

Review

Mechanism of vascular endothelial dysfunction causing atherosclerosis: vascular endothelial glycocalyx

Running title: vascular endothelial glycocalyx damage in atherosclerosis

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Abstract: In atherosclerosis patients, vascular endothelial dysfunction is commonly observed with damage of vascular endothelial glycocalyx, an extracellular matrix-bound to and encapsulating the endothelial cell lining the blood vessel wall. Unfavorable lifestyle; smoking and physical inactivity, also induces glycocalyx degradation. Moreover, the vascular endothelial glycocalyx is damaged by various unfavorable disease conditions like as dehydration, acute infectious disease, trauma, sepsis, ARDS, Kawasaki disease, preeclampsia, gestational diabetes mellitus, hypertension, diabetes, chronic kidney disease, atherosclerosis, stroke, dementia, microvascular angina, acute coronary syndrome, and heart failure. The vascular endothelial glycocalyx has been shown to be important not only as a physical cytoprotective barrier for vascular endothelial cells but also as a mechanism that regulates intracellular cell signaling. Therefore, vascular endothelial glycocalyx has great potential to explore new strategies for assessing the benefit conditions of our healthy vasculature.

Keywords: vascular endothelial function; inflammation; oxidative stress; cardiovascular disease prevention; disease management

1. Vascular endothelial cell dysfunction

The blood vessels that extend all over the body consists of one layer of vascular endothelial cells. Vascular endothelial cells are known to produce various physiologically active substances and are responsible for homeostasis in the living body. If one person's blood vessels are connected together, the length will be two and a half rounds of the earth, and if all the vascular endothelial cells forming the inside of the blood vessel are put together into one, it is comparable to the largest organ which is larger than the liver.

Vascular endothelial cells have various functions such as inflammation/anti-inflammation, coagulation/fibrinolysis system, promotion/inhibition of angiogenesis, etc. By regulating these balance, vascular endothelial cells are responsible for maintaining homeostasis in the body (Fig. 1). Healthy vascular endothelial cells exhibit a good vascular endothelial function. Coronary risk factors like as smoking, hypertension, diabetes, dyslipidemia, obesity, and physical inactivity are major factors that cause vascular endothelial dysfunction and further promote atherosclerosis progression (Fig. 2). The

comprehensive disease management, such as smoking cessation and improvement of lifestyle-related diseases, makes it possible to maintain a good vascular endothelial function.

Figure 1. Vascular endothelial dysfunction.Vascular endothelial dysfunction is equal to vascular endothelial cell dysfunction which leads to vascular failure, which cannot keep the balance of good vascular conditions.

