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# Associations of plasma von Willebrand factor ristocetin cofactor activity and 5-hydroxyindole acetic acid concentrations with blood flow in lower-leg arteries in Japanese type 2 diabetic patients with normal ankle-brachial index

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ABSTRACT

*Aims:* To evaluate the associations of circulating levels of proinflammatory molecules and endothelial factors with blood flow in lower-leg arteries in diabetic patients with normal ankle-brachial index (ABI>0.9).
*Methods:* We enrolled 123 type 2 diabetic patients with normal ABI and 30 age-matched nondiabetic subjects consecutively admitted to our hospital. Flow volume and resistive index, an index of peripheral vascular resistance, at the popliteal artery were evaluated using gated two-dimensional cine-mode phase-contrast magnetic resonance imaging. An automatic device was used to measure ABI and brachial-ankle pulse-wave velocity (baPWV) for evaluation of arterial stiffness. Plasma soluble intercellular adhesion molecule-1 (sICAM-1) and monocyte chemoattractant protein-1 (MCP-1) concentrations, serum high-sensitivity C-reactive protein (hsCRP) levels, plasma von Willebrand factor ristocetin cofactor activity (VWF), and plasma vasoconstrictor serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA) concentrations were measured.
*Results:* Diabetic patients had higher baPWV ($P<.0001$), resistive index ($P<.0001$), sICAM-1 ($P<.0001$), MCP-1 ($P=.0224$), log hsCRP ($P<.0001$), VWF ($P<.0001$), 5-HIAA ($P=.0015$), and lower blood flow ($P<.0001$) than nondiabetic subjects. VWF ($P=.0019$) or 5-HIAA ($P=.0011$), but not sICAM-1, MCP-1, and log hsCRP, was negatively correlated with blood flow in diabetic patients. A multivariate analysis revealed that the significant independent determinants of blood flow were hypertension, use of renin–angiotensin system inhibitors, VWF and 5-HIAA ($r^2=0.198$, $P<.0001$) in diabetic patients.
*Conclusions:* Plasma VWF and 5-HIAA concentrations are associated with blood flow and are involved in the pathogenesis of impaired peripheral circulation due to higher arterial stiffness and greater vascular resistance in lower-leg arteries in diabetic patients with normal ABI.



## 1. Introduction

Lower-extremity arterial disease is a major cause of ischemic limb, foot ulcers, and leg amputation in diabetic patients (Faglia et al., 2009; Gorogawa et al., 2006). Diabetic patients are known to have two distinct types of insufficient arterial blood flow to the lower limbs associated with the vessel wall properties. The diabetic condition promotes atherosclerotic plaque formation in the vessel wall and leads to peripheral artery disease (PAD), resulting in reduced blood supply to lower limbs during exercise or at rest. To help identify high-risk patients with PAD, the ankle-brachial index (ABI) is generally used (American Diabetes Association, 2003). The diabetic condition also causes higher arterial rigidity and greater vascular resistance to blood flow, resulting in reduced blood supply in the lower-leg arteries even though the individual has a normal ABI (> 0.9) (Suzuki et al., 2001). It has been reported that waveform analysis at the popliteal artery provides a powerful tool for identifying impaired peripheral circulation caused by either occlusive arterial disease or increased arterial stiffness and peripheral vascular resistance in diabetic patients using gated two-dimensional cine-mode phase-contrast magnetic resonance imaging (2D-cine-PC MRI) (Suzuki et al. 2001). In Japanese patients with diabetes, elderly patients (>65 years) had a higher prevalence of PAD (12.7%) compared with younger patients (<65 years) (4.0%) (Maeda et al., 2008). Prevalence of diabetic patients with low ABI (<0.90) and intermittent claudication is similar to that of diabetic patients with normal ABI and reduced blood flow in lower-leg arteries, indicating that increase in arterial stiffness and vascular resistance to blood flow may be one of the major causes of lower-extremity arterial disease in Japan (Suzuki et al., 2003).

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Endothelial cells actively regulate vascular tone and permeability, the balance between coagulation and fibrolysis and interaction with platelets (Davignon & Ganz, 2004). Vascular endothelium is resistant to interaction with circulating platelets under normal circumstances. An adhesive glycoprotein, von Willebrand factor (VWF), is synthesized by endothelial cells and stored in intracellular granules. When endothelial cells are injured, this molecule mediates platelet adhesion to the inflamed endothelial cells and participates in thrombus formation to arrest hemorrhage at the sites of vascular injury (Ruggeri & Mendolicchio, 2007). Serotonin is a monoamine neurotransmitter mainly synthesized in the enterochromaffin cells of the gastrointestinal mucosa and is released into the portal blood. This molecule is either rapidly stored in platelets for use in vasoconstriction to stop bleeding or metabolized by the liver and kidney to 5-hydroxyindole acetic acid (5-HIAA) (Tyce, 1990). Associations of these endothelial factors with peripheral circulation in lower-leg arteries among diabetic patients with normal ABI are not fully understood.

In the present study, we attempted to clarify whether circulating levels of VWF or 5-HIAA are associated with blood flow in lower-leg arteries in type 2 diabetic patients with normal ABI using gated 2D-cine-PC MRI.

