Picturing Vascular Health in Patients with Type 2 Diabetes Mellitus: Review based on the Special Lecture 2021

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Abstract:
Professor Hamburg’s lecture focuses on understanding what is happening to the endothelium in patients with diabetes. The many previous cohort studies have shown that vascular dysfunction predicts cardiovascular disease and that it really reflects an early event in the pathogenesis of atherosclerosis, myocardial dysfunction, hypertension, and stroke. According to her own studies and the recent researches reveal that there is a particular association between diabetes mellitus and microvascular dysfunction reflected by abnormal Peripheral Arterial Tonometry ratio and reactive hyperemia. And further, she carried out a series of studies using the ability to collect endothelial cells from patients to show that endothelial phenotype is altered in patients with diabetes, with loss of insulin signaling, loss of nitric oxide, and activation of inflammation. And it is suggested that this is a platform to understand how interventions might benefit the endothelial cells in patients with diabetes at cardiovascular standpoint.

Key words: Vascular function, Diabetes mellitus, Insulin resistance, Endothelial cell

Introduction
The reasons why the endothelial function is focused in diabetic patients is that diabetes is one of the many factors which can act as a barometer for vascular health. In this review from professor Naomi Hamburg’s lecture at the 6th annual meeting of Japanese Society of Vascular Failure, her investigation group tries to elucidate what is happening to the endothelium in the patient with diabetes mellitus, and a multiple phase of endothelium over the clinical course of diabetes and to discuss on broad spectrum from the pathophysiology of endothelial dysfunction to the future therapeutic consideration for this important condition in diabetes. Especially, their work focuses on understanding what is happening to the endothelium in patients with diabetes. In her lecture, the barometer for vascular function implies both healthy condition of endothelium where endothelium normally products nitric oxide (NO) and the endothelial dysfunction where NO production decreases and vasoconstriction are induced. The factors other than diabetes include aging, smoking, hypertension, dyslipidemia, physical inactivity, infection/inflammation, novel tobacco products, alcohol consumption and genetics. The status of these factors influences endothelial function which comprises NO production, anti-thrombotic activity and anti-inflammatory conditions. When these factors cause endothelial dysfunction, NO production decreases and vasoconstriction is induced. The vessels are prone to prothrombotic condition as well as aggravated inflammation. The endothelial dysfunction subsequently promotes atherogenesis, this will lead to plaque activation / rupture, worsened thrombosis and vasospasms. These malfunctions end up to coronary events and stroke (Figure 1).

The reasons why we measure the vascular function are as follows; 1) to clarify how vascular function becomes abnormal in the atherosclerotic disorders, for example in patients with diabetes, 2) to use the vascular function as biomarker of cardiovascular toxicity, 3) to understand better how new drugs or treatments improve vascular health, 4) to identify mechanisms that may lead to novel therapies and/or to understand better the mechanisms of therapies that have been developed, 5) to potentially think about what is the individ-
Figure 1. A Barometer for Vascular Health
NO, nitric oxide; EC, endothelial; HF, heart failure

Methodology of measuring endothelial function and its role of prediction for cardiovascular events