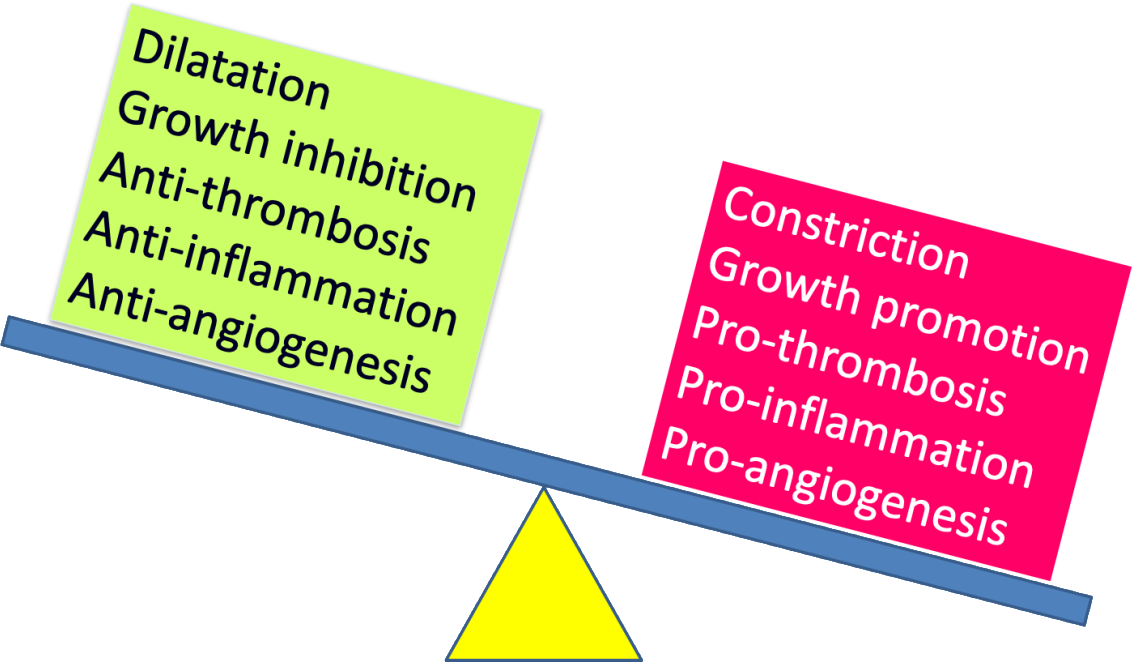
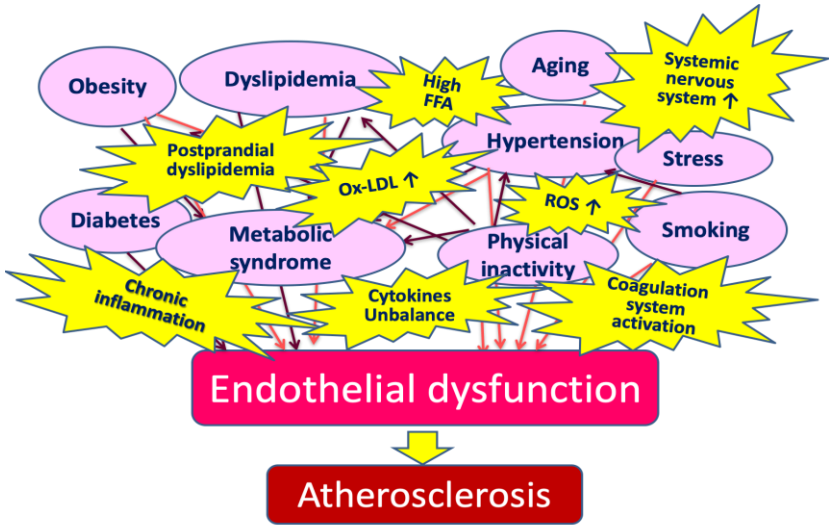


Figure 2. Endothelial dysfunction induces atherosclerosis progression.The vascular endothelial function might have potential prognostic value for the early detection of atherosclerosis. FFA, fatty free acids; ROS, reactive oxygen species, Ox-LDL, oxidized-low density lipoprotein.

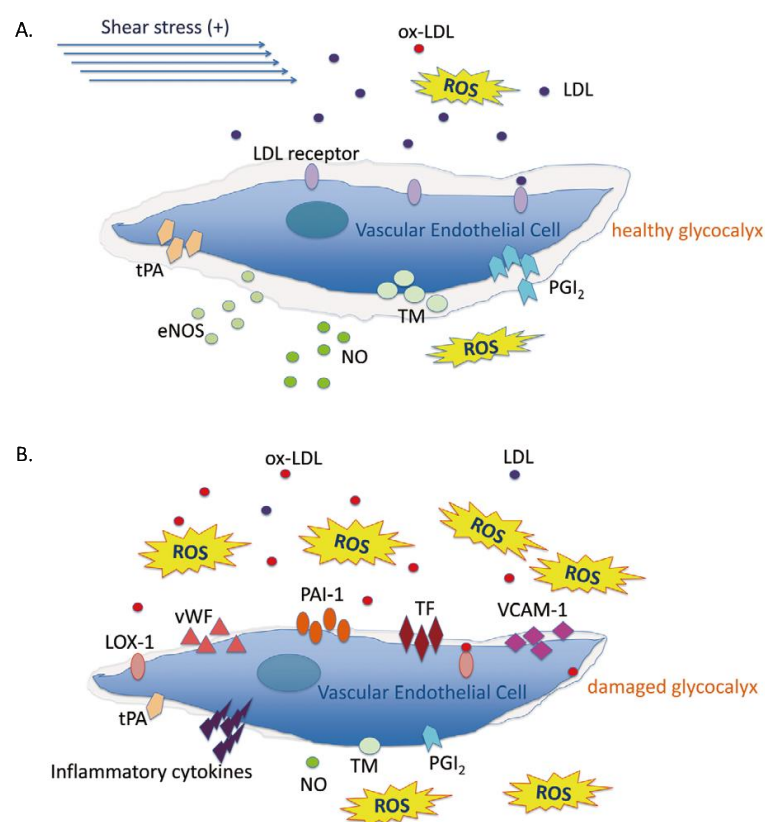


Vascular endothelial function test has been used as an indicator to predict the onset of future cardiovascular events and determine the effectiveness of comprehensive disease management in cardiovascular medicine. Although it has said that “humans grow older with blood vessels”, the vascular endothelial function is an integrated index showing the health of blood vessels, and maintaining a good vascular endothelial function leads to the health of the living body. To elucidate these precise mechanisms, we should research more about the molecular mechanisms of vascular endothelial cell function.

