by receptor-mediated endocytosis or transcytosis.[33] In endocytosis, the endocytic LDL receptor (LDLR) binds and transports LDLs to the intracellular lysosomal system, where LDL components are degraded to metabolic products. The upregulation of Cav-1 expression was found to increase the number of internalized LDLs.[28] An overload of LDLs within endothelial cells disturbs endothelial functions leading to the progression of atherosclerosis.

The transcytosis of LDLs is mediated by caveolae in cell membranes, similar to endocytosis.[34] Plasma LDL traverses cells without passing the lysosomal system and reaches the opposite barrier in its intact form.[33] Thus, transcytotic receptors, including activin receptor-like kinase-1 (ALK-1) and scavenger receptor class B type 1 (SRB1), located in caveolae, directly bind to LDL for cellular transportation.[35–38] *Cav-1*$^{-/-}$ mice had a reduced efficacy of LDL transcytosis resulting in the prevention of atherosclerosis.[39] The content of Cav-1 in caveolae is positively related to the rate of LDL transcytosis, and the upregulation of Cav-1 expression corresponds to the elevation of LDL transcytosis.[35] Overall, Cav-1 in caveolae, which modulate the assembly of ALK-1 and SR-B1 into cell membranes, functions as an atherosclerosis-promoting factor by affecting the uptake of plasma LDL into the intima.[40]

The molecular mechanism by which oxLDL causes endothelial dysfunction has not been fully elucidated. However, the oxLDL-dependent perturbation of cholesterol homeostasis affects caveolae functions leading to the modulation of atherosclerosis-relating signaling pathways.[33] oxLDLs are present within arterial walls as well as circulating blood, particularly in CVD patients.[41] Circulating oxLDL is bound to LOX-1 present in caveolae and then incorporated into endothelial cells.[42] oxLDL influences the localization of Cav-1 and its related signaling molecules in caveolae by affecting the content and distribution of cholesterol in cell membranes.[33] In oxLDL-dependent endothelial dysfunction, caveolae are the target for changes in the composition and dynamics of membrane lipid bilayers. Cav-1 expression was upregulated in endothelial cells treated with oxLDL by a time-dependent mechanism.[43] The downregulation of Cav-1 decreased the uptake of oxLDL into vascular endothelial cells.[44] These findings suggest that Cav-1 regulates the uptake of oxLDL into endothelial cells by contributing to the activity or expression of LOX-1.

Shear stress-dependent regulation of eNOS activity and activation of transcription factors. Endothelial nitric oxide synthase (eNOS) was the first protein found to be associated with caveolae.[45] Although the presence of caveolae is required for maximal eNOS activity, Cav-1 inhibited the eNOS activity by directly interacting with eNOS proteins in caveolae.[46,47] Two cytoplasmic domains, the scaffolding domain (amino acids 61–101) and to a lesser extent the C-terminal tail (amino acids 135–178), are responsible for its interaction with eNOS.[48] This interaction negatively regulates NO production to Cav-1 in endothelial cells.

