Dear readers,

I am honored to have been chosen as the first editor-in-chief of “Vascular Failure”, which serves as the official journal of “Japan Society of Vascular Failure” founded in 2016. “Vascular Failure” is the online peer-reviewed journal that is available to every physician and scientist free of charge, as the editorial team hopes the journal significantly contributes to the management of patients with vascular failure, which will be defined as the following paragraph.

Vascular science includes a wide range of scientific fields from morphological features to functional changes. For example, the most well-known vascular disorder is atherosclerosis. In atherosclerosis, chronic multifactorial injuries cause atheromatous and/or fibrous plaques that can progress to overt diseases of stroke or heart attack. The normal vascular physiologic state consists of normal endothelial and smooth muscle function, as well as proper control and regulation of inflammation, oxidative, and neurohormonal stress. The founding members of our society, Kouichi Node and Teruo Inoue, defined a new clinical entity of “Vascular Failure” as “the integration of all of these vascular abnormalities.” They continued, “Vascular failure is not an anatomical disease but rather a comprehensive syndrome of abnormal vascular function. Vascular failure extends from risk factors to established atherosclerotic disease with arterial stenosis, and further to calcification of the vessel wall or serious vascular events that may be caused by plaque rupture and thromboembolic occlusion.” (J Hypertens. 2006).

Even if the concept of vascular failure is not an anatomical disease and the journal mostly publishes clinical studies, its aims and scope are to publish papers on a wide range of studies, including molecular, cellular, tissue and gross anatomical studies, on normal and experimental animals. Such studies on humans are also acceptable. In addition, functional morphology, biochemical, physiological, and interventional studies are considered for publication as long as they contribute to improve the management of “Vascular Failure”.

I sincerely hope that many excellent manuscripts from many authors are submitted to “Vascular Failure” in the near future.

Bonpei Takase, MD, PhD
Editor-in-chief
“Vascular Failure”
Vascular failure and recent anti-diabetic drugs

Jun-ichi Oyama, MD, PhD and Koichi Node, MD, PhD

Abstract:
Type 2 diabetes mellitus (T2DM) is highly prevalent and is a critical risk factor for cardiovascular (CV) disease, increasing both morbidity and mortality. T2DM is one of the most important classical CV risk factors that promote atherosclerosis. Therefore, it is important for both patients with T2DM and their doctors to identify vascular dysfunction at an early stage of atherosclerosis. Recently, new therapies based on the actions of the incretin hormones and blockade of sodium glucose transporter (SGLT) 2 have become widely used, because they offer advantages over conventional treatments by achieving glycemic control and/or possible reducing CV risks. Many experimental studies have suggested that glucagon-like peptide (GLP)-1 and dipeptidyl peptidase (DPP)-4 inhibitors exert cardioprotective effects on atherosclerosis and cardiac dysfunction both in vitro and in vivo. However, thus far, there is little clinical evidence supporting the efficacy of incretin therapy in patients with CV disease. In contrast, the SGLT2 inhibitor empagliflozin achieved a remarkable reduction in CV-related mortality in a large clinical study. The present review focuses on the effects of GLP-1-related therapies and SGLT2 inhibitors on clinical indices of endothelial function.

Key words:
Vascular failure, Endothelial function, GLP-1, DPP-4 inhibitor, SGLT2 inhibitor

Introduction
Type 2 diabetes mellitus (T2DM) is one of the most important risk factors for the development of cardiovascular (CV) disease, because it promotes both systemic atherosclerosis and lifestyle-associated diseases. Cardiovascular complications are an important cause of morbidity and mortality in patients with diabetes, and endothelial dysfunction is an early indicator of developing cardiovascular complications. Recently, several new classes of antidiabetic drugs, including glucagon-like peptide (GLP)-1, dipeptidyl peptidase (DPP)-4 inhibitors, and sodium glucose cotransporter 2 (SGLT2) inhibitors, which have different mechanisms of action from those of conventional antidiabetic agents, have become widely used. In this article, we review the effects of these agents, with a focus on the significance of endothelial function.

1. Endothelial function

1.1 Regulation of the vascular system
Blood vessels are composed of three layers of tissue and modulate vascular tone and blood flow by constricting or relaxing in response to physical, neurological, and chemical stimuli. The endothelium is a flat monolayer of cells that covers vascular lumina and plays a role as a barrier between blood and the vessel wall, preventing the aggregation of platelets and invasion of macrophages. Vasodilator molecules are released in response to shear stress. Vascular endothelial cells can produce and secrete several vasodilators, including nitric oxide (NO), prostaglandin I2, C-type natriuretic peptide, and endothelium-derived hyperpolarizing factor (EDHF). The cells also produce several vasoconstrictors, including endothelin, angiotensin II, prostaglandin H2, and thromboxane A2. NO in particular plays a significant role in atherosclerosis. Normally, endothelial NO synthase (eNOS) is activated by shear stress caused by blood flow and various agonists that stimu-
late mechanically or bind with receptors on vascular endothelial cells. NO can be produced from L-arginine, and when released, it is transferred to vascular smooth muscle cells in the immediate vicinity by diffusion. It then activates soluble guanylate cyclase in the cell, relaxes vascular smooth muscle, and inhibits chemotaxis and platelet aggregation by increasing the amount of cyclic guanosine 3',5'-monophosphate (cGMP)\(^{13}\).

Thus, normal vascular endothelium functions to regulate and maintain vascular homeostasis through multiple and complex physiological functions, including the release of vasactive factors that regulate vascular tone, blood fluidity, and coagulation, while limiting vascular smooth muscle cell proliferation and inflammation\(^ {14}\).

### 1.2 The concept of vascular failure

Clinically, abnormalities of vascular endothelium are critical in relation to atherosclerosis, hypertension, and diabetes. Oxidative stress, inflammation, obesity, hyperlipidemia, and insulin resistance are major contributors to endothelial dysfunction in cardiovascular disorders. Endothelial dysfunction is strongly associated with aging, atherosclerosis, diabetes, hyperlipidemia, hypertension, and obesity\(^ {15}\).

Imbalances in the production of endothelium-derived relaxing and contracting factors are important contributors to endothelial dysfunction\(^ {16}\). Decreased synthesis and/or increased degradation of NO can contribute to impairment of its bioavailability as a consequence of enhanced production of reactive oxygen species (ROS)\(^ {17}\).

Tetrahydrobiopterin (BH4) binds to NOS as a cofactor and suppresses superoxide production\(^ {18}\). However, insufficient BH4 directs NOS to produce superoxide rather than NO\(^ {19}\), and increased oxidative stress can oxidize BH4, resulting in the uncoupling of eNOS and reduced NO production\(^ {11,12}\). Moreover, the absence of BH4 increases oxidative stress through transfer of electrons to molecular oxygen, forming oxidant species that further consume NO and increase oxidative stress\(^ {2,11,12}\). Given that superoxide converts NO into peroxynitrite, generates hydroxyl radicals, and injures cells, the balance of production and the existence of NO, superoxide, and related factors in vascular endothelial cells play important roles in vascular endothelial injury.

Elevated levels of asymmetric dimethylarginine (ADMA) may reduce NO production, because ADMA is an endogenous inhibitor of eNOS through competition with L-arginine\(^ {20}\). Endothelial dysfunction can affect vasoconstrictor levels, including endothelin-1 and angiotensin II, and other vasodilators, including endothelial-derived hyperpolarizing factor (EDHF) and prostacyclin\(^ {21}\). Therefore, increased ROS, inflammatory, and vasmotor factors and deficiency of NO bioavailability are hallmarks of endothelial dysfunction\(^ {22}\). Dysfunctional endothelial cells may permit increases in leukocyte adhesion, permeability, inflammatory cell infiltration, and lipid deposition, thereby aggravating atherosclerotic plaque formation\(^ {14,15}\). In addition, the proliferation, migration, and apoptosis of endothelial cells are closely related to endothelial dysfunction in cardiovascular disorders\(^ {16,17}\). Because atherosclerosis is characterized as a response to chronic multifactorial injury and inflammation, which leads to the formation of atheromatous or fibrous plaques, vascular endothelial dysfunction occurs in the early stages of the disease and is closely related to it mechanistically.

In addition to endothelial dysfunction, smooth muscle dysfunction, metabolic abnormalities of the vessel wall including inflammation, oxidative stress, and neurohormonal imbalance occur at various stages of atherosclerosis. The concept of “vascular failure” is defined as the integration of these vascular abnormalities\(^ {20}\). Vascular failure is not an anatomical disease but a syndrome of abnormal vascular function beyond endothelial dysfunction, because the vasculature is not merely a system of conduits, but an endocrine organ that regulates the total blood supply to end-organs by contracting and relaxing. Vascular failure encompasses several divergent concepts from risk factors to established atherosclerotic disease with arterial stenosis, and further to calcification of the vessel wall or serious vascular events including plaque rupture and thrombo-embolic occlusion. Therefore, active intervention to correct risk factors is important to treat the condition. Comprehension and therapeutic strategies against vascular failure may be expected to prevent and treat vascular lesions.

**Table 1.** Endothelial function

<table>
<thead>
<tr>
<th>Position</th>
<th>Method</th>
<th>Stimulus</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial artery</td>
<td>SPG</td>
<td>Ach</td>
<td>High specificity</td>
<td>Invasive</td>
</tr>
<tr>
<td>Brachial artery</td>
<td>FMD</td>
<td>Reactive hyperemia</td>
<td>Non-invasive</td>
<td>Low specificity</td>
</tr>
<tr>
<td>Finger</td>
<td>RH-PAT</td>
<td>Reactive hyperemia</td>
<td>Non-invasive</td>
<td>Invasive</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>Flow-wire</td>
<td>Ach</td>
<td>Low specificity</td>
<td>Complicated</td>
</tr>
<tr>
<td>Blood/Urine</td>
<td>Bioactive substance</td>
<td>None</td>
<td>Simple</td>
<td>Supportive tool</td>
</tr>
</tbody>
</table>

**Notes:**
- **SPG:** strain-gauge plethysmography; **FMD:** flow-mediated dilatation; **RH-PAT:** reactive hyperemia peripheral arterial tonometry; **ACh:** acetylcholine
- **Table 1.** Endothelial function

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1.3 Measurement of endothelial function and its clinical utility

Table 1 lists the methods available for evaluating endothelial function. Indirect measures in the peripheral circulation assess the vasodilatory responses of conduit and resistance arteries to stimuli that increase NO release. Endothelial function can be assessed by invasive and non-invasive methods. Invasive angiographic quantification of the changes in vascular diameter in response to intra-coronary infusion of muscarinic receptor agonists such as acetylcholine (ACh) is used to evaluate endothelium-dependent vasodilation. In contrast, endothelium-independent relaxation is measured using an NO donor such as sodium nitroprusside or nitroglycerin, because NO directly acts on vascular smooth muscle cells. Invasive venous occlusion strain-gauge plethysmography (SPG) can be used to evaluate blood flow changes in the forearm circulation following hyperemia or after intraarterial infusion of ACh. However, invasive methods are technically difficult and cannot be used routinely.

Thereafter, flow-mediated dilatation (FMD) is measured in the brachial artery, using high-resolution ultrasonography as a non-invasive measure of endothelium-dependent function. FMD quantifies the transient changes that occur in the diameter of the brachial artery in response to shear stress generated by hyperemia following an induced period of local ischemia. FMD is calculated as the percentage change in brachial artery diameter from baseline in response to the increase in blood flow. Non-invasive ultrasound FMD of the brachial artery is now widely used, although results vary owing to technical issues, and the procedure is not easy to standardize among institutions.

For the assessment of pre-clinical disease, procedures for measuring endothelial function that are simple, non-invasive, reliable, reproducible, and inexpensive are desirable. Recently, reactive hyperemia peripheral arterial tonometry (RH-PAT) has become commercially available to evaluate endothelial dysfunction. RH-PAT is an automated, quantitative, non-invasive, clinical procedure for the digital measurement of peripheral arterial tone, allowing the examination of the entire conduit arterial tree. RH-PAT correlates positively with FMD and inversely with various cardiovascular risk factors and has been shown to have good reproducibility.

1.4 Predictive value of endothelial dysfunction

Endothelial dysfunction not only represents the present status of vascular function but also acts as an independent predictor of future cardiovascular events. Endothelial dysfunction is thought to be reversible and useful as a screening test for pre-arteriosclerosis changes in the vessel wall. Risk of future cardiovascular disease can be assessed by screening for endothelial dysfunction, enabling early intervention to prevent the development of complications. As therapeutic interventions, drug and supplemental therapy, and modifications of lifestyle habits are available, treatment of vascular endothelial dysfunction is of potential value in the primary prevention of atherosclerosis. Therefore, it is important to undertake intensive screening for endothelial dysfunction in the early stages of arteriosclerosis.

2. Diabetes and endothelial dysfunction

2.1 Incretin-related agents on endothelial function

In vitro experiments

Several studies in vitro have found that GLP-1 attenuated tumor necrosis factor-alpha (TNF-α)-mediated induction of plasminogen activator inhibitor-1 expression, advanced glycation end-product (AGE)-induced upregulation of vascular cell adhesion molecule (VCAM)-1, and ROS-induced protein kinase A activation-mediated cell senescence all upregulated the expression of eNOS in human umbilical vein endothelial cells (HUVEC). In cardiac microvascular endothelial cells, glycototoxic-induced ROS production and apoptosis were decreased by GLP-1 with an increase in cAMP/PKA activity. On the other hand, GLP-1 promoted angiogenesis in a dose-dependent manner and restored oxidized LDL-induced loss of cell viability accompanied by a decrease in intracellular NO activity. GLP-1 also improved the proliferation and differentiation of endothelial progenitor cells by increasing vascular endothelial growth factor (VEGF) generation. The proliferation of human coronary artery endothelial cells was promoted by exendin-4, a GLP-1R agonist, through eNOS-, PKA- and PI3K/Akt-dependent pathways via the GLP-1 receptor. Exendin-4 also restored eNOS-induced ROS production due to lipotoxicity and protected against lipoprotein-induced cell apoptosis.