2. Endothelial glycocalyx and atherosclerosis

The barrier of extracellular polymeric compounds consists of glycoproteins that form a 200-500 nm glycosylated gel layer that covers vascular endothelial cells. The gel layer is called vascular endothelial glycocalyx. In a healthy vascular endothelial cell covering with enough amount of glycocalyx, cells are well organized with a good vascular endothelial function such as producing nitric oxide (NO), anticoagulant factors, and a sufficient amount of reactive oxygen species (ROS) to maintain vascular homeostasis (Fig. 3A).

Figure 3. Vascular endothelial glycocalyx covering vascular endothelial cells. A. A healthy vascular endothelial cell covering with enough amount of glycocalyx. Endothelial cells covered with glycocalyx are well maintained in endothelial function producing nitric oxide (NO), anticoagulant factors, and a sufficient amount of reactive oxygen species (ROS) to maintain vascular homeostasis. B. An unhealthy vascular endothelial cell partly covered thinned glycocalyx. In vascular endothelial cells in which vascular endothelial glycocalyx is damaged by hyperglycemia or oxidized LDL, excessive ROS are produced, and NO production is reduced, which promotes inflammatory cytokines releasing and coagulation. LOX-1, lectin-like oxidized LDL receptor; VCAM-1, vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; TF, tissue factor; vWF, von Willebrand factor; ox-LDL, oxidized low-density lipoprotein; eNOS, endothelial NO synthase, MMPs, matrix metalloproteases; tPA, tissue plasminogen activator; PGI₂, prostacyclin; TM, thrombomodulin.



The glycocalyx is composed of proteoglycans and glycosaminoglycans. In the group of proteoglycans, there are syndecans such as syndecan-1 (CD138) and syndecan-4, glypican, mimecan, perlecan, and biglycan. In the glycosaminoglycans, hyaluronan (including hyaluronic acid and hyaluronate), heparin, heparan sulfate, chondroitin, dermatan sulfate, keratan sulfate, are included. The vascular endothelial glycocalyx plays important roles to cover physically vascular endothelial cells, maintain cell-cell adhesion of vascular endothelial cells, and control the cell signaling among vascular endothelial cells, which may affect cancer metastasis, angiogenesis, and vascular permeability. The vascular endothelial glycocalyx is necessary to maintain the function of vascular endothelial cells which is also necessary to keep homeostasis in the body¹.

The vascular endothelial glycocalyx is involved in the control of intracellular signal transduction such as proliferation/differentiation/apoptosis of vascular endothelial cells, cytoskeleton, thrombus formation, inflammatory/anti-inflammatory control, and immunity. Attention has been focused on the relationship with the pathogenesis of arteriosclerosis, and it has been suggested that it is associated with the progression of atherosclerotic diseases such as plaque formation and progression, thrombus formation, plaque rupture, and the pathogenesis of cardiovascular events. The vascular endothelial glycocalyx is impaired by endotoxin, inflammation, hypernatremia², oxidized-LDL³, hyperglycemia, excessive fluid infusion, dehydration, and reduction of vascular wall shear stress. These stimulations systemically induce shedding of vascular endothelial glycocalyx and the glycocalyx layer is thinned under these conditions (Fig. 3B). In vascular endothelial cells partly covered by damaged endothelial glycocalyx, inflammatory cytokines are released a lot, excessive ROS are produced, and NO production is reduced, which promotes atherosclerosis progression. Importantly, vascular endothelial glycocalyx damage has already been reported in patients with diabetes^{4, 5}, inflammatory disease⁶, Kawasaki disease⁷, sepsis⁸, ischemia/reperfusion injury⁹, trauma¹⁰, acute lung injury (ALI)/acute respiratory distress syndrome (ARDS)¹¹, preeclampsia¹², familial hypercholesterolemia¹³, chronic heart failure¹⁴, microvascular angina¹⁵, and cardiogenic shock¹⁶. It has been suggested that vascular endothelial glycocalyx damage is involved in the onset/deterioration mechanism of these diseases. In these patients, the fragmented vascular endothelial glycocalyx is detected at high concentrations in their blood and urine, and their vascular endothelial glycocalyx layer is thinned. In addition, coarse and fragile glycocalyx layer-induced by vascular endothelial glycocalyx damage is more frequently observed in these patients. Furthermore, by improving the pathological condition through therapeutic intervention, etc., these vascular endothelial glycocalyx damage can be ameliorated. Therefore, as a target for preventive measures for the onset and exacerbation of these diseases and new therapeutic strategies, vascular endothelial glycocalyx research is going on progressing.

