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Abstract

Background

Diabetic autonomic neuropathy (DAN) is thought to reduce skin nutritive perfusion through increase of arteriovenous shunting flow, resulting in foot ulceration. However, the correlation between skin tissue oxygenation and DAN has not been fully elucidated. Transcutaneous oxygen tension ($TcPO_2$) is a reliable indicator of skin nutritional microcirculation. The aim of this study was to evaluate the influence of DAN on skin microcirculation by using $TcPO_2$ measurements.

Methods

The resting $TcPO_2$ (REST-$TcPO_2$) and post-exercise $TcPO_2$ (Ex-$TcPO_2$) of the calf and dorsalis pedis regions were measured simultaneously in 52 patients (104 limbs), including 41 diabetes patients. All patients underwent angiography, and the presence of arterial stenosis was evaluated.

Results

$TcPO_2$ levels were compared among the groups of patients with no neuropathy, sensory neuropathy alone, and DAN. In both the calf and dorsalis pedis regions, Ex-$TcPO_2$ levels in diabetes patients with DAN were significantly lower than those in diabetes patients without any neuropathy. However, there was no difference in REST-$TcPO_2$ levels among these groups. We then performed multiple regression analysis to evaluate the influence of DAN on each $TcPO_2$ after adjustment for multiple clinical factors. DAN was a significant determinant of REST- and Ex-$TcPO_2$ in the calf region, and it was independent of arterial stenosis and sensory neuropathy. In contrast, DAN was not an independent determinant of REST- and Ex-$TcPO_2$ in the dorsalis pedis region.

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Conclusions

We, for the first time, showed that DAN has significant effects on skin microcirculation of the calf region but not of the dorsalis pedis region.

Key Words: $TcPO_2$; Diabetes mellitus; Autonomic neuropathy; Peripheral neuropathy

Introduction

In diabetes patients, foot ulceration is the most common precursor to lower limb amputation[1-3]. Approximately 45-60% of all diabetic ulcerations are purely neuropathic components[4]. In general, diabetic peripheral sensory neuropathy (DPN) primarily causes unperceived trauma and leads to ulceration[4,5], while diabetic autonomic neuropathy (DAN) often results in dry skin, resulting in increased susceptibility to bacterial infection[6,7].

In patients with diabetes mellitus, despite normal peripheral arterial circulation, reduced levels of tissue oxygenation have been reported[8,9]. One possible explanation of this finding may be the effects of DAN. The autonomic nervous system plays a role in regulating peripheral blood flow via sympathetic nerve fibers that regulate flow through their action on arteriovenous shunts (A-V shunts) physiologically dedicated to body temperature homoeostasis[10]. Autonomic neuropathy is thought to result in vasodilatation with increased flow through the A-V shunts. This increased A-V shunt flow may impair normal nutritive perfusion and microvascular responses to skin injury, a hypothesis known as the capillary steal phenomenon[11,12]. However, despite the potential association between autonomic neuropathy and impaired A-V shunt flow, it has not been actually shown that autonomic neuropathy reduces nutritive capillary flow, resulting in impaired tissue oxygenation.

Transcutaneous oxygen tension ($TcPO_2$) is a simple indicator of skin microcirculation and tissue oxygenation. It has been reported that $TcPO_2$ is directly related to skin oxygen delivery and the prognoses of leg ischemia and intractable ulcers[13-16]. We also reported that $TcPO_2$ levels in a region are clearly dependent on the angiosome and that post-exercise $TcPO_2$ (Ex-$TcPO_2$) is strongly reduced by stenosis of the perfusing arteries, while resting $TcPO_2$ (REST-$TcPo_2$) is affected by multiple clinical factors. Thus, $TcPO_2$ is a reliable measure to assess the functional status of skin blood flow and the factors affecting skin and tissue oxygenation.

The present study was performed to evaluate the effects of diabetic neuropathy, especially autonomic neuropathy, on skin microcirculation and tissue oxygenation by using $TcPO_2$ measurements independent of macroangiopathy and other clinical factors.

