Arterial wall stiffness is associated with peripheral circulation in patients with type 2 diabetes

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Abstract

The prevalence of peripheral vascular disease (PVD) in diabetic patients is manyfold higher than that of age- and sex-matched nondiabetic subjects. This study was designed to evaluate the relationship between quantitatively determined peripheral circulation in the lower extremities and arterial wall thickness or stiffness in 68 patients with type 2 diabetes. Peripheral circulation during treadmill-exercise was monitored by transcutaneous oxygen tension (TcPO2) and was expressed as percentage of post-exercise TcPO2 adjusted by that of pre-exercise (TcPO2 index). Arterial wall thickness (intima-media thickness; IMT) and stiffness (stiffness b) were measured by ultrasonography. TcPO2 index was negatively (r = 0.350, P = 0.0007) correlated with stiffness b, not with IMT, of the femoral artery. In patients without insulin therapy (n = 52), both fasting plasma insulin concentration (r = 0.323, P = 0.0023) and HOMA IR, an insulin resistance index, (r = 0.281, P = 0.0084) were negatively correlated with TcPO2 index. Multiple regression analyses showed that association of stiffness b of the femoral artery or HOMA IR with the TcPO2 index was independent of other factors including age, smoking index, ankle brachial pressure index and IMT of femoral artery. Thus, arterial wall stiffness of femoral artery appears to be a major determinant of peripheral circulation in patients with type 2 diabetes.

Keywords: Atherosclerosis; Peripheral vascular disease; Transcutaneous oxygen tension (TcPO2); Stiffness index b; Insulin resistance

1. Introduction

Diabetes mellitus is associated with accelerated atherosclerotic macrovascular disease affecting arteries that supply the heart and brain, that are major causes of morbidity and mortality in diabetic patients [1–3]. The prevalence of peripheral vascular disease (PVD) in diabetic patients is also manyfold higher than that of age- and sex-matched nondiabetic subjects [4,5]. Assessment of peripheral circulation is clinically important since patients with PVD have increased mortality from cardiovascular disease [6].

Measurement of transcutaneous oxygen tension (TcPO2) is a noninvasive and quantitative method for assessment of the peripheral circulation. TcPO2 has been proven to be accurate in predicting the level of amputation in patients with critical leg ischemia [7,8]. Diabetic patients were shown to have lower TcPO2 in lower extremities than control subjects with equivalent degrees of PVD [9].

Arterial ultrasound is now commonly used to assess arterial wall thickness (intima-media thickness; IMT) and stiffness b as an arterial stiffness parameter. In cross-sectional studies, IMT has been shown to be associated with occurrence of myocardial infarction [10,11]. Arterial stiffness is a predictor of risk for cardiovascular mortality in patients with end-stage renal diseases [12,13]. These parameters have been shown to

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be increased in patients with type 2 diabetes [14]. Moreover, a recent report from our laboratory showed that stiffness $\beta$ in the femoral artery is closely associated with ischemic symptoms of the lower extremities in patients with type 2 diabetes [15]. However, the relationship between arterial stiffness and quantitatively measured peripheral circulation in the lower extremities is not clear. The aim of this study is to evaluate the relationship between $\text{TcPO}_2$ and arterial wall thickness or arterial stiffness in patients with type 2 diabetes.

2. Research design and methods

2.1. Subjects

The subjects were 68 patients (26 men and 42 women) with type 2 diabetes who were hospitalized in Osaka City University Hospital for treatment or attending an educational course on diabetes. To negate the effects of occlusion or severe stenosis in the lower extremities, patients with a history or any symptoms of PVD and patients with an ankle-brachial pressure index (ABI) lower than 0.9 were excluded from the study. Patients had a diagnosis of diabetes mellitus as defined by the American Diabetes Association criteria [16]. Informed consent was obtained from all patients enrolled in the study. The BMI was calculated by dividing the body weight (kg) by the square of height (m). Information on smoking habits was obtained by a self-administered questionnaire. Smoking index was calculated by multiplying the number of cigarettes per day by years.

2.2. Biochemical analyses

Blood was drawn after an overnight fast. Plasma concentrations of glucose were measured by the glucose oxidation method, hemoglobin A1c (HbA1c) by high-pressure liquid chromatography, and plasma insulin levels by immunoradiometric assay (Insulin RIA Read II Kit; Dainabot, Tokyo). Serum concentrations of total cholesterol, triglyceride and HDL cholesterol were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 740; Hitachi, Tokyo). The homeostasis Model Assessment (HOMA) index, an insulin resistance index (IR), was calculated as (fasting plasma insulin; unit) $\times$ (fasting plasma glucose; unit)/22.5 [17].

2.3. Measurement of ankle-brachial pressure index (ABI)

The systolic blood pressure in the posterior tibial artery was measured by an 8 MHz continuous-wave Doppler probe (Fukuda Denshi Co. Ltd. Tokyo, Japan) in the supine position. ABI was calculated by dividing the systolic blood pressure at the ankle with that in the brachial artery.