In vivo or ex vivo experiments

Exendin-4 increased NO production, improved endothelium-dependent vasodilation, and reduced the expression of NF-κB via the cAMP or AMPK-eNOS pathways in aortas isolated from obese rats. Exendin-4 also inhibited monocyte adhesion and attenuated the formation of atherosclerotic lesions in apolipoprotein E-deficient (ApoE−/−) mice. Liraglutide improved endothelial function via GLP-
1R, increased the eNOS level, and reduced ICAM-1 expression in the aortic endothelium in mice\textsuperscript{61}. Liraglutide also inhibited the progression of atherosclerotic plaques, and improved plaque stability in ApoE\textsuperscript{-/-} mice, although the extent of endothelial vasodilation induced by ACh was unchanged\textsuperscript{57}. Sitagliptin protected the endothelial function of the renal artery in spontaneously hypertensive rats, and exendin-4 ameliorated endothelial dysfunction in renal arteries from hypertensive patients in an \textit{ex vivo} study\textsuperscript{48}. However, it has been reported that triglyceride-induced endothelial dysfunction was not restored by exendin-4 from rat conduit arteries in \textit{ex vivo}\textsuperscript{50}.

**Clinical data**

Table 2 lists the effects of GLP-1 and GLP-1-related drugs on endothelial function. GLP-1 treatment enhanced endothelium-dependent and endothelium-independent responses to ACh and sodium nitroprusside (SNP) during infusion of insulin in patients with metabolic syndrome by SPG\textsuperscript{51-56}. Several studies have indicated that GLP-1 analogues improved endothelial function\textsuperscript{51,56-60}. However, Kelly et al. demonstrated that exendin-4 did not improve the responses of RH-PAT compared to metformin in patients with impaired glucose tolerance\textsuperscript{67}. In addition, exendin-4 and liraglutide did not improve the FMD responses in patients with T2DM\textsuperscript{51,56,61-63}. Surprisingly, Ayaori et al. reported that sitagliptin and alogliptin led to a deterioration in the FMD response in patients with T2DM\textsuperscript{60}. Two papers reported that sitagliptin and trelagliptin did not improve FMD or RH-PAT responses in patients with T2DM when given for three months\textsuperscript{61,62}. However, sitagliptin, alogliptin, vildagliptin, and tenegliptin improved endothelial dysfunction in most studies\textsuperscript{46,67}. We reported that long-term therapy with sitagliptin failed to improve FMD relative to conventional treatment\textsuperscript{61}. Adding sitagliptin to conventional antihyperglycemic drugs for two years in patients with T2DM did not alter endothelial function measured by FMD. Antihyperglycemic agents including metformin, pioglitazone, and an α-glucosidase inhibitor have been found to improve glycemic control in patients with T2DM\textsuperscript{61,66,68-70}. Based on lowering glucose levels with anti-diabetic drugs, reduction of hyperglycemia may improve endothelial dysfunction clinically. DPP-4 inhibitors may also improve endothelial dysfunction, but possibly not more than conventional agents.

It is generally accepted that correcting blood sugar may improve endothelial dysfunction in T2DM. However, its effectiveness beyond the glycemic control is still debated as all studies included fewer than 50 patients, and some of the trials were non-randomized. Therefore, a carefully designed large randomized trial will be required to clearly define the impact of these agents on endothelial function.

### 2.2 SGLT2 inhibitors and endothelial function

**Preclinical studies**

New anti-diabetic agents target sodium-glucose co-transporter 2 (SGLT2), the main glucose transporter in the kid-

**Table 2. Characteristics of studies included in this review**

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects</th>
<th>Methods</th>
<th>Medication (n)</th>
<th>Control (n)</th>
<th>Duration</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 related drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tesaro M et al. (50)</td>
<td>MetS</td>
<td>SPG</td>
<td>GLP-1 (5)</td>
<td>Saline</td>
<td>30min</td>
<td>Improved (conditional)</td>
</tr>
<tr>
<td>Basu A et al. (51)</td>
<td>T2DM</td>
<td>SPG</td>
<td>GLP-1</td>
<td>Placebo</td>
<td>240min</td>
<td>Improved</td>
</tr>
<tr>
<td>Nystrom T et al. (52)</td>
<td>T2DM</td>
<td>FMD</td>
<td>GLP-1</td>
<td>Saline</td>
<td>115min</td>
<td>Improved</td>
</tr>
<tr>
<td>Koska J et al. (53)</td>
<td>T2DM</td>
<td>RH-PAT</td>
<td>Exendin-4</td>
<td>Saline</td>
<td>210min</td>
<td>Improved</td>
</tr>
<tr>
<td>Ceriello A et al. (54)</td>
<td>T2DM</td>
<td>FMD</td>
<td>GLP-1 (12)</td>
<td>Saline (12)</td>
<td>2h</td>
<td>Improved</td>
</tr>
<tr>
<td>Irace C et al. (55)</td>
<td>T2DM</td>
<td>FMD</td>
<td>Exendin-4 (10)</td>
<td>Glimepiride</td>
<td>16w</td>
<td>Improved</td>
</tr>
<tr>
<td>Ha SJ et al. (56)</td>
<td>Volunteer</td>
<td>FMD</td>
<td>Exenatide (20)</td>
<td>Placebo (20)</td>
<td>30min</td>
<td>Improved</td>
</tr>
<tr>
<td>Kelly AS et al. (57)</td>
<td>IGT</td>
<td>PAT</td>
<td>Exendin-4</td>
<td>Metformin</td>
<td>6m</td>
<td>No change</td>
</tr>
<tr>
<td>Hopkins ND et al. (58)</td>
<td>ObeseT2DM</td>
<td>FMD</td>
<td>Exendin-4 (n=9)</td>
<td>None (0)</td>
<td>6m</td>
<td>No change</td>
</tr>
<tr>
<td>Nomoto H et al. (59)</td>
<td>T2DM</td>
<td>FMD</td>
<td>Liraglutide (16)</td>
<td>Liarglultide</td>
<td>14w</td>
<td>No change</td>
</tr>
<tr>
<td>DPP-4 inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ayaon M et al. (60)</td>
<td>T2DM</td>
<td>FMD</td>
<td>Sitagliptin (66)</td>
<td>Voglibose (24)</td>
<td>6w</td>
<td>Worsened</td>
</tr>
<tr>
<td>Ida S et al. (61)</td>
<td>T2DM</td>
<td>FMD</td>
<td>Sitagliptin (17)</td>
<td>Conventional</td>
<td>2y</td>
<td>No change</td>
</tr>
<tr>
<td>Kubota Y et al. (63)</td>
<td>T2DM</td>
<td>FMD</td>
<td>Trelagliptin (27)</td>
<td>None (0)</td>
<td>12w</td>
<td>No change</td>
</tr>
<tr>
<td>Noda Y et al. (64)</td>
<td>Healthy volunteer</td>
<td>FMD</td>
<td>Sitagliptin (40)</td>
<td>None (0)</td>
<td>12w</td>
<td>Improved</td>
</tr>
<tr>
<td>van Poppel PC et al. (65)</td>
<td>T2DM</td>
<td>SPG</td>
<td>Vildagliptin</td>
<td>Acarbose</td>
<td>4w</td>
<td>Improved</td>
</tr>
<tr>
<td>Nakamura K et al. (66)</td>
<td>T2DM</td>
<td>FMD</td>
<td>Sitagliptin (31)</td>
<td>Voglibose (35)</td>
<td>12w</td>
<td>Improved (in both groups)</td>
</tr>
<tr>
<td>Hashikata T et al. (67)</td>
<td>T2DM</td>
<td>RH-PAT</td>
<td>Tenegliptin (31)</td>
<td>None (0)</td>
<td>3m</td>
<td>Improved</td>
</tr>
</tbody>
</table>

MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; SPG, strain-gauge plethysmography; GLP-1, glucagon-like peptide-1; FMD, flow-mediated dilatation; RH-PAT, reactive hyperemia peripheral arterial tonometry
ney, which is located in the S1 and S2 segments of the proximal tubule and responsible for reabsorption of >90% of glucose in primary urine. SGLT2 inhibitors lower blood glucose by inhibiting the reabsorption of filtered glucose, thereby increasing urinary glucose excretion, which is independent of insulin secretion and action. Generally, SGLT2 inhibitors achieve an emission of 60-100 g/day glucose into urine, equivalent to a caloric loss of 240-400 kcal/day, which can ameliorate systemic glucotoxicity and insulin resistance. In addition to the glycemic pathway, SGLT2 inhibitors are associated with non-glycemic effects, including hemodynamic, metabolic, renal, and neurohormonal effects that decrease blood pressure, weight, visceral adiposity, hyperinsulinemia, arterial stiffness, albuminuria, serum uric acid levels, and oxidative stress.

SGLT2 inhibitors increase urinary glucose excretion and reduce the serum glucose level through energy loss, and can lead to improved metabolism throughout the body. There are few data on pharmacological cardiovascular effects in vivo. Empagliflozin reduced atherosclerosis in vivo by decreasing inflammation and insulin resistance in ApoE-/- mice. Canagliflozin attenuated obesity-induced inflammation in mice. Ipragliflozin ameliorated impaired phosphorylation of Akt and eNOS, reduced ROS generation, monocyte chemotactic protein (MCP)-1, VCAM-1 and ICAM-1, and ameliorated endothelial dysfunction in diabetic mice. Oelze et al. reported that empagliflozin improved endothelium-dependent relaxation in diabetic rats. Thus, on the basis of experimental data, SGLT2 inhibitors may ameliorate endothelial dysfunction through the correction of glucose and other metabolic abnormalities.

Clinical study

Currently, the SGLT2 inhibitors including canagliflozin, dapagliflozin, empagliflozin, ipragliflozin, luseogliflozin, and tofogliflozin are available clinically in Japan. Recently, the EMPA-REG OUTCOME trial showed that empagliflozin greatly improved clinical outcomes in diabetic patients with a high risk of CV. Therefore, it may be possible to determine whether other SGLT2 inhibitors can also improve endothelial function. However, there are no pertinent clinical data currently available regarding endothelial function and we are planning to undertake such studies. The results of our studies will be available in 2018.

3. Limitations

Our study has a limitation. It is a comprehensive review and not a systematic review according to PRISMA guidelines.

4. Conclusions

Vascular failure and subsequent cardiovascular disease are often fatal, and early prevention of cardiovascular complications by ensuring strict glucose control is essential in patients with T2DM. The current findings add to a growing body of evidence that we might be entering a new era of cardiovascular diabetology with new anti-diabetic drugs. On the other hand, several studies contradicted the beneficial effects on the vasculature of incretin-related agents. For the time being, a definite relationship between treatment with glititin or gliflozins and endothelial function remains uncertain and needs to be proven. Future large-scale clinical studies and their sub-analyses will provide evidence on whether treatment with glititin or gliflozins provides clinical benefits for vascular protection beyond glycemic control in patients with T2DM at increased risk of cardiovascular disease.

Conflicts of Interest

JO has no conflicts of interest regarding the content of the manuscript. KN has received honorariums from Boehringer Ingelheim, Daiichi Sankyo, Astellas, MSD, Takeda, Mitsubishi Tanabe, and Sanofi; research grants from Sanwa Kagaku Kenkyusho, Astellas, Takeda, Boehringer Ingelheim, Bayer, Teijin, and Mitsubishi Tanabe.

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YoSAMA J et al.


Recent clinical trial of central hemodynamics

Kazuo Eguchi, MD, PhD

Abstract:
Non-invasive measures of central hemodynamics, such as augmentation index (AI), and central blood pressure (BP) have emerged as a novel and more sophisticated method than brachial BP measurements. For the evaluation of cardiovascular risks and efficacy of medications, central hemodynamics have been shown to be better parameters than peripheral BP. We have introduced these measures of central hemodynamics in our clinical trials and found that 1) patients with type 2 diabetes (DM) had lower rAI but a higher central PP compared to that in the non-diabetes patients, which suggested a proximal conduit-predominant arterial stiffening causing reduced reflection coefficients at the systemic reflection sites: 2) An increased wave reflection caused by the stiffened aorta could be a key factor in the pathophysiology of hypertensive disorders of pregnancy: 3) Central BP compared to brachial clinic BP and home BP during antihypertensive treatment is better in predicting the measures of target organ damage: 4) A very aggressive antihypertensive therapy guided by home morning BP was effective for the change in the central SBP, and was correlated with the change in urinary albumin and PWV: 5) When beta-blockers were additionally used for the treatment of hypertension, bisoprolol achieved a greater reduction in pulse rate and improved baroreflex sensitivity and vascular stiffness, whereas celiprolol reduced the central BP. In conclusion, central hemodynamics might be useful for the evaluation of intra-individual changes, such as drug efficacy or that of some interventions, reflecting the pathophysiological mechanisms of cardiovascular diseases.

Key words:
Central hemodynamics, Augmentation index, Central blood pressure, Cardiovascular diseases

Introduction
Since the Conduit Artery Function Evaluation (CAFE) study, a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)\(^1\), in 2006, non-invasive measures of reflection wave, such as augmentation index (AI), and central blood pressure (BP) have been evaluated more easily in the clinical practice. These measures are expected to be used as a novel and more sophisticated method than brachial BP measurements and to gain more popularity. For the evaluation of cardiovascular risks and efficacy of medications, central hemodynamics have been shown to be better parameters than peripheral BP. However, there have been many problems pointed out\(^2\), including the excessive expectation on this new methodology. We have introduced these measures of central hemodynamics in our clinical trials, and the presentation of these data is still ongoing. In this review, I summarized the results of AI and central BP in our recent clinical studies, while considering the future perspectives on this issue.