The vascular endothelial glycocalyx is observed to be impaired from the early stage of arteriosclerosis due to exposure to coronary risk factors for the development of arteriosclerosis such as smoking, hypertension, diabetes, and dyslipidemia¹⁷. On the other hand, it has been revealed that vascular endothelial glycocalyx is dramatically improved due to lifestyle changes such as smoking cessation¹⁸. In addition, vascular endothelial glycocalyx damage is also caused by a decrease in shear stress, and it is known that vascular endothelial glycocalyx damage is responsible for vulnerable plaque formation in the area of arterial bifurcations and strong flexion. *In vivo* and *in vitro* models of decreased shear stress caused vascular endothelial glycocalyx degradation, and plaque formation was strongly induced at the same site¹⁹.

3. Molecular biological mechanism of vascular endothelial glycocalyx disorder in arteriosclerosis

In arteriosclerosis model animals and patients with atherosclerotic diseases, vascular endothelial glycocalyx damage is induced and elevated blood levels of syndecan-1 and hyaluronan were observed. Increasing glycocalyx damage results in further thinning of the vascular endothelial glycocalyx layer. It is known that the reduction of shear stress also causes thinning of vascular endothelial glycocalyx through the inactivation of AMP-activated protein kinase (AMPK) and the activation of hyaluronidase 2 which induces shedding of hyaluronan and exfoliates vascular endothelial glycocalyx¹⁹. Syndecan-1 is known as a molecule that regulates shear stress-related signals. In syndecan-1-deficient mice, signals that induce inflammation and arteriosclerosis are activated, and vascular permeability is enhanced, and leukocyte cell adhesion on vascular endothelial cells is activated²⁰. On the other hand, it has been

confirmed that these signals are suppressed by the re-expression of syndecan-1 and the progression of arteriosclerosis is suppressed. It is deeply involved in pathological conditions such as vascular hyperpermeability associated with acute inflammation and is used in many clinical studies as a major biomarker that reflects vascular endothelial glycocalyx damage.

Factors that directly damage vascular endothelial glycocalyx include reactive oxygen and nitrogen species (RNOS), uric acid²¹, oxidized LDL³, matrix metalloproteinases (MMPs), heparanase, sialidase, and these stimuli reduce vascular endothelial function. Administration of 4-methylumbelliferone (4-MU), a hyaluronan synthesis inhibitor, to high-fat diet-fed ApoE knockout mice showed impaired vascular endothelial glycocalyx from the early stage, increased blood pressure, and acetylcholine-dependent vasorelaxation was decreased, and accelerating formation of aortic plaque with increased macrophage accumulation was clearly observed²².

On the other hand, antithrombin, TNF- α inhibitor²³, allopurinol²¹, sphingosine-1 phosphate (S1P)²⁴, MMP inhibitors, hyaluronic acid, and steroids are reported to protect vascular endothelial glycocalyx. Since these substances are expected to exert anti-inflammatory and anti-oxidative actions, suppressive effects of both inflammation and excessive oxidative stress are expected to affect not only vascular endothelial cells but also vascular endothelial glycocalyx. It is also possible that these substances may act protectively against vascular endothelial glycocalyx.

As a morphological study, there is a report of a high-fat diet-induced arteriosclerosis model using ApoE knockout mice²⁵. The vascular endothelial glycocalyx layer covering the plaque site was significantly thinned in the arteriosclerosis-induced mouse model, but even in the same individual, the vascular endothelial glycocalyx at the plaque-free site was not thinned. However, its thickness was similar to that of the vascular endothelial glycocalyx in normal high-fat diet-fed control mice²⁵. Furthermore, vascular endothelial cell apoptosis was detected at a higher rate in plaque-forming sites than in non-plaque-forming sites, and shedding of the glycocalyx, promotion of plaque formation, and high expression of MMP-9 were simultaneously detected in the same sites. The vascular endothelial glycocalyx may contribute to plaque stabilization as well as maintaining the good vascular endothelial function, and the disorder is likely to play an important role in triggering plaque rupture in acute coronary syndrome.