Mechanical forces derived from wall shear stress, which origi-

doi: 10.3164/jcbn.24-30

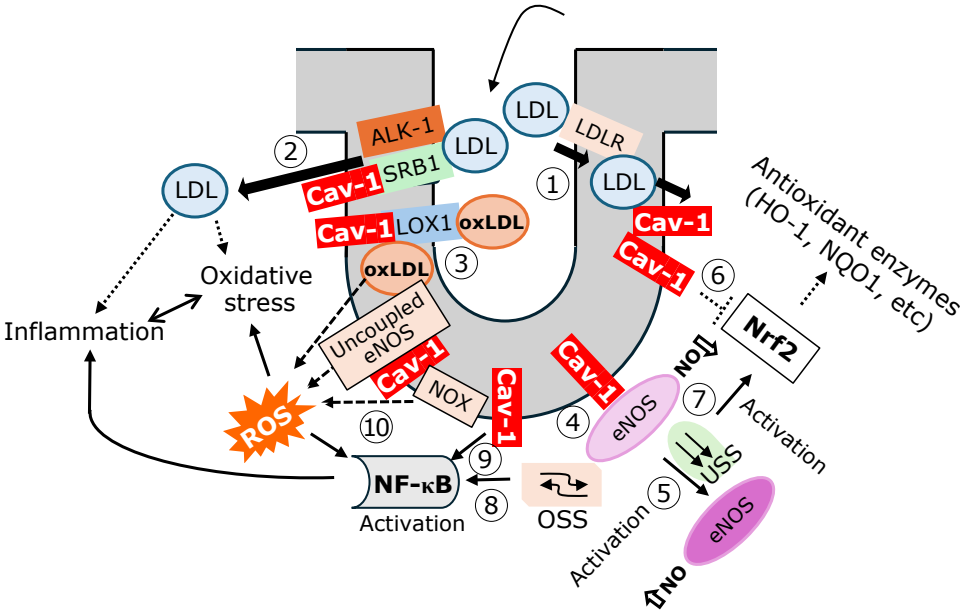

Fig. 2. Potential involvement of caveolae and Cav-1 in endothelial dysfunction leading to atherosclerosis. LDL, low-density lipoprotein; oxLDL, oxidized LDL; LDLR, LDL receptor; SRB1, scavenger receptor class B type 1; ALK-1, activin receptor-like kinase-1; LOX-1, lectin-like oxidized LDL receptor-1; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; NOX, NADPH oxidase; USS, unidirectional shear stress; OSS, oscillatory shear stress; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor-E2-related factor 2; ROS, reactive oxygen species; HO-1, heme oxygenase-1; NQO1, NAD(P)H quinone oxidoreductase-1. ①Plasma LDLs are subject to continual endocytosis in caveolae by binding to LDLR. Endocytic LDLs are overloaded in endothelial cells, which disturbs normal endothelial functions.[28] ②Plasma LDLs are subject to transcytosis by binding to ALK-1 or SRB1. LDL accumulates in the intima, inducing proinflammatory effects on endothelial cells. ③oxLDLs are subject to endocytosis in caveolae by binding to LOX-1. Endocytic oxLDL induces oxidative stress by generating ROS in endothelial cells. ④Cav-1 inhibits eNOS activity by directly interacting with eNOS proteins in caveolae.[46,47] ⑤USS stimulates caveolae to promote the dissociation of eNOS from Cav-1, resulting in the release of NO.[53] ⑥Nrf2 interacts with Cav-1 to suppress Nrf2 activation.[54] ⑦USS stimulates caveolae to activate Nrf2, which promotes the gene expression of antioxidant enzymes such as HO-1 and NQO1.[48] ⑧OSS stimulates caveolae to induce NF-κB activation resulting in the promotion of inflammation-inducing signaling pathways.[52] ⑨Cav-1 accelerates NF-κB activation by modulating upstream signaling pathways.[55] ⑩NOX and uncoupled eNOS in caveolae produce ROS, which disturb the homeostatic redox balance inducing oxidative stress.[56,57]

nates from the blood flow, have pathophysiological functions in vascular endothelial cells. Wall shear stress is highly dependent on the vessel diameter and location in the vasculature. Hemodynamics derived from wall shear stress can convert mechanical forces to biochemical signals through mechanosensors by interacting with vascular vessels.[49,50] Caveolae transform with the bend or stretch of vessels by mechanical forces resulting in modulation of the membranous scaffolding and activation of downstream effectors.[51] It is therefore evident that caveolae have an essential role in the process of mechano-sensing, responses to mechanical signals, and mechano-transduction, the conversion of mechanical signals to biochemical signals, leading to the activation of downstream signaling pathways.

Endothelial cells respond differently to unidirectional shear stress (USS) versus oscillatory shear stress (OSS).[52] When endothelial cells are exposed to USS, NO is immediately released by the dissociation of eNOS from Cav-1 related to stretching of the vascular wall.[53] It is therefore likely that Cav-1 regulates the blood pressure by controlling eNOS activity in response to the type and degree of shear stress. Interestingly, Cav-1 was reported to inhibit the expressions of antioxidant enzymes through direct interactions with a transcription factor, nuclear factor-E2-related factor 2 (Nrf2) in lung epithelial cells.[54] In endothelial cells, USS induces the activation of Nrf2, which promotes the gene expression of antioxidant enzymes such as heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase-1 (NQO1).[52] In contrast, OSS induces pathological effects such as activation of the inflammatory gene regulator, nuclear factor kappa B (NF-κB).[52] Thus, vascular inflammation and disease progression including atherosclerosis are partly a consequence of mechanical force generated by rheological changes in blood flow.

Role of Cav-1 in signaling pathways and endothelial dysfunction. Cav-1 may have a regulatory effect on inflammation in vascular endothelial cells by modulating inflammation signaling pathways.[55] Compared with *ApoE*$^{-/-}$ mice, *Cav-1*$^{-/-}$ and *ApoE*$^{-/-}$ mice had decreased endothelial cell activation and vascular cell adhesion molecule-1 (VCAM-1) expression in the aorta.[7] Furthermore, defects in Cav-1 diminished atheroma injury in *ApoE*$^{-/-}$ mice, in association with a decrease in NF-κB-mediated inflammation. Therefore, Cav-1 has a proatherogenic role in the initial stage of atherosclerosis by enhancing inflammation in vascular endothelial cells.

Modification of reactive oxygen species (ROS)/redox signals in caveolae. The release of a variety of ROS, such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$), in caveolae has a critical role in the cell signaling cascade in cardiovascular systems.[56,57] In this system, caveolae accommodate two key cellular sources of ROS, NADPH oxidase (NOX) and "uncoupled" eNOS, which is the irregular form of the eNOS homodimer.[58] Of note, various proteins acting as receptors, ion-channels, and redox-signals accumulate close to these two ROS-generating proteins. In vascular endothelial cells, caveolae have an essential role in the maintenance of normal cell homeostasis by controlling the production of ROS. However, dysregulated ROS might react with various key proteins in cell signaling at the initial stage of atherosclerosis.