Recent Clinical Studies
Twelve prospective cohort studies assessed the relationship between central hemodynamics and prognosis (Table 1). Among them, 10 studies demonstrated a significant relationship between central BP and cardiovascular risk; however, central hemodynamics, including central systolic BP (SBP) and pulse pressure (PP) were not predictors of cardiovascular disease (CVD) in the Framingham study\(^3\). Vlachopoulos et al.\(^4\) performed a meta-analysis on central hemodynamics and prediction of cardiovascular events and all-cause mortality and showed that there was a trend for the central PP to predict CVD events better than the brachial PP. Central hemodynamic parameters are affected by many confounding factors, such as body size, heart rate, and cardiovascular medications; thus, the impact of central hemodynamics on cardiovascular diseases may vary on the basis of the characteristics of the subjects. Even in such varying populations, ranging from the general population to hemo-
dialysis patients, central hemodynamics were effective in predicting CVD events. However, in treated and/or untreated hypertensive subjects, the clinical significance of central hemodynamics has not been established because of many confounding factors. Therefore, we performed the “Antihypertensives and Blood Pressure of Central artery in Japan II (ABC-J II) Study” to evaluate the predictive values of central BP for cardiovascular events in treated Japanese hypertensives and Blood pressure of Central artery in Japan II (ABC-J II) Study by analyzing aortic stiffness and wave reflection in diabetes mellitus (DM). We sought to clarify whether rAI is truly lower in patients with DM than in those without DM who have cardiovascular risk factors. In the ABC-J II study, the brachial systolic blood pressure (cSBP) and central BP were measured in 5,631 participants with antihypertensive medications at 27 institutions in Japan; a total of 3,564 subjects were analyzed. The main results are not published yet; however, this study will provide some information on the significance of central BP in the clinical practice.

Our Own Studies

1. Why the Radial Augmentation Index (rAI) is Low in Patients with Diabetes Mellitus (DM): The Japan Morning Surge Home Blood Pressure (J-HOP) study

rAI, a marker of wave reflection, has been reported to be paradoxically lower in patients with DM than in those without DM, although the atherosclerotic change is usually progressed in DM. We sought to clarify whether rAI is truly lower in patients with DM than in those without DM who have cardiovascular risk factors.

This study was a subanalysis of the J-HOP study, a prospective observational study, which evaluated the predictive values of home BP for cardiovascular events in Japanese subjects with any of the cardiovascular risk factors, such as hypertension, impaired glucose tolerance or DM, dyslipidemia, smoking (including those with chronic obstructive pulmonary disease), chronic renal disease, atrial fibrillation, metabolic syndrome, or sleep apnea syndrome. Among the 4,000 subjects in the J-HOP study, radial applanation tonometry was performed in 1,787 subjects, consisting of 449 patients with DM and 1,338 without DM. rAI was determined as follows: [late systolic shoulder pressure amplitude (PP2) − radial PP (rPP)]. The late systolic shoulder BP (SBP2) and PP2 of a radial pressure wave were used as estimates of the central SBP and central PP (cPP), respectively. Although the age (65.8±9.8 vs. 65.8±12.1 yrs) and mean brachial SBP (141±16 vs. 141±17 mmHg) were similar between the DM and non-DM groups, the rAI was significantly lower in the DM group (83.3±14.1 vs. 87.3±15.7%, p <0.001), but clinic PP (62±14 vs. 59±14 mmHg, p<0.001) and cPP (51±15 vs. 49±15 mmHg, p=0.019) were significantly greater in the DM group than in the non-DM group, but cSBP were similar (Figure 1). In the multivariable analyses adjusted for covariates, the significant determinants of rAI were the estimated glomerular filtration rate (eGFR) (β=0.17, P<0.001) in the DM group and the log-transformed homeostatic model assessment of insulin resistance (HOMA-IR) (β=−0.15, P<0.001) in the non-DM group. Similar trends were also observed for central SBP and cPP. It was concluded that the lower rAI in the DM group was associated with a higher cPP compared to that in the non-DM group, which suggests a proximal conduit-predominant arterial stiffening caused reduced reflection coefficients at the systemic reflection sites. Even when the renal dysfunction is only moderate, the related increase in cPP may overcome the increase in augmentation pressure in the DM group. Therefore, in DM, cPP may be more important than central SBP.

2. Changes in Central Hemodynamics in Hypertensive Pregnancy from before to after Delivery

Hypertensive disorders of pregnancy (HDPs) can cause pregnancy-associated complications, such as preterm birth, a small-for-gestational-age (SGA) infant, intrauterine growth restriction, and placental abruption. Although various causal factors of HDP have been examined over many years, the factor that best predicts HDP is still undetermined. Augmen-
Comparisons of central hemodynamics between the diabetes mellitus (DM) and the non-DM groups. Radial augmentation index (rAI) was significantly lower in the DM group, and central PP was significantly greater in the DM group than in the non-DM group, but central BP was similar between the groups. Values are mean ± SEM. [Reference 7]

Changes in central pulse pressure (PP) and aortic augmentation index (AIx) before and after delivery. AIx@75 (AIx adjusted by heart rate 75 bpm) and central PP were higher in the HDP group than in the control group, but both parameters declined after delivery until they were similar to the controls. [Reference 9]

AIx@75 and central PP were higher in the HDP group than in the control group; however, both parameters decreased after delivery until the levels became similar to those of the control group. AIx@75 and central PP were higher in the HDP group than in the control group; however, both parameters decreased after delivery until the levels became similar to those of the control group. AIx@75 and central PP were higher in the HDP group than in the control group; however, both parameters decreased after delivery until the levels became similar to those of the control group. AIx@75 and central PP were higher in the HDP group than in the control group; however, both parameters decreased after delivery until the levels became similar to those of the control group.
reflection caused by the stiffened aorta could be a key factor in the pathophysiology of HDP.

The central hemodynamic parameters were elevated in the patients with HDP but returned to normal after delivery. Opposite findings were observed in the control group. Because the CO and TPR did not significantly change before and after delivery, increased body fluid or vasoconstriction might not be the main factor. Other factors that increased the functional stiffness of the mid to large arteries, such as angiogenic factors, sex hormones, or nitric oxide sensitivity, could have also influenced the central hemodynamics in the patients with HDP. In addition to endothelial dysfunction, which plays a central role in the pathogenesis of HDP, the changes in the characteristics of central hemodynamics were clarified in this study.

**3. Correlation of Central Blood Pressure to Hypertensive Target Organ Damage (TOD) During Antihypertensive Treatment: The Japan Morning Surge-Target Organ Protection (J-TOP) Study**

This study aimed to determine whether central BP compared to brachial clinic BP and home BP during antihypertensive treatment is better in predicting the measures of TOD, such as urinary albumin/creatinine ratio (UACR) and left ventricular mass index (LVMI). This study was a subanalysis of the J-TOP study, an open-label randomized multicenter trial investigating the effects of the time of administration of candesartan, an angiotensin II receptor blocker (ARB)\(^1\)

In 180 hypertensive patients (aged 68.7±12.1 y) during the 6-mo treatment with either bedtime or awakening dosing of candesartan (plus diuretics as needed), significant reductions were found in the central SBP, UACR, and LVMI (all P<0.001). In the multivariable analyses, the decrease in the central SBP was associated with those of the log-transformed UACR (β=0.24, P<0.01) and LVMI (β=0.23, P=0.04), independent of the decrease in both clinic and home SBP. The goodness-of-fit of the association between the reduction in the SBP and UACR (P<0.01) or LVMI (P=0.04) improved by adding the central SBP to the SBP measurement. Therefore, the change in the central BP could be an important therapeutic target during antihypertensive treatment, in addition to peripheral clinic and home BP. In the treatment of hypertension, therapies that lower central BP, in addition to clinic and home BP, may be effective in protecting against TOD.

**4. Aggressive Blood Pressure-Lowering Therapy Guided by Home Blood Pressure Monitoring Improves Target Organ Damage in Hypertensive Patients With Type 2 Diabetes/Prediabetes**\(^2\)

This study tested the hypothesis that a very aggressive antihypertensive treatment guided by home morning BP monitoring (i.e., home morning BP <125/75 mmHg) is effective in improving the measures of TOD in patients with type 2 DM/prediabetes. We enrolled 60 patients with uncontrolled hypertension (i.e., home morning SBP >135 mmHg) and DM/prediabetes and performed clinic, home, and ambulatory BP monitoring at baseline and after 6 mo. Irbesartan (ARB), amlopidine [calcium channel blocker (CCB)], and indapamide (diuretic) were used in accordance with a titration schedule from steps 1 to 5 for a target home BP level <125/75 mmHg. The flow-mediated vasodilation (FMD), rAI, pulse wave velocity (PWV), and UACR, as a surrogate marker of TOD, were also measured at baseline and after 6 mo. The mean age of the patients was 62.6±9.4 y, and 51.7% were men.

Compared with baseline, clinic (clinic SBP: from 147±18 mm Hg to 125±15 mm Hg, P<0.001), home (home morning SBP: from 145±17 mm Hg to 128±11 mm Hg , P<0.001), and ambulatory SBP (24-hour SBP: 138±13 mm Hg to 125±11 mm Hg , P<0.001) measures, as well as DBP measures, were significantly lowered after sixth months. Central SBP (i.e. SBP2) also decreased from 132±20 to 112±13 mmHg (P<0.001) (Figure 3). FMD increased significantly, and rAI (Figure 3), PWV, and UACR significantly decreased using the treatments. Therefore, a very aggressive antihypertensive therapy guided by home morning BP was effective for the
surrogate end points in the patients with DM/prediabetes. The change in the central SBP was not correlated with the change in the FMD but was correlated with the change in urinary albumin and PWV. Because of the limited number of subjects, whether central BP measures are superior to clinic or home BP measures is unknown; however, in cases of large fluctuations in clinic or home BP measures, central BP measures would be effective in predicting the actual antihypertensive effects.

5. Effects of Celiprolol and Bisoprolol on Blood Pressure, Vascular Stiffness, and Baroreflex Sensitivity

The antihypertensive effect of beta-blockers, such as atenolol, has been shown to be insufficient, and the protection against cardiovascular events was inferior to those of other drugs. Therefore, beta-blockers were expelled from among the first choice of hypertensive medications and were even deemed harmful in patients without heart diseases. On the other hand, 3rd-generation beta-blockers, such as nebivolol, carvedilol, and celiprolol, have been reported to not influence metabolism but have significant effects on vascular functions in patients with essential hypertension. In this study, we investigated whether a vasodilating beta-blocker, celiprolol, and non-vasodilating beta-blocker, bisoprolol, would have metabolic effects and differential effects on BP and vascular functions, such as endothelial function and vascular stiffness.

We enrolled 102 hypertensive subjects (mean age: 59±14 y) treated with medications other than beta-blockers. The subjects were randomized to receive an add-on treatment with either celiprolol 100-200 mg (C group) or bisoprolol 2.5-5 mg (B group) and followed up for 3 mo. In addition to clinic, home, and ambulatory BP monitoring, the FMD, rAI, brachial-ankle PWV (baPWV), UACR, and BRS were measured at baseline and at the end of the study.

Compared to the baseline values, home and 24-h BPs significantly decreased in the third mo in both groups (all P<0.05). Pulse rates (PR) and baPWV decreased (P<0.001), and BRS increased significantly only in the B group (P=0.02). rAI was unchanged in the C group but significantly increased in the B group (P<0.001). Central BP significantly decreased in the C group (P=0.003) but was unchanged in the B group. FMD significantly increased in both groups (both P<0.01) (Figure 4).

Bisoprolol achieved a greater reduction in PR and improved BRS and vascular stiffness, whereas celiprolol reduced the central BP. Thus, in treating hypertensive patients,
an add-on use of celiprolol may be favorable in the uncomplicated stage of hypertension. On the other hand, bisoprolol may be useful in hypertensive patients with cardiac or vascular diseases who have advanced atherosclerotic changes and sympathetic nervous system activation. Recently, the decrease in PR with ivabradine was associated with an increase in central SBP in patients with coronary artery disease. However, in our study, such an effect was not observed in the hypertensive subjects, and the treatments did not cause a cardiovascular overload.

**Conclusion**

The clinical studies that evaluated central hemodynamics were described. There are several confounding factors that influence these measures; thus, central hemodynamics are not always suitable for cross-sectional studies. However, central hemodynamics might be useful for the evaluation of intra-individual changes, such as drug efficacy or that of some interventions. The concept of central hemodynamics is attractive when considering the pathophysiological mechanisms of cardiovascular diseases. Further studies, especially prospective outcome studies, are needed to establish the clinical significance of central hemodynamics.

**Conflicts of Interest**

None.

**References**


Effects of purified eicosapentaenoic acid on red blood cell distribution width and vascular endothelial function in patients with coronary artery disease

Emi Tajima, MD, Shichiro Abe, MD, Ryo Watanabe, MD, Yota Koyabu, MD, Fumiya Saito, MD, Hiroyuki Kaneda, MD, Masashi Sakuma, MD, Shigeru Toyoda, MD and Teruo Inoue, MD

Abstract:

Background: Highly purified eicosapentaenoic acid (EPA) is a promising agent for the secondary prevention of coronary artery disease. Red blood cell distribution width (RDW), a measure of red blood cell size heterogeneity, has been shown to be associated with the risk of cardiovascular events in patients with coronary artery disease. In the present study, we investigated whether EPA affects red blood cell size heterogeneity and vascular endothelial function in patients with coronary artery disease. Methods and Results: Among the 30 patients with coronary artery disease with an EPA/arachidonic acid (AA) ratio <0.4, 19 patients were administered with EPA (1800 mg/d) (EPA group); the remaining 11 patients received no intervention (control group). During a follow-up period of 36±14 mo, the EPA/AA ratio increased in the EPA group (P<0.001) but did not change in the control group. The flow-mediated dilatation (FMD) value also increased in the EPA group (P<0.05) but not in the control group. The RDW value did not change in either group. In 15 patients with a baseline RDW value greater than the median (12.8), 8 in the EPA group had a decreased RDW value (P<0.05), while 7 in the corresponding control group had an unchanged RDW value. In all patients, the change in FMD was negatively correlated with that in RDW (R=−0.39, P<0.05). Conclusions: EPA improved red blood cell size heterogeneity as well as vascular endothelial function in patients with coronary artery disease. The improvements were correlated with each other.