Hyaluronic acid, which is one of the important components of vascular endothelial glycocalyx, and its binding protein, CD44, are strongly expressed in atherosclerotic lesions. The hyaluronic acid/CD44 signal activates vascular smooth muscle migration and proliferation which promotes plaque formation and increased intercellular matrix synthesis¹⁷. Further, as a mechanism of hyaluronic acid inducing atherosclerosis, hyaluronic acid activates Toll-like receptors (TLRs) to enhance the accumulation of macrophages in atherosclerotic lesions and promote Th1 cell polarization, thereby promoting macrophage immunity to promote an inflammatory response through these cells²⁶. On the other hand, hyaluronic acid existing abundantly in the vascular endothelial glycocalyx layer exerts to suppress adhesion of leukocytes and platelets to the vascular endothelial cells. hyaluronic acid controls an inflammatory reaction in the extracellular matrix. Hyaluronic acid is known to exhibit antithrombotic and anti-inflammatory properties and is expected to suppress atherosclerosis progression by targeting the hyaluronan synthase and its receptors^{27, 28}.

Biglycan, a member of the class I family of small leucine-rich proteoglycans, is abundantly existing in the vascular endothelial glycocalyx layer and atherosclerotic plaque. Circulating biglycan is increased in patients with acute coronary syndrome, and also detected in the atherosclerotic plaque in ApoE knockout mice. In ApoE/biglycan double knockout mice, thrombin, platelet, and adhesion molecules were activated, as well as an increase in inflammatory cytokines and macrophage accumulation in the aortic wall²⁹. Therefore, the loss of biglycan is considered to be closely involved in the atherosclerosis progression and plaque formation especially in the thinning layer of vascular endothelial glycocalyx.

4. Vascular endothelial function and glycocalyx

At the very early stage of atherosclerosis, vascular endothelial function is reduced by exposure to coronary risk factors such as hypercholesterolemia, hyperglycemia, hypertension, obesity, excessive oxidative stress, and chronic inflammation. Vascular endothelial dysfunction is observed not only in patients with early-stage of atherosclerosis but also in patients with heart failure and hypertension.

Therefore, vascular endothelial function attracts attention as an independent prognostic factor for cardiovascular disease.

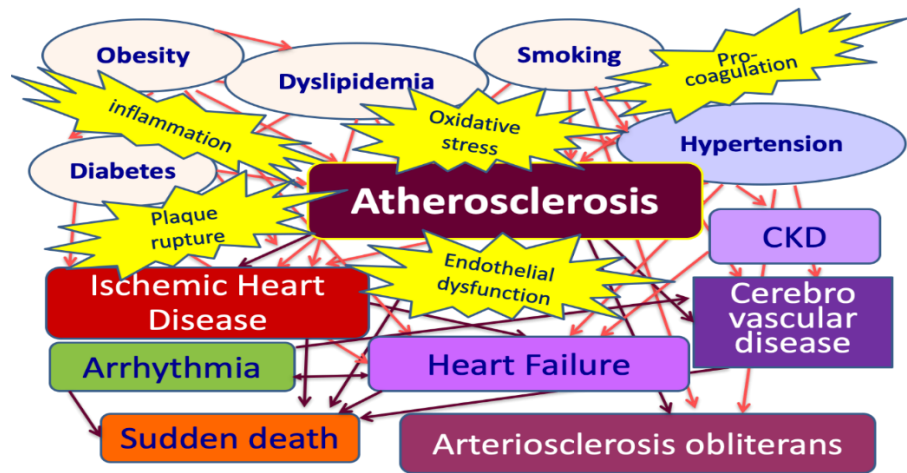
Before vascular endothelial dysfunction, vascular endothelial glycocalyx damage accompanied by a decrease in endothelium-dependent vasodilation reaction and increased vascular permeability occurs. Therefore, the vascular endothelial glycocalyx is considered a major determinant of vascular endothelial function and can be measured by the perfused boundary region (PBR) of sublingual microcirculation.