Senescent endothelial cells and Cav-1. In rodent studies, senescent endothelial cells were shown to accumulate in atherosclerotic lesions during aging and induce the infiltration of inflammatory cells via a senescence-associated secretory phenotype.[58] Cav-1 expression is upregulated by aging and the promo-

tion of oxidative stress.[28] Smoking has been associated with the development of atherosclerosis by inducing oxidative stress in the vascular system.[60] Endothelial cells isolated from chronic smokers with premature atherosclerosis display senescent features including the elevation of Cav-1 expression compared with endothelial cells isolated from nonsmokers.[61] Cellular senescence in endothelial cells isolated from patients with severe coronary artery disease was accelerated by oxidative stress-associated risk factors for CVD and Cav-1 expression was elevated in these cells.[62] Cav-1 is thought to be involved in oxidative stress-induced premature senescence, which causes CVD.[31]

Behavior of Dietary Polyphenols in the Vascular System

Classification of polyphenols and their bioavailability. Polyphenols are secondary metabolites ubiquitously present in the plant kingdom. For humans, polyphenols have long been recognized as preferred food ingredients. In recent years, their impact on health has attracted much attention in relation to their preventive effect on uncommunicable diseases including CVD.[63] More than 8,000 species of polyphenols are present in the plant kingdom, and they are broadly divided into polymeric tannins and monomeric polyphenols.[64] Polymeric tannins are categorized as hydrolysable tannins (ellagitannins and gallotannins) and non-hydrolysable condensed tannins (proanthocyanidins). Monomeric polyphenols consist of five subgroups: phenolic acid derivatives, flavonoids, lignans, stilbenes, and curcumins.

Figure 3 summarizes the behavior of polyphenol subclasses in the digestive tract. Only some monomeric polyphenols can be delivered to the blood via intestinal absorption by epithelial cells in the small intestine.[65] Chlorogenic acid, a typical phenolic acid derivative, is converted to caffeic acid, ferulic acid, and other phenolic acids, and then partly absorbed from the small intestine.[66] Flavonol-type flavonoids such as quercetin are mostly present as *O*-glycoside derivatives in plant-derived foods.[67] Their glucose-attached derivatives are partly absorbed from the small intestine after hydrolysis to aglycone.[68] These are then converted to conjugated metabolites by the action of phase II enzymes before circulation.[69] Therefore, *O*-glucosides are mostly present as their conjugated metabolites such as sulfates and glucuronides in the blood. Other monomeric polyphenols are partly absorbed from the small intestine and exist as their conjugated metabolites in the blood. Among flavan-3-ols, (−)-epicatechin gallate (ECg) and (−)-epigallocatechin gallate (EGCg), which possess a galloyl group, are present in the circulation in their intact forms without conjugation.[70] Residual monomeric polyphenols move to the large intestine in their intact forms and polymeric tannins reach the large intestine without absorption in the small intestine.[71] These polyphenols transferred into the large intestine are finally excreted with the feces. However, they are partly catabolized by the action of intestinal microbiota.[65] Flavonol glycosides can be subjected to de-glycosylation and ring-fission reactions resulting in their aglycones, various phenolic acid derivatives and other degradation products.[68] For example, 3,4-dihydroxyphenylacetic acid (3,4-DHPAA) is yielded from rutin (quercetin 3-*O*-rutinoside) as its major catabolite.[72] Equol is a unique catabolite of daidzin (daidzein 7-*O*-glucoside) and daidzein.[73] Protocatechuic acid (3,4-hydroxybenzoic acid: PCA) is the main product from an anthocyanin, cyanidin 3-*O*-glucoside.[74] Flavan-3-ols including EGCg and ECg yield γ-valerolactones as microbiota-derived catabolites.[75] Enterolactone and enterodiol were detected in human plasma as the catabolites of lignans.[76] Reductive products, such as tetrahydrocurcumin and 3,4'-dihydroresveratrol, are the main catabolites of curcumin and resveratrol, respectively.[77,78] Ellagic acid is thought to be converted to urolithin by intestinal microbiota after the hydrolysis of ellagitannins.[79] Gallotannins are hydrolyzed to monomeric gallic acid leading to the production of pyrogallol.[80] Intestinal microbiota are thought to convert procyanidins to ring fission products including γ-valerolactones through the cleavage of carbon-carbon covalent bonds.[81] Some of these catabolites have functions in blood vessels after their transfer to the circulation via absorption from the large intestine. For example, ring-fission products from anthocyanins and hydrolysis products from ellagitannins were shown to contribute to the anti-atherosclerotic effects of dietary polyphenols.[82,83] However, the absorption of these catabolites in the large intestine and their transfer into the circulation is still ambiguous. Transfer from the digestive tract to the blood is a critical point required for dietary polyphenols to exert their functions by targeting caveolae and Cav-1 in vascular endothelial cells. Of note, polyphenols present in the circulation are restricted to monomeric polyphenols or their conjugated metabolites as well as microbiota-derived catabolites of monomeric polyphenols and polymeric tannins.