Key words:
Coronary artery disease, Eicosapentaenoic acid, Red blood cell distribution width, Flow-mediated vasodilation, Eicosapentaenoic acid/arachidonic acid ratio

Introduction

Recent evidence has suggested that n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), contribute to cardiovascular risk reduction1,2). The Japan Eicosapentaenoic acid Lipid Intervention Study (JELIS) has shown that administration of a highly-purified EPA agent in addition to statins is a promising treatment to prevent coronary events in hypercholesterolemic patients with coronary artery disease, peripheral artery disease, metabolic dyslipidemia, and/or impaired glucose metabolism3). Although EPA evidently prevents cardiovascular events, its mechanism is not understood. EPA has various pleiotropic actions, such as anti-inflammatory effects4), improvements in vascular endothelial dysfunction5), and cardio-protective effects6) in addition to anti-platelet actions7) that are well established. Nevertheless, it is unclear whether the apparent effects of EPA depend on these pleiotropic effects.

Red blood cell distribution width (RDW), measured using a blood analyzer, is a parameter of anisocytosis, i.e., the size heterogeneity of peripheral red blood cells. Currently, RDW is primarily used to analyze the type of anemia and to aid in the differential diagnosis of various leukemic conditions8). Recently, RDW has been shown to be associated with coronary artery disease9), peripheral artery disease10), and stroke11). An elevated RDW value may be associated with an increased risk of cardiovascular events in patients with coro-
Red blood cell size heterogeneity and vascular endothelial dysfunctions, dyslipidemia, smoking, and metabolic syndrome with atherosclerotic risk factors, such as hypertension, diabetes, dyslipidemia, smoking, and metabolic syndrome.

The Tochigi Ryomo EPA/AA Trial in Coronary Artery Disease (TREAT-CAD) is our ongoing clinical trial, in which we are investigating the serum EPA/arachidonic acid (AA) ratio in patients with coronary artery disease living in Tochigi prefecture and assessing the efficacy of intervention with highly purified EPA in patients with a low EPA/AA ratio. In the TREAT-CAD trial, we recruited patients with confirmed or suspected coronary artery disease who had undergone diagnostic coronary angiography and registered participants after obtaining informed consents. We measured the serum EPA/AA ratio in all registered patients. Patients who had taken EPA or DHA as agents or dietary supplements were excluded from the registry. We then performed an intervention study using EPA in the patients with angiographically verified coronary artery disease, defined as the presence of at least 1 stenotic lesion (>75% stenosis), in the following manner. The patients with coronary artery disease with an EPA/AA ratio <0.4 were randomly assigned to receive either 1800 mg/d EPA (EPA group) or no intervention (control group). The patients with EPA/AA ratios ≥0.4 were observed without EPA administration (observation group). From the patients recruited from the TREAT-CAD intervention arm, we selected 30 patients (25 men and 5 women, aged 67±9 y) with an EPA/AA ratio <0.4, in whom both RDW and FMD values could be measured at baseline and after a follow-up period of 36±14 mo for the present study. The study was initiated at least 2 wk after the percutaneous coronary intervention (PCI) in the patients with chronic coronary artery disease and 4 wk after its onset in the patients with acute coronary syndrome. Of these 30 patients, 19 (16 men and 3 women, aged 67±9 y) were included in the EPA group and the remaining 11 (9 men and 2 women, aged 67±11 y) in the control group. All patients were administered statins at the initiation of this study as a baseline treatment. In all patients, the serum EPA/AA ratio was measured again at the end of the follow-up period. The study was approved by the ethics committee of Dokkyo Medical University.

Measurement of EPA/AA ratio

The serum fatty acids were assayed using gas chromatography (SRL, Tokyo, Japan). Briefly, the total plasma lipids were extracted in accordance with the procedure by Folch, which underwent hydrolysis to release free fatty acids. The free fatty acids were then esterified using potassium methoxide/methanol and boron trifluoride/methanol. The methylated fatty acids were analyzed using a GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an omega-wax-250 capillary column (SUPELCO, Sigma-Aldrich Japan, Tokyo, Japan). Reproducibilities (i.e., coefficients of variation) of the measurements of serum EPA and AA using this method have been reported to be 4.4% and 3.8%, re-

Table 1. Baseline characteristics of the EPA group and control group

<table>
<thead>
<tr>
<th>Description</th>
<th>EPA group (n=19)</th>
<th>Control group (n=11)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age: y</td>
<td>67±9</td>
<td>67±11</td>
<td>0.85</td>
</tr>
<tr>
<td>Male sex: n (%)</td>
<td>16 (84)</td>
<td>9 (82)</td>
<td>0.86</td>
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<tr>
<td>Affected vessels: n (%)</td>
<td></td>
<td></td>
<td>0.56</td>
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<tr>
<td>Single-vessel disease</td>
<td>10 (53)</td>
<td>7 (64)</td>
<td></td>
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<tr>
<td>Multi-vessel disease</td>
<td>9 (47)</td>
<td>4 (36)</td>
<td></td>
</tr>
<tr>
<td>Basal disease: n (%)</td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Chronic coronary artery disease</td>
<td>6 (32)</td>
<td>4 (36)</td>
<td></td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
<td>13 (68)</td>
<td>7 (64)</td>
<td></td>
</tr>
<tr>
<td>Risk factor: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (58)</td>
<td>7 (64)</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (37)</td>
<td>4 (36)</td>
<td>0.98</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>8 (42)</td>
<td>7 (64)</td>
<td>0.26</td>
</tr>
<tr>
<td>Smoking</td>
<td>13 (68)</td>
<td>7 (64)</td>
<td>0.73</td>
</tr>
<tr>
<td>White blood cell count: μl</td>
<td>7242±2013</td>
<td>7290±2037</td>
<td>0.94</td>
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<tr>
<td>Red blood cell count: x10^12 μl</td>
<td>438±47</td>
<td>443±62</td>
<td>0.79</td>
</tr>
<tr>
<td>Hemoglobin: g/dL</td>
<td>13.6±1.4</td>
<td>13.3±2.3</td>
<td>0.63</td>
</tr>
<tr>
<td>Hematocrit: %</td>
<td>40.0±3.9</td>
<td>40.0±5.8</td>
<td>0.90</td>
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<tr>
<td>Platelet count: x10^12 μl</td>
<td>22±52</td>
<td>24±88</td>
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<tr>
<td>Albumin: g/dL</td>
<td>4.0±0.4</td>
<td>4.0±0.4</td>
<td>0.52</td>
</tr>
<tr>
<td>LDL cholesterol: mg/dL</td>
<td>75±20</td>
<td>88±25</td>
<td>0.22</td>
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<tr>
<td>HDL cholesterol: mg/dL</td>
<td>41±11</td>
<td>46±11</td>
<td>0.73</td>
</tr>
<tr>
<td>Triglyceride: mg/dL</td>
<td>117±52</td>
<td>120±49</td>
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</tr>
<tr>
<td>Hemoglobin A1C: %</td>
<td>6.3±0.8</td>
<td>6.0±1.7</td>
<td>0.25</td>
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<tr>
<td>EPA/AA ratio</td>
<td>0.24±0.06</td>
<td>0.27±0.10</td>
<td>0.38</td>
</tr>
<tr>
<td>RDW: %</td>
<td>12.9±0.7</td>
<td>13.3±0.9</td>
<td>0.16</td>
</tr>
<tr>
<td>FMD: %</td>
<td>4.7±2.4</td>
<td>5.8±3.8</td>
<td>0.35</td>
</tr>
<tr>
<td>Use of ACE inhibitors/ARBs</td>
<td>19 (100)</td>
<td>10 (91)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

LDL, low-density lipoprotein; HDL, high-density lipoprotein; EPA, eicosapentaenoic acid; EPA/AA, EPA/arachidonic acid; RDW, red blood cell distribution width; FMD, flow-mediated dilatation; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker.
Eicosapentaenoic acid and red cell distribution width

Figure 1. Changes in the EPA/AA ratio (A) and FMD value (B).
The EPA/AA ratio significantly increased during the follow-up period compared to the baseline value in the EPA group but did not change significantly in the control group. The FMD value significantly increased during the follow-up period compared to the baseline value in the EPA group but did not change significantly in the control group. The data were expressed as mean±SDs.

EPA, eicosapentaenoic acid; EPA/AA, EPA/arachidonic acid; FMD, flow-mediated dilatation

Figure 2. Changes in the RDW values in all patients (A) and in those whose baseline RDW values exceeded the median value of 12.8 (B).
The RDW value did not change significantly during the follow-up period in either the EPA or control groups. However, in the 15 patients whose baseline RDW values exceeded the median of 12.8, the value decreased significantly during follow-up in the EPA group but did not change in the control group. The data were expressed as mean±SDs.

RDW, red blood cell distribution width; EPA, eicosapentaenoic acid
In all patients, the change in the FMD value (value at the end of follow-up minus baseline value) was negatively and significantly correlated with that in the RDW value but not with that in the EPA/AA ratio. FMD, flow-mediated dilatation; RDW, red blood cell distribution width; EPA/AA, eicosapentaenoic acid/ arachidonic acid.

Measurement of the RDW value and other laboratory parameters

Venous blood samples were drawn, and the mean corpuscular volume (MCV) of the red blood cells was calculated using a fully automated blood cell counter (SYSMEX 1000 hematology analyzer; TOA Medical Electronics, Kobe, Japan). The RDW value was calculated as the standard deviation of MCV divided by MCV × 100 (%). The levels of low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol were determined using the homogenous assay. The triglyceride level was measured using an enzymatic technique. Glycosylated hemoglobin A1c (HbA1c) was measured using a high-performance liquid chromatography.

Statistical analysis

First, the normality of the distribution of continuous variables was assessed using the Shapiro-Wilk test. Since all continuous variables were normally distributed, the values were expressed as mean ± SDs, and inter- and intra-group comparisons were performed using the Student’s unpaired and paired t-tests, respectively. The chi-square test was used for an inter-group comparison of categorical variables. The Pearson correlation coefficient was used to assess the relationship between 2 parameters. A P value of <0.05 was considered significant.

Results

Baseline characteristics

The baseline characteristics were compared between the EPA and control groups (Table 1). Age, sex, affected vessel...
number, basal coronary artery disease, risk factors and laboratory data such as blood cell count, serum albumin level and parameters for lipid and glucose metabolism were comparable between the 2 groups. The baseline values of EPA/AA ratio, RDW and FMD were also comparable between the 2 groups.

**Changes in the EPA/AA ratio and RDW and FMD values**

The EPA/AA ratio significantly increased at the end of the follow-up period compared with the baseline value in the EPA group (0.24±0.06 to 1.04±0.37, P<0.001); no significant change was observed in the control group (0.27±0.10 to 0.33±0.14) (Figure 1A). The FMD value significantly increased during the follow-up period compared with the baseline value in the EPA group (4.7±2.4 to 5.6±2.2, P<0.05); no significant change was observed in the control group (5.8±3.8 to 6.1±2.6) (Figure 1B). The RDW value did not change significantly in either the EPA group (12.9±0.7 to 12.8±0.7%) or the control group (13.3±0.9 to 13.6±0.7%) (Figure 2A). When the patients were divided into 2 groups on the basis of the median value of the baseline RDW (12.8%) and when the change in the RDW value was assessed in the 15 patients with a baseline RDW value >12.8%, the value decreased significantly during the follow-up period in the EPA group (n=9, 8 men and 1 women, aged 65±7 y) (13.5±0.5 to 13.1±0.5%, P<0.05) but did not change in the corresponding control group (n=7, 5 men and 2 women, aged 66±13 y) (13.7±0.8 to 14.2±2.9%) (Figure 2B).

**Correlations between the changes in the EPA/AA ratio and RDW and FMD values**

In all patients including the EPA and control groups, the change in the value (value at the end of follow-up minus baseline value) of FMD was negatively and significantly correlated with the change in the value of RDW (R=-0.39, P<0.05) (Figure 3A), but not with that in the EPA/AA ratio (R=0.26) (Figure 3B). The changes in the EPA/AA ratio and RDW value were not correlated with each other (R=0.13).

**Discussion**

The data of the present study were collected from 30 patients with coronary artery disease among the candidates of the TREAT-CAD trial, in whom both baseline and follow-up values of RDW and FMD could be obtained. The major finding was that EPA increased the FMD value during 36±14 mo of observation in all patients and decreased the RDW value in those who had a high baseline RDW value. Additionally, the change in the value of FMD in all patients was correlated with that in the value of RDW.

RDW is a simple measurement of red blood cell size heterogeneity, which is easily and inexpensively calculated from the MCV, and most hematological analyzers automatically provide the RDW value within the complete blood cell count. Many atherosclerotic cardiovascular diseases, including coronary artery disease, peripheral artery disease, and stroke are often associated with an elevated RDW value. Lippi et al. found in a study of consecutive patients admitted to an emergency department with chest pain that the RDW value was higher in patients with acute coronary syndrome than in those with non-acute coronary syndrome. Cemin et al. found that RDW was a significant predictor of acute myocardial infarction among patients admitted to an emergency department due to chest pain of suspected cardiac origin. Isik et al. showed that patients with angiographic coronary artery disease had significantly higher RDW values than those without coronary artery disease, and that the RDW value was an independent predictor of not only the presence of coronary artery disease but also its severity as demonstrated by the SYNTAX (Synergy Between PCI With Taxus and Cardiac Surgery) score. In addition, Zalawadiya et al. observed in the National Health and Nutrition Examination Surveys that an elevated RDW value was a powerful and independent predictor of future risk of coronary artery disease onset.

Although the elevated RDW value in cardiovascular diseases has been previously described many times, its mechanism is not established. One possibility is that hypoxia in cardiovascular diseases promotes erythropoietin secretion, thereby stimulating erythropoiesis and increasing the release of enlarged red blood cells from the bone marrow. It is also possible that the elevated RDW value is due to a slight reduction in red blood cell turnover. Since the size of the red blood cells gradually decreases with age, a reduction in red blood cell turnover might allow smaller cells to persist in the circulation. In addition, the chronic inflammatory state that accompanies cardiovascular diseases may be a powerful erythropoiesis modulator. Proinflammatory cytokines may inhibit erythropoietin secretion and red blood cell maturation, thereby enhancing the elevation of RDW values. However, it remains unclear whether the association between the elevated RDW value and cardiovascular diseases is causal or a consequence of the disease. Currently, it seems reasonable to hypothesize that RDW may be an epiphenomenon rather than a direct player in the pathogenesis of cardiovascular diseases.