2.4. Measurement of $\text{TcPO}_2$

$\text{TcPO}_2$ was measured with an electrochemical transducer (PO-850; Sumitomo Electric Hightechs), the probe for which was heated to 44 $^\circ$C and fixed on the foot with double-sided adhesive rings and contact liquid supplied by the manufacturer. The probe was placed on the dorsum of the foot in the first intermetatarsal space. The test began with measurement of $\text{TcPO}_2$ after resting for at least 10 min. $\text{TcPO}_2$ was monitored during treadmill-exercise on a 12% slope at 2.4 km/h velocity for 5 min [18]. The $\text{TcPO}_2$ index was expressed as percentage of post-exercise $\text{TcPO}_2$ adjusted for pre-exercise $\text{TcPO}_2$. The coefficient of variation for the $\text{TcPO}_2$ index was 6.6% ($n=5$).

2.5. Ultrasonography

Ultrasonographic scanning of the carotid artery and femoral artery was performed with an ultrasonic phase-locked echotracking system, which was equipped with a high-resolution real-time 10 MHz linear scanner (SSD 650 CL, Aloka Co Ltd). The site of the most advanced atherosclerotic lesion was examined in longitudinal and transverse projections to record the IMT [19,20]. IMT was defined as the distance between the leading edges of the lumen–intima interface and the media–adventitia interface of the far wall. The stiffness index $\beta$ [21,22], an index of the elasticity of the arterial wall, was calculated as In(PS/Pd) $\times$ Dd/(Ds $-$ Dd), where Ps and Pd were the systolic and diastolic blood pressures, andDs and Dd the systolic and diastolic inner diameters of the artery. The coefficient of variation for IMT and stiffness index were 3.6 and 4.1%, respectively, as described previously [19,21]. The ultrasonographers were blinded to the results of $\text{TcPO}_2$ index.

2.6. Statistical analysis

All values are expressed as mean $\pm$ S.D. Statistical analyses were performed with the help of STATVIEW 5.0 software (SAS Institute, Cary, NC). Simple or multiple regression analysis was performed to evaluate relationships among the factors. Multiple regression analysis was performed to assess the combined influence of variables on $\text{TcPO}_2$ index. The following factors were considered as independent variables: age, gender, smoking, ABI, HOMA IR, IMT and stiffness index $\beta$ of femoral artery. $P$ values < 0.05 were considered significant.
Table 1
Clinical characteristics of type 2 diabetes subjects (n = 68)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57 ± 12.0</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>26/42</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>8.3 ± 7.2</td>
</tr>
<tr>
<td>Smoking index (cigarette-years)</td>
<td>308 ± 507</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>23.9 ± 3.5</td>
</tr>
<tr>
<td>ABI</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>TcPO2 index (%)</td>
<td>98 ± 13</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>8.3 ± 3.1</td>
</tr>
<tr>
<td>Fasting plasma insulin (mmol/l)</td>
<td>45.0 ± 25.2</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>2.7 ± 1.9</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.7 ± 2.3</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.20 ± 0.91</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.48 ± 1.02</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.29 ± 0.39</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131 ± 19</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 ± 10</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. or number. ABI, ankle-brachial pressure index; HbA1c, hemoglobin A1c; HDL, high density lipoprotein. Fasting plasma insulin and HOMA IR represent data of subjects not receiving insulin (n = 52).

3. Results

Table 1 shows the clinical profiles of the patients studied. Even though patients with ischemic symptoms of the lower limbs were excluded from the study, 32 patients (47%) were observed to have decreased TcPO2 values during treadmill exercise.

We performed simple linear regression analyses of the associations between the TcPO2 index and clinical factors. Age (r = −0.005), duration of diabetes (r = 0.185), BMI (r = 0.002), smoking index (r = −0.182), systolic blood pressure (r = 0.061), diastolic blood pressure (r = 0.128), fasting plasma glucose (r = −0.116), HbA1c (r = −0.010), serum cholesterol (r = −0.163), serum triglyceride (r = 0.042) and serum HDL-cholesterol (r = −0.004) were all not significantly correlated with TcPO2 index. In this study population, TcPO2 index was not correlated with ABI (r = 0.171, ns). Both fasting plasma insulin concentration (r = −0.323, P = 0.0023) and HOMA IR (r = −0.281, P = 0.0084) were negatively correlated with TcPO2 index in patients not receiving insulin therapy (n = 52) (Fig. 1).

We next examined the relationships between TcPO2 index and arterial wall thickness (IMT) or arterial stiffness (stiffness β). TcPO2 index was negatively (r = −0.350, P = 0.0007) correlated with stiffness β of the femoral artery, while it did not show significant correlation with IMT of the femoral artery (r = 0.100, ns) (Fig. 2). Stiffness β (r = −0.053, ns) and IMT (r = −0.033, ns) of the carotid artery were not significantly correlated with TcPO2 index. In this study population, femoral IMT was positively correlated with total cholesterol (r = 0.324, P = 0.026) or triglyceride (r = 0.559, P < 0.001). Its negative association with HDL-cholesterol was borderline significance (r = −0.274, P = 0.063). In contrast, femoral stiffness β was not significantly correlated with these lipid parameters.