Mechanism of action of polyphenols for the prevention of endothelial dysfunction. Polyphenols have direct antioxidant activity by scavenging ROS, which is related to the reducing property of the phenolic hydroxyl group in their structures. However, transient concentrations of polyphenols and their metabolites after oral intake are normally less than 4 μM in the blood,[84] which is much lower than plasma concentrations of the antioxidant vitamins, vitamin E and vitamin C.[65] Furthermore, monomeric polyphenols are largely present as their conjugated metabolites, whose antioxidant activity is diminished by the conjugation of a phenolic hydroxyl group.[85] It is therefore unlikely that polyphenols have direct antioxidant activity in endothelial cells unless they are present at higher levels within these cells. They seem to have indirect antioxidant activity and anti-inflammation activity through the modulation of a redox-sensitive signaling pathway in association with the modulation of transcription factors such as NF-κB and Nrf2.[86,87] Several mechanisms related to the preventive effect of polyphenols against endothelial dysfunction have been proposed using *in vitro* cultured cell experiments and *in vivo* animal experiments. Polyphenols may inhibit the expressions of proinflammatory enzymes such as cyclooxygenase-2 (COX-2), inducible NOS (iNOS), 5-lipoxygenase (5-LOX), and the inflammatory cytokines, IL-1β, IL-6, and TNF-α, by suppressing the nuclear transfer of NF-κB.[3,10] Polyphenols may suppress the adhesion of monocytes to the endothelium by inhibiting the expressions of E-selectin, monocyte chemotactic protein-1 (MCP-1), VCAM-1, and intercellular adhesion molecule (ICAM-1). However, polyphenols may enhance an antioxidant enzyme, HO-1, by stimulating Nrf2 activation.[88,89] They may also reduce oxidative stress in endothelial cells by suppressing NOX-dependent ROS production.[90] Taken together, the action of polyphenols in endothelial cells seems to occur at caveolae, so that a greater understanding of the relationship between caveolae and polyphenols is required to determine the mechanism and assess the preventive effects of polyphenols on endothelial dysfunction.

Cav-1 as a Target for Polyphenols against Endothelial Dysfunction

Dasari *et al.*[91] demonstrated Cav-1 expression in normal mammary epithelial cells was upregulated by H_2O_2 through the p38 mitogen-activated protein kinase (MAPK)/Sp-1 pathway resulting in the induction of premature senescence. Alternatively, lipopolysaccharide (LPS)-dependent Cav-1 expression was activated by the NF-κB pathway in human lung microvascular endothelial cells.[92] The overexpression of Cav-1 suppressed the H_2O_2-induced activation of Nrf2 and its subsequent transcriptional activity in colon cancer cells.[93] Therefore, Cav-1 can attenuate the cellular antioxidant capacity through direct interactions with Nrf2. The exacerbation of inflammation in endothelial cells driven by oxidative stress is associated with the pathology