Recently, Takahashi et al. reported the effect of purified EPA on RDW in patients with coronary artery disease. In that study, EPA was found to have the potential to reduce RDW values in patients with coronary artery disease, and the effect was greater in diabetic patients with elevated high sensitivity C-reactive protein levels. In the present study, EPA also reduced the RDW values in the patients whose baseline RDW values exceeded the median value of 12.8, while the effect was not significant in other patients with coronary artery disease. Regarding the possible mechanisms of RDW value reduction by EPA, Takahashi et al. suggested that it occurs through a process in which EPA is taken up into, and stabilizes, the red blood cell membrane.
A higher intake of saturated fatty acids increases the cell membrane content of total saturated fatty acids and is associated with an increased incidence of coronary artery disease. It has also been reported that EPA taken up into the red blood cell membrane improves cell deformability by stabilizing the membrane. Furthermore, an increased red blood cell deformability might be associated with low RDW values. Therefore, it is suggested that the reduction of cardiovascular events by the treatment with EPA may be mediated by the reduction in the RDW value via the stabilization of the red blood cell membrane.

Although our result of RDW value reduction by EPA only confirms previous data by Takahashi et al., our finding of a negative correlation between the changes in the RDW and FMD values is a new observation. It is well known that EPA improves vascular endothelial function. In addition to that effect, the potential of EPA to improve red blood cell size heterogeneity has been demonstrated in the present study. Regarding the causal relationship between FMD and RDW, we hypothesized at first the possibility that the heterogeneity of red blood cell size could affect vascular endothelial function. However, there have been no reports that discuss this possibility. Therefore, it seems plausible that both increased FMD and reduced RDW values are consequences of EPA treatment without a causal relationship. In contrast, we observed that neither the change in the FMD nor RDW values was correlated with that in the EPA/AA ratio. This result suggests that the improvement in both vascular endothelial function and red blood cell size heterogeneity by EPA may be a part of its pleiotropic effects.

This study has several potential limitations. First, the overall sample size was small (n=30), and the sample size for the subgroup analysis of the patients with baseline RDW values above the median value was even smaller. Second, a previous study reported that high RDW value as greater than 14% was associated with atherosclerosis. In our study, however, the RDW values were relatively low in most of subjects, so we examined the effects of EPA in patients with baseline RDW exceeded the median value of 12.8. Similar examination in the population with still higher RDW such as baseline value >14% would be promising. Third, the RDW value is considered to be affected by various confounding factors such as age, gender, hemoglobin, albumin, smoking, etc. Although the parameters for these factors were similar between the EPA and control groups, RDW value should be analyzed under adjustment by these confounding factors. Finally, the number of patients in the EPA group (n=19) differed from that in the control group (n=11). The study was performed as a retrospective subanalysis of the TREAT-CAD trial, and the data were collected in the relatively few patients in whom both baseline and follow-up values of RDW and FMD could be obtained. In the TREAT-CAD trial, RDW was not an essential parameter but was measured incidentally. A prospective trial with a large sample size targeting the RDW is warranted. Nevertheless, we believe that the present study provides novel information on the effects of EPA on the secondary prevention of coronary artery disease.

Conflicts of Interest
T.I. and S.A. received honoraria as lecture fees from Mo-chida Pharmaceutical Co., Ltd., Tokyo, Japan.

References


Respiratory diseases and vascular failure

Tomohiro Handa, MD, PhD\(^1\) and Kiminobu Tanizawa, MD, PhD\(^2\)

Abstract:

Patients with chronic respiratory diseases (e.g., chronic obstructive pulmonary disease, interstitial pneumonia, and sleep apnea syndrome) have common risk factors for atherosclerosis, including advanced age, smoking, chronic inflammation, and continuous or intermittent hypoxia. Previous epidemiological studies have revealed that patients with these diseases have an increased risk of atherosclerosis and vascular events. These comorbidities are also associated with poor survival; however, the impact of the treatment for vascular diseases on the prognosis of patients with respiratory diseases remains unclear. Further investigation is required to elucidate the mechanisms and establish treatment strategies for vascular failure associated with respiratory diseases.

Key words:
Chronic obstructive pulmonary disease, Coronary artery disease, Endothelial dysfunction, Interstitial pneumonia, Sleep apnea syndrome

1. Introduction

Atherosclerosis is considered an inflammatory disease initiated by endothelial dysfunction followed by adhesion of leukocytes or platelets to the endothelium, formation of cytokines and growth factors, migration and proliferation of smooth-muscle cells, and thickening of the artery wall. Smoking, hypertension, glucose intolerance, hyperlipidemia, and obesity are known risk factors for atherosclerosis, and chronic inflammation and hypoxemia can also accelerate the disease. Patients with chronic respiratory diseases, such as chronic obstructive pulmonary disease (COPD), bronchial asthma, interstitial lung disease (ILD), and sleep apnea syndrome (SAS) share common risk factors for atherosclerosis, including advanced age, smoking history, chronic inflammation, oxidative stress caused by intermittent hypoxia, and enhanced sympathetic activity (Figure 1). It is recognized that these respiratory diseases are associated with an increased risk of developing atherosclerosis and vascular events (Table 1). This article summarizes the current knowledge regarding the pathophysiology, epidemiology, and clinical impact of vascular failure characterized by respiratory diseases.

2. COPD

2-1. COPD and comorbidity

COPD is characterized by an airflow limitation that is not fully reversible. The airflow limitation is typically progressive and associated with an abnormal inflammatory response to noxious particles or gases within the lungs. COPD is the fourth leading cause of death in Japan and is often observed in combination with other respiratory diseases, such as interstitial pneumonia (combined pulmonary fibrosis and emphysema [CPFE]) or bronchial asthma (Asthma-COPD Overlap Syndrome [ACOS]).

COPD is recognized as a systemic disease, since it is often accompanied by a variety of comorbidities, including cardiovascular disease, osteoporosis, depression, malnutrition, diabetes, and lung cancer. More than 95% of patients with COPD have at least 1 comorbidity, and over 50% have 4 or more coexisting diseases. COPD is more prevalent in smokers, men, and patients who also have other risk factors for atherosclerosis (e.g., chronic inflammation and hypoxemia). Therefore, atherosclerosis is also an important comorbidity associated with COPD.
2-2. Endothelial dysfunction and the development of atherosclerosis in COPD

The endothelium acts to maintain vascular homeostasis through multiple complex interactions with cells within the vasculatures. Moreover, it regulates vascular tension by controlling vasodilators and vasoconstrictors and maintains blood fluidity through the production of factors that regulate platelet activity and the coagulation and fibrinolytic system. In addition, the endothelium can produce cytokines and adhesion molecules that regulate the inflammatory process. Endothelial dysfunction is considered to be an initial step in the development of atherosclerosis, followed by morphological changes, such as plaque formation and further progression, resulting in cardiovascular events. Flow-mediated dilation (FMD) and reactive hyperemia peripheral arterial tonometry (RH-PAT) are widely used to assess vascular endothelial function. Vascular assessments using aortic pulse wave velocity (PWV) and carotid ultrasonography have revealed that increased arterial stiffness and atherosclerosis are observed even in mild cases of COPD compared to healthy individuals or smokers without COPD.

2-3. Cardiovascular events in COPD

A large cohort study which enrolled patients with COPD treated by the Veterans Administration Medical System found that the prevalence of coronary artery disease (CAD), congestive heart failure, and atrial fibrillation (33.6%, 24.4%, and 14.3%, respectively) were significantly higher in this patient population than in the matched non-COPD cohort (27.1%, 13.5%, and 10.4%; p<0.001). Another epidemiological study from Canada also found an increased risk of angina pectoris (odds ratio [OR]: 1.61; confidence interval [CI]: 1.47-1.76) and acute myocardial infarction (OR: 2.23).
Cardiovascular events are a major cause of death in patients with COPD. In the Lung Health Study of 5,887 patients with a mild to moderate airflow limitation, cardiovascular disease accounted for ~25% of the causes of death\(^2\). Another study from Sweden found that in patients with severe COPD undergoing long-term oxygen treatment, circulatory diseases accounted for 16% of the causes of death, which is the 2nd most frequent cause of death following respiratory etiologies\(^3\). This study also demonstrated that the mortality ratio associated with respiratory diseases and lung cancer decreased by 2.7% and 3.4% per year, respectively, whereas those associated with cardiac disease and CAD increased by 2.8% and 2.7% per year, respectively\(^4\).

2-4. Ethnic differences in the vascular events associated with COPD

It has been reported that there are ethnic differences regarding the prevalence of cardiovascular comorbidities associated with respiratory diseases. For COPD, the prevalence of cardiovascular diseases is 16%-22% in Japan and 10%-30% in western countries. The mortality rate for cardiovascular problems in patients with COPD is 4%-29% in Japan and 18%-39% in western countries\(^5\). These differences might be attributable to the variations in COPD pathophysiology, ethnic or genetic disparities in cardiovascular diseases, or environmental aspects, including lifestyle and socioeconomic factors\(^6\).

2-5. Mechanisms of vascular failure in patients with COPD

Smoking is a strong risk factor for atherosclerosis in patients with COPD; however, some epidemiological studies have shown that the link between COPD and atherosclerosis could not be explained only by smoking. For instance, the National Health and Nutrition Examination Survey (NHANES) demonstrated that the low forced expiratory volume in 1s (FEV\(_1\)) was associated with an increased risk of cardiovascular mortality, even after adjusting for other risk factors (e.g., smoking, hypertension, obesity, and diabetes)\(^7\). In NHANES, patients in the lowest FEV\(_1\) quintile exhibited the highest risk of cardiovascular mortality (relative risk: 3.36) compared with those in the highest FEV\(_1\) quintile. In addition, a meta-analysis of large cohort studies reported that a reduced FEV\(_1\) was associated with an increased cardiovascular mortality, even after statistical adjustments for smoking status (pooled risk ratio [RR]: 1.77)\(^8\). Systemic inflammation may be a common pathophysiology that can explain the association between COPD and atherosclerosis. In addition, COPD is characterized by both chronic lung inflammation as well as systemic inflammation associated with a variety of inflammatory markers in the blood. For instance, data from the NHANES III study revealed a significant increase in the overall cardiovascular mortality rates for individuals with high leptin and C-reactive protein (CRP) levels (RR, 1.54). Moreover, elevated leptin and CRP levels were also associated with increased all-cause mortality rates (hazard ratio [HR]: 1.80) in men, while no such association was found among women\(^9\). Another study found that patients with COPD with suspected pulmonary hypertension exhibited higher levels of serum CRP and tumor necrosis factor-alpha (TNF-\(\alpha\))\(^10\). However, it remains unclear whether such systemic inflammation in patients with COPD contributes to the development of atherosclerosis.

2-6. Potential preventive treatment for cardiovascular events in patients with COPD

The Understanding Potential Long-Term Impacts on Function with Tiotropium (UPLIFT) study followed up 5,993 patients with COPD from 37 countries over 4 years and demonstrated that tiotropium had a long-term effect on the improvement of quality of life (QOL) and prevention of acute exacerbation\(^11\). It is important to note that the incidence of cardiac events, especially congestive heart failure and myocardial infarction, was significantly lower in the tiotropium group than in the placebo group. Additionally, in the UPLIFT trial, preceding treatments (including inhaled corticosteroids) were continued; however, tiotropium was not intended to suppress inflammation; this result may suggest that medical interventions for COPD might prevent the development of cardiovascular events in patients with COPD. Other studies have shown that medical treatment for atherosclerosis and cardiac disease improved the prognosis of COPD. A retrospective study of 854 patients with COPD admitted for acute exacerbation in a Norwegian hospital found that patients being treated with statins had a significantly lower risk of mortality compared with those without statins (HR: 0.57), and the effect of statins was further enhanced by the combined use of inhaled corticosteroids (HR: 0.39)\(^12\). Furthermore, it was reported that the combined use of statins and an ACE inhibitor or angiotensin receptor blocker (ARB) decreased the risk of admission (relative risk 0.66) and all-cause mortality (RR: 0.42) due to COPD\(^13\). In the establishment of a treatment strategy for COPD, further investigation is necessary regarding the benefit of medical intervention for vascular disease.

3. Bronchial asthma

3-1. Pathophysiology of bronchial asthma

Bronchial asthma is also an obstructive lung disease; however, it is characterized by airway inflammation, airway hyperresponsiveness, and often reversible airflow obstruc-
tion. The essential pathology of bronchial asthma is chronic airway inflammation dominated by eosinophils, in which continuous airway inflammation causes airway injury followed by remodeling and an irreversible airflow limitation.

3-2. Bronchial asthma and vascular failure

Previous epidemiological studies have shown that bronchial asthma is also a respiratory disease associated with an increased risk of atherosclerosis. According to a surveillance of 150,000 people who enrolled in a large managed care organization in Northern California, bronchial asthma was found to be a risk factor for the admission or death due to CAD after adjusting for cardiovascular risk factors in women (RR: 1.22), but not in men.

An epidemiological study of 262 patients with severe asthma who underwent daily treatment with oral steroids for more than 1 year found that there was an enhanced mortality due to CAD (RR: 1.9), especially among women (RR: 2.5). A study of hospitalized patients with asthma from Australia also showed an increased mortality ratio due to CAD in elderly patients. The Atherosclerosis Risk in Communities Study (ARIC) conducted in the United States found that patients with asthma were at an increased risk of stroke (HR: 1.50-1.55). Moreover, participants who had experienced a wheezing attack were at a greater risk of stroke than those who had not; however, unexpectedly, the risk of CAD was not increased in participants with a history of asthma. Although the reason for the lack of an association between asthma and CAD in this study remains unclear, confounding factors not included in the analysis (e.g., socioeconomic background during childhood) may have affected the results.