## 2. Patients and methods

### 2.1. Patients

One hundred twenty-three type 2 diabetic patients and 30 nondiabetic subjects ranging in age from 45 to 75 years consecutively admitted to our hospital between May 2006 and March 2009 were recruited for the study. All diabetic patients were admitted for strict glycemic control or assessment of diabetic complications including eye, renal, neurological, and circulatory disorders. Diabetic patients taking antiplatelet agents for the primary prevention of cardiovascular disease and diabetic patients with clinical history of cerebrovascular disease, coronary artery disease, or PAD were excluded from the study. Patients who had abused alcohol or had foot edema caused by heart failure, liver cirrhosis, severe nephropathy (serum creatinine $>177\,\mu mol/l$), malignant neoplasm, autoimmune disorder, acute illness, or urinary tract infections were excluded from the study. Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) were used for the treatment of hyperlipidemia (LDL cholesterol $\geq 3.35$ mmol/l) in diabetic patients. All diabetic patients with hypertension (>140/90 mm Hg) received renin–angiotensin system (RAS) inhibitors such as angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) for the management of high blood pressure. A 75-g glucose tolerance test was performed in our outpatient clinic for the diagnosis of patients with normal glucose regulation, impaired glucose tolerance, and diabetes mellitus (Alberti & Zimmet, 1998). Individuals with normal glucose tolerance were used as nondiabetic subjects in this study. The study was approved by the ethics committee of our institution, and informed consent was obtained from all patients before the examinations done during their stay in our hospital.

### 2.2. Clinical methods

Blood samples were drawn before breakfast in the morning after a 12-hour overnight fast. The HbA1c (%) is estimated as an NGSP equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (JDS; %) + 0.4%, considering the relational expression of HbA1c (JDS; %) measured by the previous Japanese standard materials and measurement methods and HbA1c (NGSP) (NGSP [%] = 1.019 × JDS [%] + 0.30) and the coefficient of variance of 2%–3% in the measurement of HbA1c (The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, 2010). Blood pressure was measured by a sphygmomanometer with the patients in the sitting position after 5 min of rest. Three readings separated by 2 min were taken, and the average was used for analysis. An automatic device (BP-203RPE; Colin, Komaki, Japan) was used to measure both ABI and brachial-ankle pulse-wave velocity (baPWV) as an index of arterial stiffness. A trained ophthalmologist carried out fundus ophthalmoscopies and classified diabetic patients as without retinopathy or as having simple, preproliferative, or proliferative retinopathy. Diabetic patients were classified by the measurement of urinary albumin excretion in 24-h urine collection as having normo-, micro-, or macroalbuminuria when at least two of three specimens were at diagnostic threshold of less than 30, 30–300, or greater than 300 mg/24 h, respectively. Estimated glomerular filtration rate (eGFR) was calculated by the modification of diet in renal disease formula with Japanese ethnic factor of 0.881 as follows: eGFR (ml/min per 1.73 $m^2$) $= 0.881 \times 186.3 \times Age^{-0.203} \times SCr^{-1.154}$ (if female $\times 0.742$), where SCr is serum creatinine (mg/dl) (Imai et al., 2007). Diabetic patients were screened for distal symmetric polyneuropathy using a 128-Hz tuning fork applied to the bony prominence at the dorsal surface of both great toes, just proximal to the nail bed. If the patient feels vibration for more than 10 s, vibration perception was regarded as a normal response (Boulton et al., 2005). Each subject was also classified based on smoking habits as being a current smoker or nonsmoker. Nonsmokers were defined as not having consumed tobacco for at least the previous 3 years. Plasma soluble intercellular adhesion molecule-1 (sICAM-1) and monocyte chemoattractant protein-1 (MCP-1) concentrations were measured by enzyme-linked immunosorbent assay kit (Human sICAM/CD54 or Human MCP-1 Quantikine ELISA kit; R&D Systems, Minneapolis, MN). Serum high-sensitivity C-reactive protein (hsCRP) levels were measured by a microparticle-enhanced immunonephelometric assay (CardioPhase hsCRP; Dade Behring, Newark, DE). Plasma von Willebrand factor ristocetin cofactor activity (VWF) was tested using reagents (BC von Willebrand Reagent; Dade Behring, Marburg, Germany). Plasma 5-HIAA concentrations were measured by the high-performance liquid chromatography system using a Model L-7100 pump (Hitachi, Tokyo, Japan) and a Model ECD-300 electrochemical detector (Eicom, Kyoto, Japan). An MRI scanner operating at 1.5-Tesla (Signa Horizon-LX; GE Medical Systems, Milwaukee, WI) was used for the following experimental protocols as previously described (Suzuki et al., 2001). All patients were at rest in the supine position during examinations, which were done in a temperature-controlled room at 25°C. A single slice at the popliteal artery was oriented perpendicular to the flow direction, and flow data were obtained using two-dimensional cine-mode phase-contrast magnetic resonance imaging with 80-cm/s velocity encoding triggered by peripheral gating. The accuracy and reproducibility of this methodology to measure flow volume for triphasic waveforms created from a pulsatile pump have been reported (McCauley et al., 1995). Flow data were analyzed on an Advantage Windows version 4.2 workstation (GE Medical Systems). The instantaneous flow volume at 20 equally spaced time points through the cardiac cycle was calculated from the individual velocity images by integrating the velocity across the area of the vessel. A resistive index, which is associated with arterial resistance to blood flow, has been defined as $A - B)/A$, where $A$ is the systolic peak velocity and $B$ is the end-diastolic velocity (Halpern et al., 1998).

### 2.3. Statistical analysis

Statistical evaluation was done on SPSS software version 11.0 for Windows (SPSS Inc., Chicago, IL). Normality of distribution of each variable was assessed with the Kolmogorov–Smirnov test. Comparison between the two groups was performed using the unpaired Student's $t$-test. A multiple comparison of significant differences