doi: 10.3164/jcbn.24-30

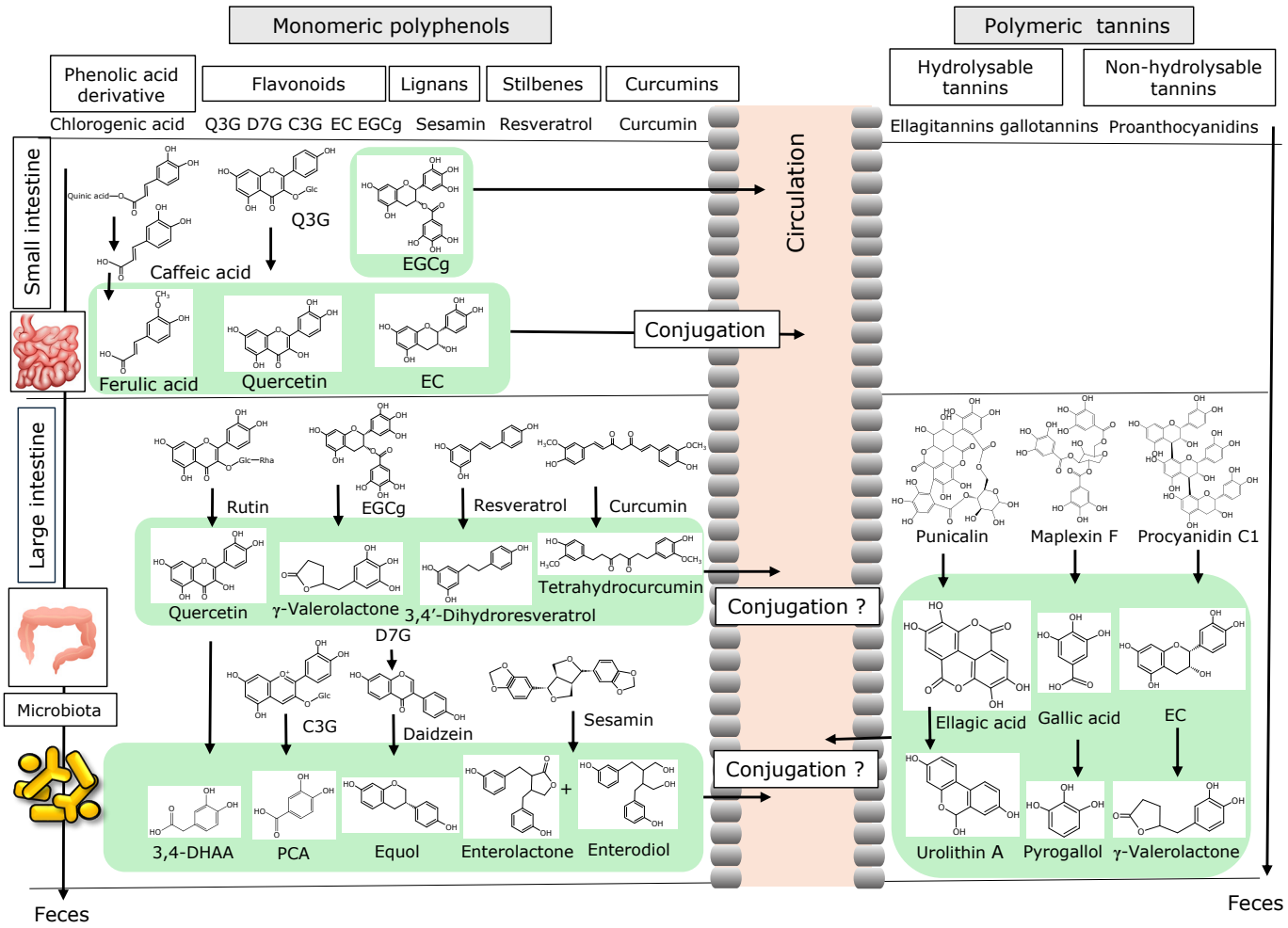

Fig. 3. Schematic diagram of the behavior of polyphenols in the digestive tract. A representative compound of each subgroup is shown in the figure as follows: phenolic acid derivatives; chlorogenic acid, flavonol glycosides; isoquercitrin (quercetin 3-*O*-glucoside: Q3G), rutin (quercetin 3-*O*-rutinoside), flavan-3-ols; (–)-epicatechin (EC), (–)-epigallocatechin gallate (EGCg), anthocyanins; cyanidin 3-*O*-glucoside (C3G), isoflavones; daidzin (daidzein 7-*O*-glucoside: D7G), lignans; sesamin, stilbenes; resveratrol, curcumins; curcumin, ellagitannins; punicalin, gallotannins; maplexin F, and proanthocyanidins; procyanidin C1. Caffeic acid is released from chlorogenic acid and then converted to ferulic acid during absorption in the small intestine.[66] Q3G is partly hydrolyzed to quercetin aglycone and then absorbed from the small intestine after conjugation.[68] Rutin is not absorbed in the small intestine, but hydrolyzed and degraded to ring-fission products such as 3,4-dihydroxyphenylacetic acid (3,4-DHPAA) by the action of microbiota in the large intestine.[68] C3G is converted to ring-fission products including protocatechuic acid (PCA) in the large intestine, although it avoids absorption in the small intestine.[74] For flavan-3-ols, EC and EGCg are partly absorbed from the small intestine with and without conjugation, respectively.[70] Most flavan-3-ols are converted to γ-valerolactones as the major catabolite in the large intestine.[75] Although some of D7G is hydrolyzed to daidzein and absorbed in the small intestine after conjugation, it is subject to hydrolysis and conversion to equol in the large intestine.[73] Sesamin is converted to enterolactone and enterodiol in the large intestine.[76] Resveratrol and curcumin are converted to reductive products, tetrahydrocurcumin and dihydroresveratrol, respectively, by the reducing activity of the microbiota.[77,78] Polymeric tannins are minimally absorbed in the small intestine. In the large intestine, they are hydrolyzed to their constitute monomer units, ellagic acid, gallic acid, and EC.[79–81] Acidic conditions in the stomach may partly release ellagic acid and gallic acid from hydrolysable tannins. Ellagic acid, gallic acid, and EC are further catabolized by the microbiota to urolithins, pyrogallol, and γ-valerolactones, respectively.

of atherosclerosis. Accordingly, the function of polyphenols on Cav-1 proteins should be examined because they possess antioxidative and anti-inflammatory properties.[15,16] The modulation of Cav-1 levels and caveolae composition by habitual diet is predicted to participate in the protection mechanism against endothelial dysfunction.[15] It is not surprising that dietary polyphenols exert anti-atherosclerotic effects by modulating Cav-1 localization and its expression in caveolae. Reports on the relationship between individual polyphenols and Cav-1 in caveolae are described in the next sections.