On the methodological points of views on measuring endothelial function in humans, there is a number of ways to measure vascular health that have been developed around for a period of time till now1. Most of these are all involving non-invasive measures that reflect vascular health in patients. One set of methodologies is ultrasound of the brachial artery, which was selected because of the similar size to the coronary arteries, about 3 to 4 millimeters. The brachial artery is measured longitudinally at rest and then, a period of 5 minutes of ischemia in the arm is induced by inflating a blood pressure cuff on the arm to super systolic pressures. After by deflating the cuff, there is a hyperemic response, this consequently increases blood flow that increases the shear stress along the endothelial surface (Flow-Mediated Dilation of the brachial artery; FMD). That is a stimulus for NO production so that the degree of dilation of the brachial artery reflects the amount of NO production. The previous study2 showed that this increase in hyperemic flow is an important measure of vascular health (endothelial function). It is in part NO-dependent and it really reflects the microvasculature in the arm which is important in cardiac and metabolic diseases like diabetes. In addition, arterial stiffness using tonometry, which looks at the speed of propagation of the pulse wave across the aorta, has been measured on assessment for vascular health. In this method, stiffer aorta transmits the pulse wave faster compared to a more elastic compliant aorta. These measures have been conducted in thousands of patients in the Framingham Heart Study. In one of the Framingham cohorts, the offspring cohort, an analysis asking the question has been performed to elucidate whether the degree of arterial stiffness and degree of endothelial dysfunction, measured by FMD, predicts the development of hypertension. As a result, some of these measures are abnormal in patients who have hypertension. However, there is a question on what about FMD measures can serve as antecedents to the development of high blood pressure. Then, professor Hamburg et al. showed that both a Carotid Femoral Pulse Wave Velocity (CFPWV), a measure of arterial stiffness, and FMD are predictive of subsequent longitudinal incident increases in systolic blood pressure, as well as the development of high blood pressure or hypertension based on standards at the time. FMD and CFPWV are really reflective of future events3,4.

About predicting for incident cardiovascular events, this is a study over 4500 individuals and multiple of the Framingham Heart Study participants. By using this cohort, the measure of FMD of conduit artery vascular function, reactive hyperemia which is a measure of microvascular vasodilation, as well as CFPWV, which measures arterial stiffness have been investigated if they predict future cardiovascular events5. As a result, each of these measures were predictive of future adverse cardiovascular events, including myocardial infarction, stroke and peripheral vascular events that were graded across the levels. A mediation analysis also had been conducted and this showed that the CFPWV or stiffness mediated the association between microvascular dysfunction and future cardiovascular events suggesting that the sequence of events here is that arterial stiffness leads to microvascular dysfunction, which in turn is indicative of future cardiovascular events sort of helping us better understand the pathogenesis.

Pathways from mal-behavior such as overeating and /or sedentary to diabetes and cardiovascular risk: interaction between abnormal vascular function and insulin resistance

When we move to specifically focus on the relation between diabetes and cardiovascular events, we really have a model that suggests what happens in the pathophysiology. It has been well known that there are certain behaviors that are associated with both development of abnormal vascular function and insulin resistance, like sedentary behavior and overeating. The thought is that these interact and then lead to future cardiovascular events as shown in Figure 2. If we can understand well this interaction between the preclinical events, insulin resistance, and abnormal vascular function,
then we can better understand how to interrupt this sequence of events. One of the earlier studies that investigated the relation between insulin resistance and vascular function by using the data from the Framingham Heart Study to look at community-based participants. It has been shown that those with insulin resistance characterized by having high homeostasis model assessment of insulin resistance (HOMA-IR) or high-fasting insulin and glucose had impaired vascular function measured by FMD when adjusting for other risk factors. Similarly, impaired microvascular function measured with the reactive hyperemia was caused by insulin resistance. The predictive value of the risk factors was 16% for the FMD and 27% for reactive hyperemia. When we added measures of insulin resistance to this basal model of risk factors, we didn’t see a lot of change in the model. This really is likely because the insulin resistance in this cohort of individuals in their 60s, travels with a lot of concomitant risk factors, like high blood pressure, lipid abnormalities and obesity, as well as diabetes. The question is if it is possible to be able to tease out in a better fashion, the association with insulin resistance and cardiovascular impairment and vascular dysfunction using other models.

For the further research purpose, methods for measuring vascular function at the fingertip have been developed. This is using a device called a Peripheral Arterial Tonometry (PAT). It senses pulsation of the fingertip with each heartbeat. Again, a similar model to FMD and reactive hyperemia can be utilized, where we have a cuff occlusion and release, and where we have cuffs on each of the fingers. In the control arm, where there was not a cuff, a fairly steady response over time can be obtained. And then, in the side where we have a cuff occlusion, in some individuals there is a marked rise indicated as the high response in the amplitude of the pulse wave after cuff release, whereas in other individual, there is a low response.