4. ILD

4-1. Pathogenesis and clinical features of interstitial pneumonia

ILD includes a wide spectrum of fibrotic lung diseases in which the interstitium of the lungs is the primary location. The clinical course of ILD is often chronic and progressive. In particular, idiopathic pulmonary fibrosis (IPF) is the most frequent and severe form of idiopathic interstitial pneumonia, with a 5-year survival rate of ~50%.

While the precise pathogenesis of IPF remains unclear, it is considered to be triggered by extrinsic stimuli, such as smoking and acid reflux which cause injury and apoptosis of alveolar epithelial cells. In response to the epithelial cell injury, increased vascular permeability, extravascular leakage of inflammatory cells, and immune activation occur, leading to myofibroblast differentiation and collagen synthesis. In IPF, these responses do not lead to complete wound healing, but result in lung fibrosis and functional impairment.

4-2. Vascular risk associated with interstitial pneumonia

Patients with interstitial pneumonia exhibit a variety of vascular risk factors, including advanced age, smoking, inflammation, chronic hypoxic stress, and treatment-related disorders (e.g., hypertension, diabetes mellitus, and dyslipidemia). In addition, the activation of the coagulation system is involved in the pathogenesis of IPF, and an increased platelet aggregation is also observed in some types of interstitial pneumonia. Furthermore, the pathogenesis of IPF shares common pathways with the process of atherosclerosis formation, which is initiated by vascular epithelial injury and characterized by fibroproliferative responses of vascular epithelial and smooth muscle cells. Therefore, several molecules have been reported to be involved in the pathogenesis of both pulmonary fibrosis and atherosclerosis.

Endothelin-1 (ET-1) induces potent vasoconstriction, and it has been shown to contribute to the development of atherosclerosis and vascular events in patients with diabetes. In the context of fibrosis, there are important biological mediators whose pathways interact closely with those of ET-1, including transforming growth factor-β (TGF-β), connective tissue growth factor, TNF-α, and a variety of cytokines, such as IL-1. These profibrotic mediators allow fibroblasts to differentiate into myofibroblasts, which contribute to the development of lung fibrosis. In addition, it was reported that ET-1 in the serum and bronchoalveolar lavage (BAL) was increased in IPF, and the level was associated with survival.

Periostin is a matricellular protein, which induces chemokines to recruit neutrophils and macrophages essential in the process of pulmonary fibrosis. Periostin is highly expressed in the lung tissues of patients with IPF, and its serum levels are associated with lung function. Periostin is also involved in the development of atherosclerosis through vascular smooth muscle cell migration.

On the other hand, some molecules have opposite effects to the development of lung fibrosis and atherosclerosis. Caveolin-1 is abundantly expressed in fibroblasts, endothelial cells, type I pneumocytes, and adipocytes; it also has a multitude of cellular functions, including membrane trafficking, endocytosis, lipid metabolism, and signal transduction during cellular proliferation and apoptosis. It was reported that caveolin-1 suppressed TGF-β1-induced extracellular matrix (ECM) production in cultured human lung fibroblasts, and its expression was reduced in the lung tissues, as well as primary pulmonary fibroblasts from patients with IPF, suggesting that its action is to ameliorate lung fibrosis. In contrast, caveolin-1 functions to accelerate the development of atherosclerosis through transporting of LDL into the vascular wall and immune modulation.

4-3. Endothelial dysfunction in interstitial pneumonia

Aihara et al. assessed endothelial dysfunction using RH-
PAT in 39 patients with chronic interstitial pneumonitis/fibrosis without any specific etiology and compared the reactive hyperemia index (RHI) with 30 age-, sex-, and body mass index-matched control subjects. RHI was significantly lower in patients with interstitial pneumonia than in control subjects. The authors found that there was a significant correlation between RHI and the diffusing capacity for carbon monoxide, the difference in the alveolar-arterial oxygen pressure, 6-min walking distance, and end-exercise oxygen saturation, suggesting a possible link between pulmonary fibrosis and endothelial dysfunction. Serum intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, not serum inflammatory cytokines, were inversely correlated with RHI. This result may suggest that molecules involved in the pathogenesis of pulmonary fibrosis and/or hypoxemia and oxidative stress caused by pulmonary fibrosis might contribute to the development of endothelial dysfunction in patients with pulmonary fibrosis. Further investigation is necessary to elucidate the mechanisms of vascular failure in patients with pulmonary fibrosis.

4-4. Vascular events in patients with interstitial pneumonia

In a population-based epidemiological study conducted in the United States, 50% of the primary cause of death for IPF was acute events, with CAD and cerebrovascular accidents accounting for 4/17 (23%) and 1/17 (6%), respectively. In contrast, cardiovascular disease accounts for 3% of the cause of death of Japanese patients with IPF, suggesting ethnic differences for cardiovascular events as recognized in COPD.

Hubbard et al. used data from a longitudinal, primary-care dataset and investigated the cumulative incidence of first-time acute coronary syndromes in 920 patients with IPF and 3,593 control subjects. They showed that there was an increased risk of acute coronary syndrome in the period before the diagnosis of IPF (OR: 1.53) and during the follow-up period (RR: 3.14). Another study enrolled 630 patients evaluated for lung transplantation for whom coronary angiography was performed at a university hospital. This study found that fibrotic lung diseases were associated with an increased prevalence of CAD compared with nonfibrotic diseases after adjusting for traditional risk factors (OR: 2.18). In particular, the risk of developing a multivessel disease was further elevated in fibrotic diseases (OR: 4.16). A similar study from Israel enrolled 100 lung transplantation candidates and revealed that the frequency of CAD was significantly higher in patients with pulmonary fibrosis (14/49, 28.6%) than those with COPD (5/51, 9.8%), despite the fact that smokers were more prevalent among the patients with COPD.

An analysis using a large UK primary care database enrolled 3,211 patients with IPF and 12,307 control subjects. It was found that the rate of first-time CAD events was significantly higher in the patients with IPF than in the control subjects (RR: 2.32); however, the incidence of stroke was only marginally higher for IPF. A high prevalence of coronary risk factors in IPF did not fully account for the increased risk of CAD, suggesting that IPF itself was a specific risk factor for CAD.

4-5. Implication of IPF treatment on vascular failure

A drug targeting the common pathway of pulmonary fibrosis and atherosclerosis might be effective for the treatment of both diseases. However, an international multicenter randomized controlled study, which investigated the efficacy of bosentan, an endothelin receptor antagonist, did not report the anti-fibrotic efficacy against IPF. Currently, 2 anti-fibrotic drugs with different mechanisms were found to be efficacious against IPF. Further investigation is necessary to elucidate the efficacy of these drugs on vascular disease in IPF.

5. SAS

5-1. Clinical features and classification of SAS

Obstructive SAS (OSAS) is characterized by repeated episodes of breathing decline (hypopneas) or cessation (apneas) during sleep due to upper airway obstructions. These events result in repetitive decreases in oxygen saturation with rapid reoxygenation causing cyclical deoxygenation/reoxygenation. SAS is classified into 2 types: 1) OSAS, in which a mechanical obstruction of the upper airway occurs intermittently and 2) central sleep apnea (CSA), in which the brain temporarily fails to signal the muscles responsible for controlling breathing, including Cheyne-Stokes respiration.

SAS is a highly prevalent disease, and a recent study from Japan showed that the frequency of mild sleep-disordered breathing (SDB) was ~30% and that of moderate to severe SDB was 8%-22% in men. The primary treatment for SAS is continuous positive airway pressure (CPAP).

5-2. Mechanisms of vascular failure in patients with OSAS

Hypopnea and apnea events in patients with OSAS can cause intermittent hypoxia, repeated changes in the intrathoracic pressure, and sleep fragmentation, which result in increased oxidative stress, inflammation, and activation of the sympathetic pathways. Increases in inflammatory cytokines and adhesion molecules lead to the activation of various immune cells (e.g., monocytes, lymphocytes, and endothelial cells), resulting in endothelial dysfunction and cardiovascular disease development.

Intermittent hypoxia is broadly defined as repeated episodes of hypoxia interspersed with episodes of normoxia, which is in contrast with continuous hypoxemia. In CAD and cerebrovascular infarction, in addition to tissue hypoxia by ischemia, the restoration of circulation also contributes to the damage caused by inflammation and oxidative stress, which is termed as reperfusion injury.
From the perspective of tissue oxygen supply, repeated apnea-related hypoxic events in OSAS (similar to hypoxia and reperfusion injury) initiate oxidative stress, which alters the expression of genes related to energy metabolism and redox responses, and induce the production of growth factors, inflammatory cytokines, and adhesion molecules. During this process, oxygen stress is considered to be more strongly enhanced by the recovery from hypoxemia to normoxia than the continuous hypoxemia. Therefore, intermittent and continuous hypoxias have different influences.

Takahashi et al. showed that serum thioredoxin, a marker of oxidative stress, was significantly increased in patients with OSAS compared with healthy subjects and that thioredoxin was correlated with both the respiratory disturbance index (RDI) and percent time of SpO₂ <90%. Oxidative stress can also contribute to the induction of heme metabolism. It was reported that an indirect serum bilirubin, a metabolite of heme metabolism, increases during sleep and can be suppressed by CPAP therapy. An animal study demonstrated that mice exposed to both chronic intermittent hypoxia and high-cholesterol diet developed atherosclerotic lesions in the aorta, whereas atherosclerosis was not observed in mice exposed to control air and high-cholesterol diet or in mice exposed to chronic intermittent hypoxia and regular diet. This result may suggest that the clinical significance of intermittent hypoxia in SAS differs depending on individual lifestyles or comorbidities.

An increased platelet aggregation and coagulation cascade can also contribute to endothelial dysfunction. Oga et al. showed that platelet aggregation was increased in moderate to severe OSAS cases and was correlated with the RDI. They also demonstrated that an increased platelet aggregation was ameliorated by CPAP treatment. Furthermore, patients with SAS are frequently obese, and many have metabolic syndrome, which greatly contributes to the development of vascular injury in patients with OSAS.

5-3. Endothelial dysfunction in OSAS

Visceral obesity and low adiponectin are known risk factors for the development of cardiovascular disease. Azuma et al. investigated the association between these factors in patients with OSAS and endothelial dysfunction using RH-PAT. They found that there was a significant negative correlation between RHI and the apnea-hypopnea index (AHI) or visceral fat area, while RHI was positively correlated with serum adiponectin. In a multivariate regression analysis, only severe OSAS remained an independent predictive factor of RHI.

5-4. OSAS and cardiovascular events

A cohort study from Spain found that untreated patients with severe OSAS had significantly increased risks of fatal (OR: 2.87) and non-fatal (OR: 3.17) cardiovascular events compared with healthy participants and that CPAP therapy reduced this risk. The Sleep Heart Health Study conducted in the United States demonstrated that hypopneas with a desaturation of at least 4% are independently associated with cardiovascular diseases.

Eguchi et al. demonstrated that nocturnal hypoxia higher than 5.6 times per hour was independently associated with a silent cerebral infarction, suggesting that OSAS is also a risk factor for vascular events in Japanese patients.

5-5. Effect of CPAP on endothelial dysfunction in patients with OSAS

A systematic review of 8 RCTs investigating the effect of CPAP therapy on endothelial dysfunction in patients with OSAS found that CPAP improved the endothelial function. In addition, the authors performed a meta-analysis on 4 RCTs involving a total of 150 patients. Compared to the control group, the CPAP therapy group had significantly improved endothelial dysfunction by 3.87% assessed using the FMD (CI: 1.93-5.80; P<0.001). The beneficial effect of CPAP on endothelial function was also confirmed by a more recent study using RH-PAT. Further investigation is necessary to elucidate whether CPAP can prevent cardiovascular events in OSAS.

6. Summary and future perspectives

Previous epidemiological studies have found an association between major chronic respiratory diseases and atherosclerosis or vascular events. The influence of vascular disease on the prognosis of respiratory diseases is not negligible; thus, the evaluation of vascular disease and its risk factors are important for the management of respiratory diseases. Further investigation is necessary regarding the effect of treatment for underlying lung diseases on comorbid vascular diseases.

Conflicts of Interest

Tomohiro Handa has no conflict of interest to disclose.

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Association of the roles of advanced glycation end products and osteocalcin between bone metabolism and vascular failure

Ippei Kanazawa, MD, PhD

Abstract:
Fragility fracture impairs the activities of daily living and quality of life of the elderly. Accumulating evidence has shown that patients with osteoporosis have an increased all-cause mortality as well as cardiovascular mortality rates. Osteoporosis and cardiovascular diseases have common risk factors, such as diabetes mellitus. Patients with diabetes have an increased risk of osteoporosis; thus, diabetes-related bone disease is now recognized as one of the complications of diabetes. Although accumulation of advanced glycation end products and oxidative stress are associated with the formation and progression of atherosclerosis, these are reported to be involved in the pathogenesis of osteoporosis, especially in diabetic patients. Moreover, recent studies have shown that osteocalcin, which is secreted from the bone into the circulation, has an endocrine function of regulating glucose and energy metabolism. In addition, osteocalcin directly affects vascular endothelial cells and smooth muscle cells and protects against oxidative stress-induced cell dysfunction. Therefore, the bone-vascular axis attracts widespread attention. In this review, I described the association between bone and glucose metabolism and vascular failure on the basis of recent evidence.