EGCg. Cav-1 is a negative regulator of NO synthesis in endothelial cells as described above. Yang *et al.*[94] showed that a high-fat diet upregulated Cav-1 expression and downregulated eNOS expression in the aorta of diet-induced obese rats. An *in vitro* experiment using bovine aortic endothelial cells demonstrated that green tea polyphenols, which contain EGCg as the main component, downregulated Cav-1 protein and its gene expression in a concentration-dependent manner.[95] Extracellular signal-regulated kinase 1/2 (ERK-1/2) activation and p38MAPK inhibition participated in the downregulation of Cav-1 expression. Although exposure of human umbilical vein endothelial cell (HUVEC) to linoleic acid induced Cav-1 and COX-2 expressions, and pretreatment with EGCg blocked these expressions in a time and concentration-dependent manner (0–24 h, 0–40 μM, respectively).[96] EGCg is likely to downregulate inflammatory factors in endothelial cells by modulating Cav-1 gene expression. Zheng *et al.*[97] found that the treatment of porcine aortic endothelial cells with EGCg at 30 μM induced the nuclear transfer

of Nrf2 and increased HO-1 expression. Interestingly, EGCg quickly accumulated in the caveolae-rich fraction, and its accumulation was associated with the displacement of Cav-1 from cell membranes to the cytosol. They also found that silencing Cav-1 by siRNA also activated the transcription of Nrf2 resulting in HO-1 expression. Thus, EGCg is likely to induce HO-1 expression through Nrf2 activation by the displacement of Cav-1 from caveolae in cell membranes to the cytosol.

Isoflavones. Male rats treated with daidzein (0.2 mg/kg per day by subcutaneous injection) for 7 days exhibited enhanced acetylcholine-induced relaxation of their aortic rings via increased eNOS activity.[98] Daidzein reduced the expression of Cav-1 in arteries with an increase of calmodulin, although it did not alter eNOS protein levels. It is therefore likely that daidzein enhances eNOS activity by affecting Cav-1 expression, resulting in the endothelial cell-dependent relaxation of blood vessels via increased NO synthesis and its release. The same treatment of daidzein to male rats increased plasma NO levels two-fold with a decrease of Cav-1 expression and enhanced acetylcholine-induced relaxation of the carotid arteries.[99] Thus, the subcutaneous administration of daidzein to rats might modulate aorta artery reactivity by targeting Cav-1 expression. In ovariectomized rats, NO production and eNOS protein levels in the myocardium were increased with a concomitant decrease in Cav-1 expression by the administration of genistein (0.5 mg/kg and 5.0 mg/kg per day by subcutaneous injection) for 6 weeks.[100] However, Cav-1 protein levels in the intact aorta of male spontaneous hypertensive rats (SHR) were unchanged by the consumption of genistein (10 mg/kg body weight per day by gavage) for 5 weeks, although eNOS activity increased with an increase in calmodulin-1 expression and decrease in NADPH-induced $O_2^{\bullet-}$ expression.[101] Genistein may directly suppress NOX overactivity without affecting Cav-1 expression, when it is administered orally.

Quercetin. Kook *et al.*[102] found that the pretreatment of human retinal pigment epithelial cells with quercetin (10–150 μM) for 24 h significantly lowered H_2O_2-mediated Cav-1 mRNA expression and protein levels. The H_2O_2-mediated activation of Cav-1 in mouse fibroblast NIH-3T3 cells was prevented by quercetin.[91] Coplanar polychlorinated biphenyls (PCBs), widespread environmental contaminants, can induce oxidative stress and activate proinflammatory signaling cascades responsible for atherosclerosis.[103] Quercetin at 10 μM reduced Cav-1 protein levels, which were enhanced by exposure to PCBs, in primary endothelial cells obtained from porcine pulmonary aorta.[104] Quercetin simultaneously reduced the PCB-dependent induction of VCAM-1, E-selectin, and P-selectin. oxLDL was reported to participate in plaque formation and endothelial dysfunction in the process of atherosclerosis. Kamada *et al.*[105] investigated the effect of quercetin aglycone and its conjugated metabolite, quercetin-3-*O*-glucuronide (Q3GluA), on the expression of Cav-1 in HUVEC exposed to oxLDL or lysophosphatidylcholine (lysoPC), the main lipid component specific to oxLDL. Interestingly, 1 μM quercetin aglycone or Q-3GluA reduced the expression of Cav-1 mRNA when cells were exposed to lysoPC, which when released from oxLDL in arterial walls, may cause endothelial dysfunction through the induction of Cav-1 expression. It is thought that quercetin-conjugated metabolites in the blood can have anti-atherosclerotic effects by suppressing caveolar Cav-1 expression.

Cav-1 inhibits the production of antioxidant enzymes such as HO-1 by interacting with the transcription factor Nrf2 in the caveolae of cell membranes as described above. Of note, quercetin induced HO-1 expression in H_2O_2-exposed endothelial cells through Nrf2 activation.[106] Matsushima *et al.*[107] recently investigated the relationship between the quercetin-dependent structural changes of caveolae and induction of HO-1 expression in NIH/3T3 cells. They found that quercetin at 30 μM lowered the level of cholesterol in caveolae lipid bilayers in association with the induction of HO-1 expression. In addition, quercetin shifted Cav-1 expression from the raft fraction to non-raft fraction in caveolae and promoted the transfer of Nrf2 protein from cell membranes to the nuclei fraction. Taken together, the protective effect of quercetin against cellular oxidative stress might be partly derived from the disorder of lipid bilayers in caveolae resulting in the expression of HO-1 by the nuclear transfer of Nrf2 released from cell membranes.