When we looked at the associated risk factors with having an impaired PAT ratio response as compared to the FMD response, interestingly, several risk factors that we think of with cardiovascular disease, like age and systolic blood pressure, were largely associated with abnormalities in FMD or conduit vessel function, but not with the microvascular function measured on the fingertip. In contrast, what was most associated with microvascular dysfunction was obesity, as well as diabetes, really suggesting that there may be especially an earlier disease, differential impacts on different circulations, reflective of the particular impact of cardiometabolic abnormalities, like diabetes on the microvasculature.

In the next study, a meta-analysis across over 16,000 participants in all three of these studies, including the ELSA-Brazil study, the Gutenberg Health Study in Germany as well as Framingham Heart Study were performed. The question in this study was about whether there is such a thing as healthy obesity with reflection on vascular function. Relative to those individuals who were normal weight without metabolic risk factors, the study showed that both those individuals who had healthy obesity and those without metabolic risk factors had impaired PAT ratio, but to a lower extent than those with unhealthy obesity, who were the most impaired. But it suggests that there is not really a category of healthy obesity. In terms of vascular function, the study suggests that both those with and without metabolic disturbances had abnormalities in microvascular function. In addition, an analysis on the impact of the PAT ratio on stroke risk and cardiovascular risk has been completed and in press in the Journal of Stroke. The study showed that in the two groups of individuals with the lowest PAT ratio where these are the most impaired, a significantly higher rate of developing stroke was observed when their prognosis was compared to those individuals with more normal PAT ratio. Again, linking this microvascular dysfunction to cerebrovascular events in the community was confirmed.

Is it possible to examine insulin resistance and vascular dysfunction separate from traditional risk factors?

The Framingham Heart Study showed that there is the association with insulin resistance and vascular dysfunction but vascular dysfunction seemed to be largely related to the risk factors that travel along with insulin resistance. So, it is interesting to find the models that can induce both insulin resistance and vascular dysfunction, as well as to find the association of these two subjects.

This is a model based on many years of data from the US Space Program, NASA, suggesting that individuals in microgravity and with inactivity develop acutely insulin resistance. And professor Hamburg’s group investigated that healthy individuals and this group had them come into the hospital for 5 days. Their insulin resistance using an oral glucose tolerance test as well as their vascular function at baseline was measured first. And then, the healthy subjects had been stayed in bed for the 5 days, from Monday through Friday. They were only allowed out of bed for 20 minutes a day in order to use the bathroom. That was the only time that they were out of bed. And then, we measured their vascular function again and their oral glucose tolerance test at the end of the 5 days. What the study showed was that there was the acute development of insulin resistance reflected in the changes in their oral glucose tolerance test. Also the study showed that there is a modest increase in their glucose response to a glucose load in the circulation. But there is a much more dramatic increase in their insulin response. Others have shown that this reflects peripheral skeletal muscle insulin resistance. When the investigators looked at microvascular function here in the arm, looking at hyperemic flow in the brachial artery, even by Day 3, there was a significant impairment compared to the baseline. That persisted out to Day 5. The plethysmography was performed to look at the hyperemic response in the leg because the authors thought that the leg is even more inactive when people are in bed. The study showed that there is a diminish-
ment of both the peak and the curve of the hyperemic response in the lower extremity after 5 days of bed rest. Also, the study did show that even just going back to normal activities after a couple of days, this impairment is restored. But it really suggested that even with acute insulin resistance, the changes in microvascular dysfunction was observed.

**What links endothelial dysfunction and insulin resistance in diabetes**

The next set of studies, professor Hamburg et al. were really interested in what links endothelial dysfunction and insulin resistance in diabetes and they tried to understand this at a cellular level. A methodology that is also used by others to assess the endothelial cell phenotype in diabetes is utilized. This involves using a standard wire, and the wire was used in placing an arterial line and stayed in the vessel. And this had been largely done this in veins. There is some interaction between the atraumatic tip of the wire and the vessel wall and some of the endothelial cells adhere to the wire. These are washed in buffer, and the endothelial cells are looked at under the microscope using immunofluorescence. This was a study that professor Hamburg’s group collaborated on with Mark Feinberg at the Brigham and Women’s Hospital that made it to the cover of Circulation Research\(^{19}\). In this study, intact nuclei were found and the DAPI stain here in blue. Stain for von Willebrand Factor to identify the endothelial cells was performed. And then, the stain for a protein of interest reflected in red was recognized.