Key words:
Bone, Osteoporosis, Osteocalcin, Advanced glycation end product, Atherosclerosis

Introduction

Both osteoporosis and cardiovascular disease (CVD) are important health problems worldwide because they deteriorate the quality of life and mortality of the patients. These diseases have been traditionally viewed as separate entities that increase in prevalence with aging. However, accumulating evidence has shown that they may be associated with each other. A previous large-scale cohort study showed that each decrease of standard deviation in bone mineral content was associated with a 43% increase in mortality in postmenopausal women. When only cardiovascular death was considered, the relative risk of dying within 17 y after menopause increased to 2.3-fold. In addition, women with bone mass in the lowest quartile had twice the risk of cardiovascular death compared with those in the highest quartile, and a prevalent vertebral fracture was independently associated with cardiovascular death with a 2.2-fold increase. Observation of postmenopausal women who received a placebo treatment in an osteoporosis treatment trial showed that subjects with osteoporosis had a 3.9-fold increased risk for CVD events compared with those with osteopenia. Moreover, subjects with vertebral fracture also had an increased risk of CVD events up to 3.0-fold compared with those without vertebral fractures, and the risk of CVD events increased incrementally as the number and severity of baseline vertebral fractures also increased. Furthermore, several clinical studies using bone mineral density (BMD) and parameters of atherosclerosis have been reported to date. BMD at the femoral neck was significantly and inversely correlated with common carotid artery intima-media thickness (IMT) and pulse wave velocity (PWV) in postmenopausal women with osteoporosis. Moreover, a similar tendency in healthy subjects independent of common risk factors for osteoporosis and CVD was found. A longitudinal study showed that a decrease in BMD was significantly associated with increased aortic calcification scores. Additionally, we previously reported that the presence of osteoporosis was a risk factor for increased IMT and aortic calcification in postmenopausal women with type 2 diabetes mellitus (T2DM).
These findings indicate that osteoporosis accompanies CVD and that these 2 diseases may be associated with each other.

Although the mechanism is not fully understood, there are common exacerbation factors of osteoporosis and CVD. Recently, advanced glycation end products (AGEs) were shown to play a pivotal role in atherosclerotic diseases and osteoporosis. Conversely, the bone has been recognized as an endocrine organ. Osteocalcin is expressed and produced specifically in the osteoblasts and has an endocrine function of regulating glucose and energy homeostasis\(^8\). Moreover, osteocalcin may be associated with CVD risk. In this review, I summarized the role of AGEs as a common factor of CVD and osteoporosis and the role of osteocalcin in the interaction between the bone and CVD on the basis of the above mentioned recent evidence.

### AGEs as a common factor of CVD and osteoporosis

There are several common factors of osteoporosis and CVD, including aging, smoking, alcohol abuse, lack of exercise, and menopause. Lifestyle-related diseases, such as diabetes, dyslipidemia, and chronic kidney disease are well known to increase CVD risk. These lifestyle-related diseases also increase osteoporotic fracture risk that may be mainly caused by a deteriorated bone quality\(^9\). AGEs are generated via the sequential nonenzymatic chemical glycoxidation of protein amino groups. AGEs accumulate in various tissues, including the bone, kidney, brain, and coronary artery with aging. Thus, AGE formation is considered as a cumulative metabolic stress marker, adversely influencing the aging process and chronic disease development and progression across the life course. Further, AGEs have a pivotal role in the development of complications in patients with diabetes, because hyperglycemia and oxidative stress accelerate AGE formation.

Blood AGE concentration is associated with CVD risk and mortality\(^{10,11}\). AGEs are also involved in the atherosclerotic process and vascular failure. AGEs increase intracellular oxidative stress generation and inflammation-related gene expression in vascular wall cells. AGEs inhibit endothelial nitric oxide (NO) synthase (eNOS) expression and NO production\(^{12,13}\), which is the most potent endogenous vasodilator and antiatherogenic factor. Moreover, AGEs impair endothelial cell repair, enhance apoptosis, and suppress the migration and tube formation of endothelial progenitor cells via Akt and cycloxygenase-2 inhibition\(^{14,15}\). AGEs also contribute to vascular calcification, the severity of which is known to be a predictor of CVD events and mortality. AGEs induce the osteoblastic differentiation and mineralization of periocytes\(^{16}\), which have the plasticity to differentiate into smooth muscle cells. Furthermore, AGEs increase oxidative stress by stimulating NADPH oxidase (Nox) expression and induce vascular smooth muscle cell apoptosis and osteoblastic transdifferentiation\(^{17,18}\) (Figure 1). Several types of AGE-binding proteins have been identified\(^{19}\). Among them, the receptor for AGE (RAGE) is reported to be a cell surface receptor that belongs to the immunoglobulin superfamily and plays an important role in the action of AGEs. Indeed, in diabetic ApoE knockout model mice, the deletion of RAGE significantly reduced the atherosclerotic plaque area, attenuated leukocyte recruitment, decreased proinflammatory mediator expression, including that of nuclear factor-κB (NF-κB), VCAM-1, and MCP-1, and reduced oxidative stress and AGE accumulation in plaques\(^{20}\). AGE-RAGE signaling elicits oxidative stress generation and evokes inflammatory and thrombogenic reactions\(^{21}\). In addition, AGEs increase RAGE expression and induce sustained NF-κB activation\(^{22}\), which is located on the RAGE promoter, linking RAGE to inflammation. Thus, a vicious cycle exists, in which AGE-RAGE signaling induces oxidative stress, subsequently enhancing further generation and accumulation of AGEs and RAGE overexpression.

Conversely, AGE-RAGE signaling is involved in the pathogenesis of osteoporosis, especially in bone quality deterioration. Although the underlying mechanism of bone quality deterioration is largely unknown, accumulation of AGEs in the bone matrix is considered an important cause\(^{23}\). Among AGEs, pentosidine is a well-characterized compound and is considered a good predictor for micro- and macrovascular complication development in diabetic patients. Serum pentosidine levels in patients with diabetes were significantly higher than those in healthy subjects. Further, spontaneous diabetic rats displayed significantly increased pentosidine cross-links in the bone, which was linked to impaired mechanical properties despite a normal bone mass\(^{24}\). As circulating pentosidine levels are significantly correlated with the pentosidine content in the cortical bone, serum and urine pentosidine levels could be used as surrogate markers for its content in the bone and bone strength. Indeed, elevated serum and urine pentosidine levels were significantly associated with fracture risks in elderly patients with T2DM\(^{25,26}\). In addition, a recent clinical study using bone biopsy in patients with T1DM showed that pentosidine content in the trabecula was significantly and positively associated with HbA1c levels and increased in patients with T1DM with fracture\(^{27}\). Therefore, pentosidine cross-link accumulation in the bone may be the major cause of impaired bone quality in patients with diabetes.

### AGEs induce osteoblast and osteocyte dysfunction

The bone tissue is constantly renewed by a balance between osteoblastic bone formation and osteoclastic bone resorption. Bone formation markers, especially serum osteocalcin, which is a marker of bone formation and produced by mature osteoblasts, significantly decreased in patients with diabetes compared to non-diabetic subjects\(^{27}\). We previously demonstrated that serum osteocalcin levels significantly increased after intensive glycemic control in T2DM, while bone-specific alkaline phosphatase (BAP),
The receptor for AGEs (RAGE) is expressed in the endothelial cells and vascular smooth muscle cells (VSMC), and AGEs act as physiological molecules. AGE-RAGE signaling enhances NADPH oxidase expression and increases oxidative stress but inhibits the expression of endogenous antioxidants and production of endothelial nitric oxide (NO) synthase (eNOS). AGEs induce apoptosis of endothelial cells and VSMC and inhibit NO production in the endothelial cells. Moreover, AGEs induce transdifferentiation of VSMC to osteoblastic cells. Thus, AGE-RAGE signaling induces endothelial dysfunction and vascular calcification. Conversely, RAGE is also expressed in osteoblasts. AGEs induce apoptosis and suppress cell growth of osteoblasts. AGEs inhibit the differentiation and mineralization of osteoblasts through endoplasmic reticulum (ER) stress and TGF\(\beta\) expression, thereby decreasing bone formation.

which is a marker of the early stage of differentiated osteoblasts, significantly decreased\(^{28}\). Moreover, the osteocalcin/BAP ratio was significantly associated with prevalent vertebral fractures in patients with T2DM\(^{29}\). These findings suggest that osteoblast maturation derangement may be associated with fracture risks in diabetic patients. Conversely, osteocytes account for 90-95% of bone cells and play multifunctional roles in orchestrating bone remodeling by regulating both osteoblast and osteoclast functions. Sclerostin is specifically expressed in osteocytes and inhibits osteoblast function and bone formation by antagonizing the canonical Wnt signaling pathway. Because elevated serum sclerostin levels were associated with increased vertebral fracture risks in patients with T2DM independent of BMD and bone turnover\(^{30}\), osteocyte dysfunction may also contribute to bone fragility.

As RAGE is expressed in osteoblasts and osteocytes, there is a possibility that AGEs directly affect bone formation and remodeling. The combination of high glucose and AGEs inhibited osteocalcin expression and osteoblastic cell line, MC3T3-E1, mineralization\(^{31}\); further, AGEs inhibited the osteoblastic differentiation or mineralization of mouse stromal ST2 cells and human mesenchymal stem cells by decreasing osterix expression, increasing transforming growth factor (TGF)-\(\beta\) expression, and suppressing endoplasmic reticulum (ER) stress proteins\(^{32,33}\) (Figure 1). Moreover, high glucose and AGEs significantly increased sclerostin expression in osteocyte-like MLO-Y4 cells\(^{34}\). In contrast, AGEs decreased the receptor activator of NF-\(\kappa\)B ligand (RANKL) expression in the cells and induced apoptosis of osteoblasts and osteocytes. In summary, AGEs directly inhibit osteoblastic differentiation and bone formation and indirectly by increasing sclerostin expression in osteocytes, as well as contribute to low turnover of bone remodeling by decreasing RANKL expression.

**Glucose metabolism regulation by osteocalcin**

Osteocalcin, which is one of the osteoblast-specific proteins and has several hormonal features, regulates glucose metabolism\(^8\). Osteocalcin knockout (Ocn\(^{-/-}\)) mice were previously generated to investigate the roles of osteocalcin in the bone tissues\(^{35}\). Although it was not reported at that time, the Ocn\(^{-/-}\) mice were obese and had an abnormal visceral fat accumulation. In 2007, it was reported that Ocn\(^{-/-}\) mice...
displayed hyperglycemia and glucose intolerance due to insulin insufficiency and resistance\(^{3}\). In these mice, pancreatic \(\beta\)-cell proliferation and insulin secretion significantly decreased, and insulin resistance from a decreased adiponectin expression in adipocytes was observed. Moreover, when Ocn expression vector-transfected COS cells were cocultured with islets or adipocytes, the insulin and adiponectin expression was significantly enhanced.

Osteocalcin has 46-50 amino acids and undergoes \(\gamma\)-carboxylation of glutamyl residues at 3 positions\(^{17,23,24}\), which facilitates the binding of osteocalcin to hydroxypyatite in the bone matrix. Further examinations have shown that an undercarboxylated form of osteocalcin (ucOC) is an active form in glucose metabolism\(^{3,25}\). Exp encodes osteotesticular protein tyrosine phosphatase (OST-PTP), which is restricted to osteoblasts, sertoli cells, and embryonic stem cells\(^{19}\). OST-PTP is a transmembrane tyrosine phosphatase, which cannot directly affect distant tissues. Because OST-PTP stimulates carboxylation of osteocalcin and decreases osteocalcin bioactivity, \(\gamma\)-mouse were examined as a model of gain of osteocalcin bioactivity\(^{16}\). In contrast to Ocn\(^{-/-}\) mice, \(\gamma\)-mouse showed hypoglycemia and low blood glucose levels after glucose injection, increased insulin expression and secretion, increased insulin sensitivity, and increased adiponectin expression in the adipose tissues. Furthermore, \(\gamma\)-mice displayed decreased fat mass and serum triglyceride levels and resistance to high-fat diet-induced obesity and diabetes and to streptozotocin-induced diabetes. The metabolic phenotype of Ocn\(^{-/-}\) mice is the mirror image of that seen in \(\gamma\)-mice. To examine whether the metabolic abnormalities in \(\gamma\)-mouse could be corrected by the inhibition of osteocalcin expression, \(\gamma\)-mice were crossed with Ocn\(^{+/+}\) mice. In \(\gamma\): Ocn\(^{+/+}\) mice, the metabolic abnormalities, such as hypoglycemia, hyperinsulinemia, and increased serum adiponectin level were completely reversed.

In addition to the direct effect of osteocalcin on insulin secretion, osteocalcin indirectly stimulates insulin expression and secretion by increasing the secretion of glucagon-like peptide-1 (GLP-1), which is an incretin released by intestinal endocrine cells. Mizokami et al. demonstrated that the treatment with ucOC significantly increased GLP-1 expression in STC-1 enteroendocrine cells in vitro and that the administration of ucOC increased serum GLP-1 and insulin levels in mice\(^{36,39}\). These effects were potentiated by an inhibitor of dipetidyl peptidase-4 and blocked by a GLP-1 receptor antagonist, suggesting that ucOC increases insulin secretion by enhancing GLP-1 secretion from intestinal endocrine cells.

The effects of recombinant osteocalcin injection on glucose metabolism in wild-type (WT) mice were previously reported\(^{40}\). Continuous intraperitoneal injection of low dose recombinant osteocalcin increased insulin secretion, pancreatic \(\beta\)-cell proliferation, insulin sensitivity, and adiponectin expression and decreased fat mass in WT mice. Moreover, recombinant osteocalcin injection prevented high-fat diet-induced obesity, fatty liver, and glucose intolerance. Further, therapeutic potential intermittent administration of recombinant osteocalcin was also tested\(^{40}\). Daily injection of osteocalcin significantly improved glucose intolerance and insulin resistance in mice fed not only with normal diet but also with a high-fat diet. In addition, hepatic steatosis induced by a high-fat diet was completely recovered in mice treated with daily osteocalcin injection. Interestingly, the oral administration of osteocalcin also improved impaired glucose tolerance in vivo\(^{39,40}\). The ucOC via oral administration reached the small intestines, remained there for at least 24 h, and entered the general circulation. Daily and long-term intermittent oral administration of ucOC significantly reduced fasting blood glucose level and improved glucose tolerance in mice without affecting insulin sensitivity. Oral administration also increased fasting serum insulin level and \(\beta\)-cell area in the pancreas. The serum GLP-1 level was increased in accordance with the presence of ucOC in the intestines and systemic circulation. In summary, these findings suggest that the intermittent injection and oral administration of recombinant osteocalcin may be useful in treating T2DM and obesity.