Icariin. Icariin is a prenylated derivative of flavonol glycosides (4'-*O*-methyl kaempferol 3,7-*O*-diglucoside) isolated from *Epmedii Herba* (*Berberridaceae*). Its pharmacological activity is thought to provide cardioprotective effects. Scicchitano *et al.*[108] evaluated the protective effect of icariin against vascular damage caused by the side-effect of an antineoplastic drug, doxorubicin. Pretreatment of rat heart-tissue derived myoblasts with icariin at 1 μM or 5 μM, prior to doxorubicin exposure, improved cell viability and reduced ROS generation, associated with the suppression of Cav-1 expression levels. Therefore, icariin may protect cells against doxorubicin-mediated cell death by suppressing Cav-1 expression levels. When SHR rats were administered icariin (10 mg/kg body weight per day by gavage) for 4 weeks, Cav-1 expression levels in their penile cavernous tissue decreased together with increased eNOS and NO levels.[109] Thus, icariin may suppress the interaction between eNOS and Cav-1 proteins resulting in the elevation of eNOS activity and NO release.

Resveratrol. Resveratrol, a natural stilbene-type polyphenol found in grapes and wine, is thought to be cardioprotective in humans.[110] Klinge *et al.*[111] found that resveratrol at 50 nM induced estrogen receptor alpha (ERα)/Cav-1-c-Src interactions, resulting in NO production through a G alpha protein-coupled mechanism in HUVEC. When male rats were fed a high-fat plus sucrose diet for 13 weeks, hyperpermeability was increased in aortas, which was attenuated when resveratrol was added to their diet (50 mg/kg body weight).[112] In addition, the application of resveratrol reversed the changes in eNOS and Cav-1 expressions in the aortas and hearts of rats fed a high-fat plus sucrose diet. This study suggests that high-fat treatment with high glucose causes endothelial hyperpermeability, which is ameliorated, at least partly, by resveratrol-related Cav-1/eNOS regulation.

Phosphorylation of Cav-1 and its inhibition by polyphenols

A variety of cellular stressors including high osmolarity, H_2O_2, and UV light stimulate the phosphorylation of Cav-1 at the Tyr^{14} site via the activation of upstream P38 MAPK and C-src kinase in NIH 3T3 cells.[113] Wang *et al.*[114] found that LPS treatment induced the phosphorylation of Cav-1 in rat and human pulmonary microvascular endothelial cells in association with an increase in transcellular permeability prior to the increase of paracellular permeability. Sun *et al.*[115] demonstrated that an H_2O_2-induced increase in Cav-1 phosphorylation was dose-dependently coupled to the increased transcytosis of albumin, decreased transendothelial electric resistance, and subsequent endothelial barrier disruption in pulmonary endothelial cells. OxLDL was also found to promote the dose-dependent phosphorylation of Cav-1 in HUVEC resulting in the intracellular accumulation of oxLDL.[116] Therefore, Cav-1 phosphorylation may disturb endothelial barrier functions resulting in oxidative stress-induced vascular hyperpermeability. Kondo-Kawai *et al.*[117] indicated that pretreatment of HUVEC with 10 μM quercetin aglycone, but not Q3GluA, for 24 h, inhibited H_2O_2-induced Cav-1 phosphorylation. Quercetin aglycone also reversed the increase of vascular permeability and decrease of vascular endothelial cadherin expression. Furthermore, quercetin aglycone suppressed LPS-stimulated Cav-1 phosphorylation in HUVEC.[118] Wogonin,

doi: 10.3164/jcbn.24-30

a naturally occurring methoxyflavone derivative extracted from the root of *Scutellaria baicalensis* Georgi, was suggested to inhibit H_2O_2-induced vascular permeability by downregulating the phosphorylation of Cav-1.[119] These results suggest that polyphenols, once transferred into the circulation, act as preventive factors against the elevation of vascular permeability by inhibiting the phosphorylation of Cav-1.

Conclusions and Perspectives

CVD is a chronic non-communicable disease that causes death globally. The habitual intake of fruit and vegetables might aid the prevention of CVD.[120] The beneficial effect of the intake of fruit and vegetables is at least partly derived from polyphenols that are ubiquitously present in plant-based foods.[3,121] Several short-term human intervention studies of polyphenols using vascular biomarkers[122] and a recent large-scale intervention study of cocoa polyphenols (mixtures of (−)-epicatechin and procyanidins)[123] indicated the validity of polyphenol intake on the prevention of atherosclerosis leading to CVD. *In vitro* studies using cultured endothelial cells and *in vivo* rodent studies strongly suggested that protection against endothelial dysfunction is a primary mechanism related to the atherosclerosis-preventive effect of polyphenols. The idea that polyphenols target caveolae and Cav-1 in endothelial cell membranes is a fascinating hypothesis that might explain the mechanism related to the protective action of polyphenols on endothelial cells, which prevents their disruption (Fig. 4). Polyphenols may affect caveolae functions by the physicochemical modification of their lipid bilayers, leading to the replacement of Cav-1. They may also attenuate enhanced Cav-1 expression related to the activation of redox-sensitive transcription factors. Their inhibitory effect on Cav-1 phosphorylation might be involved in their protective role in the barrier function of endothelial cells. However, there is insufficient evidence for these hypotheses at present.