In an atherosclerotic model using ApoE/LDL receptor-deficient mice, an increased level of biomarkers associated with various vascular endothelial dysfunction was observed as a pre-stage of atherosclerotic plaque³⁰. Specifically, circulating levels of vascular endothelial-origin biomarkers are increased like as fragmented vascular endothelial glycocalyx syndecan-1 and endothelial-cell specific molecule-1 (ESM-1), soluble vascular adhesion molecule-1 (sVCAM-1), coagulation/fibrinolysis system factor von-Willebrand-Factor (vWF), tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), vascular hyperpermeability-related molecule of soluble fms-like tyrosine kinase-1 (sFlt-1) and angiopoietin-2. Decreased endothelium-dependent vasodilation, increased vascular endothelial permeability, and decreased NO production are already observed from the very early onset of arteriosclerosis (4 weeks after birth). In the examination of vascular endothelial function by FMD (flow-mediated dilation) using magnetic resonance imaging (MRI) and vascular endothelial glycocalyx using atomic force microscope (AFM), after the appearance of microscopic abnormalities in vascular endothelial function, vascular endothelial dysfunction, thinning of the glycocalyx layer and detachment of the vascular endothelial glycocalyx were observed. This multistep vascular endothelial damage contributes to atherosclerotic plaque formation, and a more detailed examination about the vascular endothelial glycocalyx-related mechanism is underway.

5. Atherosclerotic risk factors and vascular endothelial glycocalyx

Cardiovascular diseases are mainly based on atherosclerosis progression induced coronary risk factors, like smoking, hypertension, dyslipidemia, diabetes, and obesity (Fig. 4). These risk factors induce endothelial dysfunction, oxidative stress, coagulation cascade activation, inflammation, and accumulation of lifestyle diseases to stimulate further atherosclerosis progression. To improve these risk factors, lifestyle change is known to be one of the main approaches for cardiovascular disease prevention. The main objective of the total risk management for atherosclerosis patients to prevent cardiovascular disease progression is to reduce cardiovascular events and prevent chronic heart failure, and also improve their quality of life.

Figure 4. Risk factors for atherosclerosis progression and cardiovascular disease. Cardiovascular diseases are mainly caused by atherosclerosis progression induced coronary risk factors, like smoking, hypertension, dyslipidemia, diabetes, and obesity. These risk factors induce endothelial dysfunction, oxidative stress, coagulation cascade activation, inflammation and such a kind of morbidity stimulate atherosclerosis progression. CKD, chronic kidney disease.



This section presents recent important findings regarding the relationship between each coronary risk factor and vascular endothelial glycocalyx are shown in this section.

Diabetes

A vascular endothelial glycocalyx, hyaluronan, is impaired in a hyperglycemic mouse model resulting in inadequate increased vascular permeability³¹. In a hyperglycemic model using human microvascular arterial endothelial cells (HMAECs), hyperglycemia activates TLR-2/4 signals, induces inflammation, leukocyte adhesion to HMAECs, reduces heparan sulfate expression, and increases the release of hyaluronic acid³². These signals increased the generation of excessive ROS, and contrarily antioxidants reduced TLR-2/4 gene expression and inflammatory biomarkers located its downstream. In retinal blood vessels of diabetic model rats, vascular endothelial glycocalyx of the retina was significantly thinned compared to control rats³³.

In studies using therapeutic drugs for diabetes, its effectiveness has been reported in several animal models and experiments using cultured human cells. In a diabetic mice model, metformin administration for 2 weeks improved the systemic vascular endothelial glycocalyx barrier function even if in a situation where blood glucose was not affected³⁴. Sodium glucose transporter (SGLT)-2 inhibitors are described as having a cardio/renal protective effect in recent years, it has also been reported that the incidence of cardiovascular death and exacerbation of heart failure was reduced. Empagliflozin, a SGLT-2 inhibitor, improves not only glycemic control but also reduces cardiovascular death and hospitalization for heart failure. In cultured human abdominal aortic vascular endothelial cells, empagliflozin has a protective effect on vascular endothelial glycocalyx³⁵. The protective effect of SGLT-2 inhibitors on vascular endothelial glycocalyx is considered to be one of the important mechanisms of these drugs for cardiovascular disease prevention.