Previous studies suggest that the G-protein-coupled receptor family C group 6 member A (GPRC6A) is a receptor for osteocalcin and mediates the response to osteocalcin in \(\beta\)-cells\(^{42}\). GPRC6A is an orphan receptor belonging to the G-protein-coupled receptors, which are known as seven-transmembrane domain receptors, and is ubiquitously expressed and sense amino acids and extracellular calcium\(^{43,44}\).

It is reported that GPRC6A knockout mice showed osteopenia, hyperglycemia, impaired glucose tolerance, insulin resistance, and hepatic steatosis\(^{40}\), suggesting that GPRC6A may participate in the anabolic response of multiple tissues. On the basis of the metabolic abnormalities in the GPRC6A knockout mice, Pi et al.\(^{40}\) hypothesized that GPRC6A might be involved in the function of ucOC in glucose homeostasis. To investigate the role of GPRC6A in osteocalcin function, the effects of osteocalcin on GPRC6A-expressed cells were investigated. Recombinant osteocalcin stimulated ERK activity in HEK-293 cells overexpressing GPRC6A in a dose-dependent manner but did not affect the untransfected control cells. It was confirmed that the pancreatic \(\beta\)-cell TC-6 cell line and pancreas isolated from WT mice expressed GPRC6A and that the recombinant osteocalcin treatment stimulated ERK activity in vitro and in vivo. Moreover, administration of recombinant osteocalcin significantly increased insulin expression in the pancreas as well as serum insulin levels in the WT mice but not in the GPRC6A knockout mice.

**Effects of osteocalcin on vascular cells and atherosclerosis**

As described above, osteocalcin regulates glucose and energy homeostasis (Figure 2). In addition, adiponectin and GLP-1 are known to have favorable effects on the formation
Figure 2. Schematic representation of the mechanisms regulating glucose metabolism by the bone.
Osteocalcin secreted from the bone directly stimulates insulin secretion from the pancreas and indirectly by increasing the secretion of glucagon-like peptide-1 (GLP-1) from the small intestines. Osteocalcin stimulates the expression of adiponectin in the adipose tissues, thereby increasing insulin sensitivity. Osteocalcin also enhances insulin signaling in the muscles. G-protein-coupled receptor family C group 6 member A (GPRC6A) is a receptor for undercarboxylated osteocalcin (ucOC) and is expressed in pancreatic β-cells, epithelial cells of the small intestines, adipocytes, and myotubes. 

and progression of atherosclerosis. The evidence suggests that osteocalcin may indirectly affect CVD. However, several studies have shown that osteocalcin might directly affect the blood vessels because the receptor for osteocalcin, GPRC6A, exists on endothelial cells and vascular smooth muscle cells. A previous in vitro study showed that osteocalcin prevented free fatty acid-induced apoptosis of vascular endothelial cells in the vascular tissue. A high-fat diet induced apoptosis, increased autophagic indicators, such as Atg7 and LC3-II, decreased p62, and activated ER stress sensor PERK and its downstream molecule, eIF2alpha. Osteocalcin injection improved these effects of high-fat diet on the abnormal autophagy and ER stress. Moreover, they showed similar results by using the cell lines of vascular endothelial cells and vascular smooth muscle cells. The role of osteocalcin in vascular function has been reported. Daily injections of osteocalcin can significantly improve lipid metabolism, glucose tolerance, and insulin sensitivity in ApoE-deficient mice. In ApoE-deficient mice fed with a high-fat diet, the osteocalcin-treated mice displayed an improved acetylcholine-stimulated endothelium-dependent relaxation (EDR) compared with the vehicle-treated mice. Moreover, osteocalcin-treated human umbilical vein endothelial cells displayed increased activation of the Akt-eNOS signaling pathway compared with the vehicle-treated cells. Furthermore, a similar beneficial effect of osteocalcin on the thoracic aorta was observed using an ex vivo organ culture of an isolated mouse aortic segment. These findings suggest that osteocalcin has beneficial effects against metabolic stress-induced vascular dysfunction by stimulating eNOS expression and regulating ER stress in the vascular tissues (Figure 3).

Since in vitro and in vivo studies have shown that osteocalcin plays crucial roles in glucose metabolism and has protective effects on metabolic stress-induced vascular dysfunction, of particular interest is whether osteocalcin level in the circulation is associated with atherosclerosis parameters and CVD events in humans. Indeed, the size and some amino acids of osteocalcin are different between mice and humans, and osteocalcin is encoded by a single gene in humans that is highly conserved across species, while mice contain a cluster of 3 osteocalcin genes. We have previously shown for the first time that serum osteocalcin levels were significantly and inversely associated with IMT and PWV in men with T2DM independent of conventional risk factors for CVD. In addition, we found a negative association between changes in serum osteocalcin and plaque score during glycemic control in patients with T2DM in a longitudinal study. Since then, several clinical studies on the association between osteocalcin and atherosclerosis and CVD have been reported. Some studies demonstrated that serum osteocalcin levels were inversely associated with atherosclerotic parameters and artery calcification; however, other studies reported otherwise. Sheng et al. showed that serum osteocalcin levels were significantly and inversely associated with IMT and carotid plaques in men with T2DM via
Figure 3. Protective effects of osteocalcin against metabolic stress-induced vascular failure.
Metabolic stress, such as hyperglycemia and obesity, increases endoplasmic reticulum (ER) stress and induces abnormal autophagy and insulin resistance in endothelial cells and vascular smooth muscle cells (VSMC), resulting in apoptosis of the cells and endothelial dysfunction. In contrast, osteocalcin activates phosphatidylinositol 3-kinase (PI3K)/Akt signaling and increases endothelial nitric oxide (NO) synthase (eNOS) expression. Osteocalcin has a protective effect against vascular failure induced by metabolic stress.

Figure 4. Schematic representation of the association between the bone and vascular failure.
Advanced glycation end products (AGEs) have direct harmful effects on bone metabolism and vascular cells. AGEs inhibit osteoblastic differentiation and osteocalcin secretion from the bone. AGE-suppressed osteocalcin expression induces glucose intolerance and decreases expression of adiponectin and glucagon-like peptide-1 (GLP-1), both of which have beneficial effects on vascular function. Thus, AGEs are directly and indirectly involved in the mechanisms of vascular failure.
subgroup analysis of subjects with normal glucose tolerance showed that serum osteocalcin significantly decreased in patients with coronary artery disease compared with those without and as the number of stenotic vessels increased.

Taken together, these findings suggest that osteocalcin derived from the bone plays pivotal roles in the association between osteoporosis and CVD. Osteocalcin may have direct effects on atherosclerosis and CVD events as well as indirect effects by regulating glucose and fat metabolism. However, the number of studies on osteocalcin and CVD is limited. In addition, it is still controversial whether ucOC is the active form of the endocrine factor in humans. Therefore, further large-scale studies and meta-analyses are necessary in the future.

**Summary**

In summary, it has been shown that osteoporosis and CVD are associated with each other. AGEs are involved in the pathology of osteoporosis and CVD; thus, these can be a candidate therapeutic target for both diseases. There are 2 different strategies of anti-AGE signaling. The first is to inhibit either the production and accumulation of AGEs or the signaling pathway at the level of their receptors. The second is to increase the breakdown of already existing AGEs in the atherosclerotic lesion and bone matrix. Although several existing drugs, such as statins and metformin, are reported to reduce the formation of AGEs and inhibit their signaling, further pre-clinical and clinical studies are necessary. Moreover, the bone secretes osteocalcin into the circulation, which regulates glucose and fat metabolism and directly affects the formation of atherosclerosis. Since AGEs inhibit osteocalcin expression in osteoblasts, AGEs indirectly deteriorate vascular function by affecting the interaction of the bone and vascular tissue (Figure 4). Therefore, in cases of osteoporosis and CVD, it may be beneficial to enhance the expression of osteocalcin and bone formation.

**Conflicts of Interest**

None.

**References**

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Bone metabolism and vascular failure


Effects of prostaglandin E₁ infusion on blood flow in a patient with Buerger’s disease: a case report

Takashi Umemura, MD, PhD¹, Kenji Nishioka, MD, PhD³, Kazuaki Chayama, MD, PhD³ and Yukihito Higashi, MD, PhD, FAHA²

Abstract:
A 45-year-old male patient with Buerger’s disease presented with intermittent claudication, sharp rest pain in his right foot, and several episodes of lower extremity superficial thrombophlebitis. He had no cardiovascular risk factors and his glucose levels were within normal ranges. However, he had a smoking history of more than 26 years; he had stopped smoking 3 years prior. Early treatment with antithrombotic and vasodilating agents as well as intravenous infusion of prostaglandin E₁ did not improve his condition. Intra-arterial infusion of prostaglandin E₁ once a day for 2 weeks slightly increased blood flow in the areas with previous low blood flow and improved his condition from Fontaine stage III to II.

Key words:
Buerger’s disease, Prostaglandin E₁, Laser doppler perfusion image

Introduction
Thromboangiitis obliterans (Buerger’s disease) is recognized as a segmental inflammatory obliterator arteriopathy of small- and medium-sized distal arteries, veins, and nerves. It is distinct from atherosclerosis¹², and is a rare disease that affects approximately less than 10,000 people in Japan. Although inflammatory processes caused by altered autoimmune response are thought to contribute to the pathology of Buerger’s disease, the precise pathogenesis of Buerger’s disease remains unclear. The frequency of Buerger’s disease is greater in men than in women. However, the frequency of Buerger’s disease increases in young women with increased tobacco use, while it is decreasing in men. Treatment strategies for Buerger’s disease include smoking cessation, administration of anti-platelet agents, use of vasodilators, revascularization, sympathectomy, and cell therapy. However, some cases of Buerger’s disease progress to critical limb ischemia, requiring major amputation. Therefore, it is clinically important to improve or control the progression of Buerger’s disease.

Case Report
We report the case of a 45-year-old man with a 13-year history of Buerger’s disease presenting with intermittent claudication, sharp rest pain in his right foot, and several episodes of lower extremity superficial thrombophlebitis. He had no cardiovascular risk factors, such as elevated blood pressure, cholesterol, and glucose, but had a smoking history of more than 26 years (3.2 pack-years); he had stopped smoking 3 years prior. Tests for rheumatoid factor and lupus anticoagulants, and serologic investigations had returned negative results. Early treatment with antithrombotic and vasodilating agents did not improve his condition. Although right lumbar sympathectomy improved his symptoms transiently, his symptoms deteriorated within 4 weeks after sympathectomy. Intravenous infusion of lipo prostaglandin E₁,
Figure 1. Effects of intra-arterial (A) and intravenous (B) infusion of alprostadil and long-term intra-arterial infusion of alprostadil (C) on blood flow of the right dorsum pedis in a patient with Buerger’s disease.

Figure 2. Digital subtraction angiography in the right lower leg in a patient with Buerger’s disease.

alprostadil alfadex (20 μg) for 30 minutes twice a day for 2 weeks also did not improve his condition. Laser Doppler perfusion imaging (LDPI) showed very low blood flow areas at the great, second, and third toes and multiple low blood flow areas in the dorsum pedis (Figure 1A, 1B, 1C). Alfadex was intra-arterially infused through a 24-gauge catheter into the right femoral artery. Blood flow of the right dorsum pedis immediately increased and reached a peak level at 10 minutes after intra-arterial infusion of alprostadil (0.5 ng/kg/min) for 5 minutes (Figure 1A, arrow) and returned to the baseline level at 30 minutes after alprostadil infusion. However, blood flow of the right dorsum pedis did not change during and after intravenous infusion of alprostadil (10 ng/kg/min) for 60 minutes (Figure 1B). Intra-arterial infusion of alprostadil (10 ng/kg/min) for 10 minutes once a day for 2 weeks slightly increased blood flow in the previous low blood flow areas (Figure 1C, arrow) and improved his condition from Fontaine stage III to II.

Discussion

LDPI enables non-invasive and repeated determination of not only blood flow but also distribution of blood flow in extremities*. We confirmed that ischemic extremities in patients with peripheral diseases (n=38, 34 males and 4 females) can be divided into 3 types according to observation by LDPI during and after the intravenous infusion of alprostadil (10 ng/kg/min) for 60 minutes. First, in most cases (n=32, 29 males and 3 females), intravenous infusion of alprostadil immediately increased blood flow in ischemic ex-
tremities, as shown in previous studies\(^4\)\(^5\). Second, as in this case (n=5, 4 males and 1 female), blood flow did not change during and after alprostadil infusion. Finally, in one case (n=1, male), blood flow decreased only during and after intravenous alprostadil infusion (so-called steal phenomenon and borrowing-lending phenomenon)\(^6\)\(^7\). The two latter types compared with the former type may have more serious symptoms and arteriographic findings. Indeed, our patient had rest pain (Fontaine stage III) in his lower right leg. Digital subtraction angiography showed avascular areas at the tip of the great toe and multiple occlusions of the distal arteries with collateralization around the area of occlusions in his lower right leg (Figure 2). It is expected that intra-arterial infusion of prostaglandin E\(_1\) is more effective compared with intra-venous infusion of prostaglandin E\(_1\). However, reproducibility and safety of intra-arterial infusion of prostaglandin E\(_1\) are not guaranteed yet. Future studies are needed to confirm the effectiveness and safety of intra-arterial infusion of prostaglandin E\(_1\) in patients with peripheral arterial diseases.

**Conclusion**

Although intra-arterial infusion of prostaglandin E\(_1\) is worth trying in patients for whom intravenous prostaglandin E\(_1\) infusion is not effective, we must keep in mind the preservation capacity of blood supply in collateral arteries when vasodilators are intravenously or intra-arterially administered in patients with severe peripheral arterial diseases. LDPI, a non-invasive approach for observation, is expected to play an important role in screening for safety and usefulness of the administration of vasodilators, including intravenous infusion of prostaglandin E\(_1\), in patients with peripheral arterial diseases.

**Conflicts of Interest**

None.

**References**