To examine whether the association between TcPO2 index and stiffness β of the femoral artery is independent of the other clinical factors, multiple regression analyses were performed. As shown in Table 2, the association of stiffness β of the femoral artery with TcPO2 index was independent of other factors including age, gender, smoking index, ABI and IMT of femoral artery. In patients without insulin therapy, stiffness β of the femoral artery and HOMA IR were independently and significantly associated with TcPO2 index.
We demonstrated that increased stiffness $\beta$ of the femoral artery is closely associated with quantitatively assessed peripheral circulation in patients with type 2 diabetes without PVD symptoms. Recent observations have revealed the importance of functional impairment of the arterial wall as well as arterial wall thickening (atheromatous changes) in vascular complications. Van Popele et al. [23] showed that stiffness of the aortic and carotid artery was significantly associated with arterial thickness and plaque formation. They also showed that arterial stiffness is increased in patients with PVD (ABI < 0.9) compared with that in patients without PVD. Arterial stiffness was also shown to be an independent predictor of cardiovascular mortality in patients with end-stage renal disease [12,13]. Taniwaki et al. [15] found stiffness $\beta$ of the femoral artery to be significantly associated with PVD symptoms independent of femoral artery thickness in patients with type 2 diabetes, suggesting involvement of sclerotic changes in the symptoms of PVD. Recently, Suzuki et al. [24] found that pulse wave velocity from the heart to the tibial artery was associated with reduced arterial flow volume in the popliteal artery in type 2 diabetes. Our present study further shows that quantitatively assessed peripheral circulation is also closely associated with stiffness $\beta$ of the femoral artery. Of importance, involvement of arterial stiffness in the femoral artery was observed in patients without apparent symptoms or signs of PVD, suggesting that arterial stiffness play a role in the early phase of PVD. Lack of association between stiffness $\beta$ of carotid and femoral arteries may be explained by the findings that stiffness $\beta$ of carotid and femoral arteries is related to distinct clinical factors [21]. It is to some extent surprising that classical risk factors including aging, duration of diabetes and smoking were not associated with TcPO$_2$ index in this study. One possible explanation would be that subjects in this study do not have severe PVD, and association with these risk factors may not be apparent in early phase of PVD. We used the value of TcPO$_2$ index following treadmill-exercise, since previous studies showed that TcPO$_2$ during effort clearly demonstrated ischemia in PVD patients and eliminated false-positive clinical results [18,25].

\[ R^2 = 0.17, P = 0.0128 \]

\[ R^2 = 0.31, P = 0.0012 \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\beta$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.133</td>
<td>0.329</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.064</td>
<td>0.641</td>
</tr>
<tr>
<td>Smoking index</td>
<td>0.009</td>
<td>0.951</td>
</tr>
<tr>
<td>ABI</td>
<td>0.133</td>
<td>0.193</td>
</tr>
<tr>
<td>FA-IMT</td>
<td>0.090</td>
<td>0.391</td>
</tr>
<tr>
<td>FA-stiffness $\beta$</td>
<td>-0.360</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

FA, femoral artery; ABI, ankle-brachial pressure index. $\beta$: standard regression coefficients, $R^2$: multiple coefficient of determination.

4. Discussion

In general, type 2 diabetes patients are obese and have insulin resistance. However, BMI (mean BMI 23.1 kg/m$^2$) of general Japanese population in Japan Diabetes Complication Study (JDCS) ($n = 2205$) is reported much less than that of western diabetic patients in UKPDS ($n = 2015$, mean BMI 29.4 kg/m$^2$) [26]. Even though our subjects showed much less BMI (23.9 kg/m$^2$) than Western subjects as reported [26], fasting plasma insulin concentrations or HOMA IR, a well-known marker of insulin resistance was significantly associated with peripheral circulation. Insulin resistance was reported to be associated with increased stiffness $\beta$ of both the carotid and femoral arteries in patients with type 2 diabetes [21]. A similar finding was reported for non-diabetic white women [28]. In the present study, stiffness $\beta$ of neither the carotid artery ($r = 0.005$, $P = 0.97$) nor the femoral artery ($r = -0.033$, $P = 0.83$) exhibited a significant correlation with HOMA IR. This discrepancy in findings may be due to differences in phase of atherosclerosis of subjects studied, since patients with severe PVD were excluded from this study. It is also possible that assessments of insulin resistance by HOMA IR are less sensitive than those by the glucose clamp technique used in previous studies. Although the present study did not determine the mechanism underlying the association between TcPO$_2$ index and insulin resistance, insulin-induced accelerations of blood flow in peripheral arteries may be impaired in the insulin resistance state [29,30].

It is also known that insulin resistance is associated with platelet activation, which may result in platelet aggregation and impaired microcirculation [31].
In summary, stiffness of femoral artery and insulin resistance affect peripheral circulation in type 2 diabetic patients without ischemic symptoms of the lower limbs.

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