Dyslipidemia

Oxidized LDL (ox-LDL) is considered to be a strong inducer of atherosclerosis progression, and its direct mechanism is to hurt vascular endothelial glycocalyx³. It has been reported in animal studies that approximately 60% of the vascular endothelial glycocalyx is released as the shedding glycocalyx from the surface of vascular endothelial cells 25 minutes after the administration of ox-LDL, and at the same time, activation of platelet adhesion is observed on the vascular wall³. A clinical study comparing estimated whole-body vascular endothelial glycocalyx volume showed a significant decrease in patients with familial hypercholesterolemia compared to healthy subjects¹³. Statin therapy partially improved the volume of vascular endothelial glycocalyx, but it was significantly lower than healthy subjects.

Hypertension

Hypertension, like diabetes and dyslipidemia, causes damage to vascular endothelial glycocalyx. In the retina and choroid of hypertensive rats, vascular endothelial glycocalyx was significantly degraded and exfoliated from the blood vessels, and leukocytes and platelet adhesion to vascular endothelium were accelerated at sites where vascular endothelial glycocalyx was thinned³³. In cultured vascular smooth muscle cells, glycocalyx covering the cell membrane was damaged by high-salt loading within 2-4 days, but vascular smooth muscle remodeling and cell hypertrophy were observed 6-16 days after continuous high-salt loading³⁶. Since proliferation and hypertrophy of vascular smooth muscle are deeply involved in the progression of hypertension and arteriosclerosis caused thereby, salt reduction instruction may be effective in patients with hypertension targeting vascular endothelial glycocalyx.

Although it is known that excessive salt intake causes salt-sensitive hypertension, negatively charged vascular endothelial glycocalyx neutralizes cell surface charges by retaining sodium in the plasma in the glycocalyx layer. It is also reported that vascular endothelial glycocalyx is a regulator of salt sensitivity³⁷. It is well known that the instruction of salt reduction as a treatment or prevention of hypertension is well known, too much salt induces myocardial cell hypertrophy even under conditions where blood pressure does not rise, and the secretion of aldosterone from the adrenal gland resulting hypertension and causing hypertrophy and fibrosis of the heart and blood vessels. Furthermore, it has

been clarified that excessive salt intake increases urinary excretion of potassium and calcium, which may cause renal stones and osteoporosis, but potassium deficiency also increases blood pressure. Although further studies are awaited, hypernatremia-induced vascular endothelial glycocalyx damage and the activation of vascular smooth muscle remodeling should be considered to clarify the mechanism of hypertension development and arteriosclerosis progression.

Chronic kidney disease (CKD)

The involvement of albuminuria in the early stage of diabetic nephropathy has been pointed out because the clearance of negatively charged albumin is increased due to vascular endothelial glycocalyx damage³⁸. It has been reported that glomerular vascular endothelial glycocalyx damage is caused not only by hyperglycemia and diabetic nephropathy but also by administration of adriamycin³⁹. Among the gestational diabetes mellitus, the group in which vascular endothelial glycocalyx was released more in the blood had a higher rate of developing diabetes mellitus in the future⁴⁰. From these findings, it is expected that the prognosis of the onset and exacerbation of renal damage in various diseases can be predicted by measuring the blood level of fragmented and released vascular endothelial glycocalyx.

Syndecan-1 is produced not only by vascular endothelial cells but also by glomerular epithelial cells (podocytes), and it is associated with impaired barrier function in glomerular filtration. In patients with stage 3-5 CKD, increasing levels of circulating syndecan-1 and hyaluronan were associated with decreased vascular endothelial function, and increasing levels of biomarkers related to vascular endothelial damage (sFlt-1, sVCAM-1, vWF, angiopoietin-2). Furthermore, thinning of vascular endothelial glycocalyx and increasing levels of syndecan-1 were observed in a rat 5/6 nephrectomized CKD model⁴¹.

In patients with glomerular disease, vascular endothelial dysfunction is observed in early-stage, arteriosclerosis is likely to progress more, and cardiovascular death increases⁴². A cross-sectional study of patients with initial nephrotic syndrome found a significant increase in circulating syndecan-1 levels in patients with nephrotic syndrome who retained renal function⁴³. In these patient groups, the vascular endothelial function measured by FMD was significantly lower than that in healthy subjects, and the increasing levels of syndecan-1 were closely related to the vascular endothelial dysfunction measured by FMD. Therefore, vascular endothelial glycocalyx damage accompanied by releasing its component syndecan-1 and vascular endothelial dysfunction is thought to occur at almost the same time.