The following study on inflammation and oxidative stress in the diabetic endothelium was performed\(^{15}\). This study is that characterizing the endothelial cells in patients with diabetes is investigated. This is the first set of studies looking at inflammation, that is just looking at statically in these endothelial cells. Multiple cells were quantified from each individual. The study showed that there was the activation of the NF-kappa-B pathway reflected by lower levels of I-kappa-B-alpha, which is an inhibitor of NF-kappa-B, higher levels of one of the subunits p65, and higher levels of a downstream signaling target of NF-kappa-B ICAM-1. The study results showed that there was evidence of oxidative stress reflected by higher levels of nitric tyrosine.

The next study was related on the subject where stimulated NO production is impaired in diabetes\(^{16,17}\). In this study, professor Hamburg’s group has gone on to show what happens in the endothelial cells when they take them ex vivo and stimulate them. Endothelial cell was stimulated with insulin to elucidate the NO response measured by immunofluorescence. Healthy controls have an increase in the NO stimulated by insulin to elucidate the NO response measured by immunofluorescence. Healthy controls, an increase in the degree of eNOS phosphorylation in response to acute stimulation with insulin, whereas in patients with diabetes, a decrease in eNOS phosphorylation was shown. That shows significantly different between the two. The degree of stimulation of eNOS phosphorylation associates with FMD, really linking the findings at the endothelial cell level to those at the functional level in terms of vasodilation.

**What pathways connect energy excess to endothelial dysfunction in diabetes**

Then, the next interest is what pathways connect the endothelial dysfunction to an energy excess in patients with diabetes. And the question on what pathways might be contributing to this endothelial dysfunction. To answer these questions, number of studies have been conducted. A couple of them are reviewed in the following in this review. One is looking at the activation of the non-canonical WNT signaling and the inflammatory pathway JNK in patients with diabetes\(^{16}\). This study showed that there were elevated levels of this non-canonical WNT5 that Dr. Ken Walsh had shown was important for mediating a metabolic dysfunction and vascular dysfunction in animal models of diabetes. And then the study showed that in endothelium in patients with diabetes, that there were higher levels of this signaling pathway and that they reflected the downstream consequences with higher levels of activation of JNK, with higher JNK phosphorylation. In addition, the results of this study then showed that if an inhibitor of WNT5a, Box5 is used, as a pharmacological inhibitor, the insulin response in endothelial cells taken from patients with diabetes could be restored. So, again, there is a diminished response at baseline. When the endothelial cells ex vivo with Box5 was treated, again this insulin response could be restored. Similarly, an inhibitor of JNK, SP600125 also restored the insulin response, providing functional evidence that these pathways are important in the impaired endothelial function in patients with diabetes.

**About drugs that are available for patients with diabetes**

There have been a lot of exciting developments in the treatment of diabetes reflected in improvements in cardiovascular outcomes. One of them is with liraglutide, which is the GLP-1 receptor agonist. And the effect of liraglutide on endothelial function was so much interested in patients with diabetes. And again, professor Hamburg’s group showed that if the treatment with liraglutide and insulin was applied, a marked increase in the eNOS activation, or the better response compared to without liraglutide treatment was observed, suggesting, again, that GLP-1 receptor agonist may act directly on the endothelium\(^{18}\). There was an associated diminishment in JNK activation. This may be one pathway leading to the relation between GLP-1 receptor activations and the restoration of endothelial function.
The other attractive medicines are dipeptidyl peptidase 4 inhibitor (DPP-4) and sGLT2. Especially, sodium-glucose transporter 2 inhibitor (sGLT2) have been reported to be effective on ameliorating prognosis in the patients with diabetes\(^9,20\).