In vitro cultured cell experiments frequently use polyphenols at physiologically impossible high concentrations. In the circulation, dietary polyphenols are mostly present as their conjugated metabolites or gut microbiota-derived catabolites. Therefore, the activities of their conjugated metabolites or degraded catabolites, but not their original forms, at lower concentrations should be investigated to determine their protective effects against endothelial dysfunction. Nevertheless, glucuronide-conjugated metabolites of flavonoids are subject to deconjugation by β-glucuronidase activity to yield their aglycones in association with inflammation.[87,124] β-glucuronidase activity, which catalyzes the release of aglycones from glucuronide-conjugated metabolites, was observed in the plasma of healthy volunteers[125] as well as the serum of patients undergoing hemodialysis.[126] Therefore, aglycones may accumulate in the caveolae of cell membranes and exert functions during inflammation, as deconjugated metabolites are promoted by the LPS-induced inflammatory activation of macrophages.[127] The translocation of polyphenols from the blood to endothelial cell membranes has not been elucidated yet, although caveolae may be essential for the uptake of polyphenols by endothelial cells.[128] Detailed features of the transfer of polyphenols to endothelial cell membranes and their behavior in caveolae must be clarified to evaluate their contribution to protection against endothelial dysfunction.

Senolytics, which eliminate senescent cells from the body, are expected to be a novel type of chemotherapy for CVD.[59] The combined use of quercetin with an anti-cancer drug, dasatinib, and single use of fisetin, a flavonol-type flavonoid, exhibited senolytic effects on senescent endothelial cells, and a preclinical trial of their application as a novel therapy was initiated recently.[129] However, Cav-1 might be a potential target for the prevention and early treatment of atherosclerosis, because Cav-1 in caveolae is tightly associated with the regulation of lipid transport, inflammation, and cellular signaling pathways.[30,31] Polyphenols may be an attractive tool for CVD therapy by regulating Cav-1 activity, although Cav-1 itself is essential for maintaining normal cardiovascular function.[130] Present knowledge on the role of polyphenols targeting caveolae and Cav-1 for protection against endothelial dysfunction warrants future research to determine the therapeutic suitability of polyphenols for CVD as well as its prevention by dietary means.

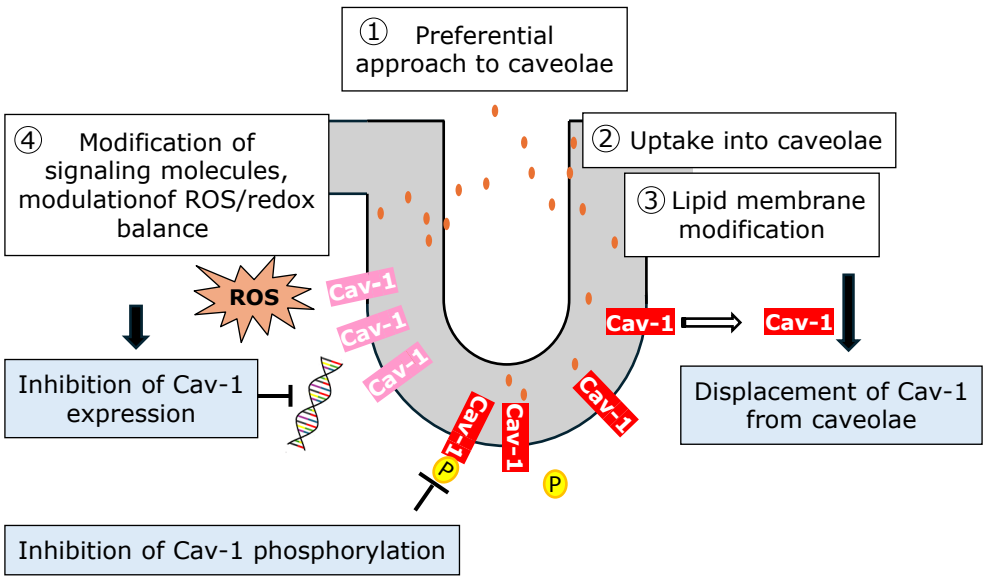

Fig. 4. Hypothetical mechanism of action of circulating polyphenols to exert protective effects against endothelial dysfunction by targeting caveolae and Cav-1. ①Circulating polyphenols preferentially approach caveolae in endothelial cell membranes. ②Polyphenols are incorporated into caveolae by endocytosis. ③Polyphenols affect the physicochemical properties of lipid bilayers in caveolae resulting in the displacement of Cav-1 from caveolae. ④Polyphenols modify signaling pathways by its direct interaction with signaling molecules or modulation of the ROS/redox balance, resulting in the inhibition of Cav-1 expression leading to a decrease in the amount of Cav-1 in caveolae. Alternatively, it results in the inhibition of Cav-1 phosphorylation, leading to the suppression of adverse effects on the barrier function of endothelial cells.

Acknowledgments

We thank J. Ludovic Croxford, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Conflict of Interest

No potential conflicts of interest were disclosed.

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doi: 10.3164/jcbn.24-30