In patients with advanced renal dysfunction, dialysis treatment may become necessary. However, dialysis treatment itself is a risk factor that strongly promotes arteriosclerosis. In patients undergoing dialysis therapy, the PBR of sublingual microvessels, as an important indicator for vascular endothelial glycocalyx damage, was enlarged. Moreover, circulating levels of hyaluronan and syndecan-1 were increased, and the activation of hyaluronidase was enhanced in patients with dialysis therapy⁴⁴. Vascular endothelial glycocalyx damage was strongly observed in dialysis patients with high inflammatory biomarkers, and it suggests that the mechanism of atherosclerosis progression and carcinogenesis associated with decreased immune function in dialysis patients may be involved in.

6. Cardiovascular diseases and vascular endothelial glycocalyx

Ischemic heart disease

Circulating syndecan-1 levels were significantly higher in 141 patients with the acute coronary syndrome than that of 45 patients with chest pain syndrome without coronary artery lesions or 24 healthy subjects, and it was significantly higher especially in male patients⁴⁵. The high levels of syndecan-1 in patients with the acute coronary syndrome were also significant after adjusting for sex, age, or present and past smoking⁴⁵. In addition, circulating levels of syndecan-1 were useful to diagnose acute coronary syndrome in these patients. On the other hand, it has been reported that the blood level of syndecan-4 was significantly higher in female patients with acute myocardial infarction⁴⁶. From these results, the measurement of circulating syndecans concentration may be useful as a diagnostic biomarker to detect destabilized coronary plaque.

Compared to the rapid increase of circulating fragmented glycocalyx levels in acute diseases, circulating levels of fragmented glycocalyx was reduced in patients with chronic pathological

conditions such as stable angina⁴⁷. In such a pathological condition, thinning of the glycocalyx layer and reduction of the blood level of fragmented glycocalyx occur simultaneously due to the decreased production and accelerated degradation of the vascular endothelial glycocalyx. Therefore, the measurement of blood glycocalyx concentration is not suitable as a prognosis predictor or a severity index in chronic disease conditions.

In sublingual microvessels, vascular endothelial glycocalyx was thinned according to the severity of atherosclerotic lesions in coronary arteries, and circulating levels of hyaluronic acid in coronary arteries was significantly high in these patients⁴⁸. In patients with ischemic heart disease and its high-risk patients, high PBR was extracted as a high-risk factor for arteriosclerotic cerebrovascular disease and ischemic heart disease, and PBR was associated with systolic blood pressure⁴⁹. High levels of PBR are observed in patients with arteriosclerotic diseases and their high-risk groups, and the PBR measurement could be effective on disease management and screening for cardiovascular diseases. Expectations are growing for the usefulness of PBR measurement as a judgment index.

Cerebrovascular disease

Atherosclerotic cerebrovascular disease is one of the age-related diseases of the central nervous system, is related to vascular endothelial dysfunction, and is considered to be the main cause of dementia. In patients with lacunar infarction with white matter lesions, the PBR was significantly increased than those in healthy individuals or those without white matter lesions⁵⁰. In a time-course analysis of endothelium-related biomarkers (chondroitin sulfate, heparin sulfate, keratan sulfate, hyaluronic acid, CD44, syndecan-1, syndecan-2, syndecan-3, syndecan-4, glypican-1, and biglycan), plasma concentrations of CD44 were significantly correlated with stroke severity in ischemic stroke patients, suggesting a relationship between CD44 and brain tissue damage and infarct size⁵¹. The measurement of PBR in arteriosclerotic diseases may be useful in predicting its severity and prognosis, but further study is necessary.

Hyaluronic acid concentrations in cerebrospinal fluid of 46 patients with cerebrovascular dementia were significantly higher compared to 45 patients with Alzheimer's disease and 26 healthy groups without dementia⁵². Regarding the hyaluronic acid in cerebrospinal fluid, vascular endothelial glycocalyx damage in cerebral blood vessels has been suggested that vascular endothelial glycocalyx damage in the microcirculation of various organs is closely related to the damage of various organs.

On a final note

It is said that vascular endothelial glycocalyx is a primitive barrier function, but the gel-like component consisting of macromolecules such as sugar chains and glycoproteins flexibly change their density and composition according to the state of the living body. Vascular endothelial glycocalyx encapsulates various substances, provides various molecules for maintaining homeostasis in the body, and regulates signals of vascular endothelial cells. Although the direct investigation of the *in vivo* function of vascular endothelial glycocalyx throughout the living body is complicated, vascular endothelial glycocalyx has great potential to explore new strategies for assessing the benefit conditions of our healthy vasculature.

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