**Glucose lowering restores endothelial cell phenotype**

Masaki et al. has recently focused on looking at the question on what about glucose-mediated modifications in the endothelial cell in patients with diabetes\(^21\). This is very elegant study. And so, the authors looked at O-GlcNac, which is, again, a modest sugar-based modification of multiple proteins that can happen in response to high glucose. The levels are higher in the endothelial cells from patients with diabetes compared to controls. There is an association between the fasting glucose level and the degree of O-GlcNac modification. There is also an association between the hemoglobin A1C, so a cumulative measure of glucose exposure and the degree of O-GlcNac modifications are correlated. Very interestingly, when what happens to the endothelial cells was investigated, and when the endothelial cells had been kept for 24 hours, not culturing them, above mentioned findings were obtained. The further, this study was looking at what happens to the insulin response in patients’ endothelial cells that was taken and left for 24 hours, kept for 24 hours in a normal glucose condition compared to high glucose. Really interestingly, the patients with diabetes have a restoration of the insulin response even after 24 hours of keeping them in normal glucose, with the abnormal response persisting with high glucose conditions, really connecting the high glucose to the impaired insulin signaling. Similarly, the study showed that if the cells were kept in normal glucose, there is some diminishment of the O-GlcNac modification that persists when they kept in high glucose. And then, a modulator of O-GlcNac, thiamet-G was used and the results showed that if O-GlcNac increased with thiamet-G in normal glucose conditions, thereby the benefit of being in normal glucose was diminished. That is to say that if these O-GlcNac modifications by treatment with an activator thiamet-G have been continued, the benefit in terms of insulin signaling with normal glucose condition compared to high glucose.

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**What therapeutic strategies improve vascular function in diabetes**

Professor Hamburg’s group really focused now in their laboratory thinking about, again, what therapeutic strategies might be available to improve vascular function in diabetes\(^22\). They did a large study that was funded by the American Heart Association, along with collaborators at the Brigham and Women’s Hospital, and the TIMI Group there. Their study is focused, again, on thinking about what is happening at the endothelial cell level. In addition to the response and protein measures that Hamburg et al. have showed here using immunofluorescence, they are now able to look at gene expression profile in these endothelial cells.

Again, professor Hamburg’s group collects the endothelial cells. They can then separate the endothelial cells from the white cells using a positive bead selection for CD144 antigen, which is reflective of endothelial cells. When they are separated, RNA sequencing to look at the differences in global gene expression between the patients with type 2 diabetes and without type 2 diabetes can be carried out. This was early work done in collaboration with a group at Vanderbilt, showing that there are pathways that are differentially expressed in patients with diabetes and healthy control subjects. So, professor Hamburg’s group’s current study, they are collecting endothelial cells from patients with diabetes and without diabetes and looking at both their coding gene expression using RNA sequencing as well as non-coding RNAs reflected by IncRNAs as well as microRNAs to really give an atlas of what is happening in terms of gene expression differences using an unbiased approach of RNA sequencing. IncRNAs as well as microRNAs are going to be linked that to the measures of vascular aging that is related with FMD, reactive hyperemia, PAT ratio, and arterial stiffness. Then, professor Hamburg’s group are going to conduct clinical studies with GLP-1 receptor agonists and SGLT2 inhibitors given to patients over a short period of time and look at how this changes the endothelial cell phenotype to really develop a more detailed understanding of the mechanisms of potential benefits on the vasculature of these novel diabetes therapies.

**Conclusions**

Overall findings from the previous and current studies from professor Hamburg’s group, and some evidence from cohort studies show that vascular dysfunction predicts cardiovascular disease, and suggest really that vascular dysfunction is an early event in the pathogenesis of both atherosclerosis, myocardial dysfunction, hypertension, and stroke now.

A particular association between diabetes mellitus and microvascular dysfunction reflected by abnormal PAT ratio and reactive hyperemia has been seen.

The series of studies using the ability to collect endothelial cells from patients to show that endothelial phenotype is altered in patients with diabetes, with loss of insulin signaling, loss of nitric oxide, and increased inflammatory activation. And that this is a platform to understand how interventions might benefit the endothelial cells, and potentially be mechanisms for overall clinical benefit in patients with diabetes from a cardiovascular standpoint.

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Conflicts of Interest
There are no conflicts pertaining to author.

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