Methods

Subjects

Among outpatients suspected of having peripheral arterial disease (PAD) at Osaka City University Hospital, 66 patients (132 limbs) who provided written informed consent were enrolled in our $TcPO_2$ study as describe previously[15]. This clinical study was approved in advance by the Ethics Committee of the Osaka City University, Graduate School of Medicine. In this analysis, patients with critical limb ischemia including 4 patients of Fontaine Ⅲ and 6 of Fontaine Ⅳ were excluded because of the extreme influence of their reduced blood flow. Furthermore, patients with thromboangiitis obliterans (Buerger's disease), which is

pathophysiologically different from diabetic macroangiopathy[17,18], were also excluded. Finally, we analyzed the data from 52 patients (104 limbs). PAD patients were classified as follows: Fontaine Ⅰ, 12 patients and Fontaine Ⅱ, 31 patients. Non-PAD patients were diagnosed as having symptoms due to diabetic neuropathy in the case of 5 patients and lumbar spinal stenosis in the case of 4 patients.

Diagnosis of diabetic neuropathy

Diabetic neuropathy was defined as previously described with slight modifications[19]. Briefly, DPN was assessed clinically by knee and ankle reflexes and by vibratory sensitivity measured at the medial malleolus. Abnormal autonomic nervous system testing was defined as a reduced change in R-R intervals measured on electrocardiograms during deep breathing or orthostatic hypotension with a blunted catecholamine response. Patients exhibiting abnormal autonomic nervous system test results or other autonomic neuropathic symptoms were rated as having DAN.

Measurement of $TcPO_2$

$TcPO_2$ was measured with a TINA TCM400 monitor (Radiometer, Copenhagen) as previously described[15]. In brief, the measurement probe was attached bilaterally to regions of the calf and dorsalis pedis, avoiding the skin above large blood vessels (e.g., superficial veins) and the skin immediately above bones. $TcPO_2$ in the calf region was measured along the median line on the dorsal side of the crus at a point one-third of the total length of the crus from the periphery. The conditions and method for measurement were identical to those described elsewhere[20-22]. The procedure for measurement is presented below in brief. Room temperature at the time of measurement was 25℃, and the probe temperature was set at 44℃. To eliminate fat and bony tissue, the measurement site was cleansed with alcohol before the ring was fixed, the contact solution was dropped onto it, and the probe was attached. The patient remained still in the supine position for 20-25 min. After the magnitude of change in $TcPO_2$ entered the range of 5 mm Hg or less per minute, the patient stood up and remained standing still for about 5 min, followed by treadmill exercise. Treadmill exercise was performed at a speed of 2.4 km/h and an inclination of 12 degrees. At the end of the exercise, the patient remained still in the supine position again for 20 min. $TcPO_2$ was automatically recorded at intervals of 10 sec during the resting and exercise periods. The mean value of $TcPO_2$ recorded during a one-minute rest period in the supine position immediately before standing served as REST-$TcPO_2$, and the minimum $TcPO_2$ recorded after exercise was recorded as the Ex-$TcPO_2$, according to our previous report[15].

Evaluation of diabetic macroangiopathy and arterial group perfusing the calf or dorsalis pedis region

Stenosed or obstructed sites of arteries were diagnosed by intravenous or intraarterial digital subtraction angiography or three-dimensional computed tomography-angiography in all subjects as described previously[15]. We found that the peripheral arteries of the lower extremities, including the common iliac artery (CIA), extra-iliac artery (EIA), common femoral artery (CFA), superficial femoral artery (SFA), popliteal artery (PA), and anterior tibial artery (ATA), affect $TcPO_2$ in the dorsalis pedis region15) (Fig. 1A). Additionally, another arterial group, ranging from CIA-EIA-SFA-PA to the posterior tibial artery (PTA), affects $TcPO_2$ in the calf region[15] (Fig. 1B). Each segment exhibiting 75% or greater stenosis on leg arteriography was rated as having significant stenosis.

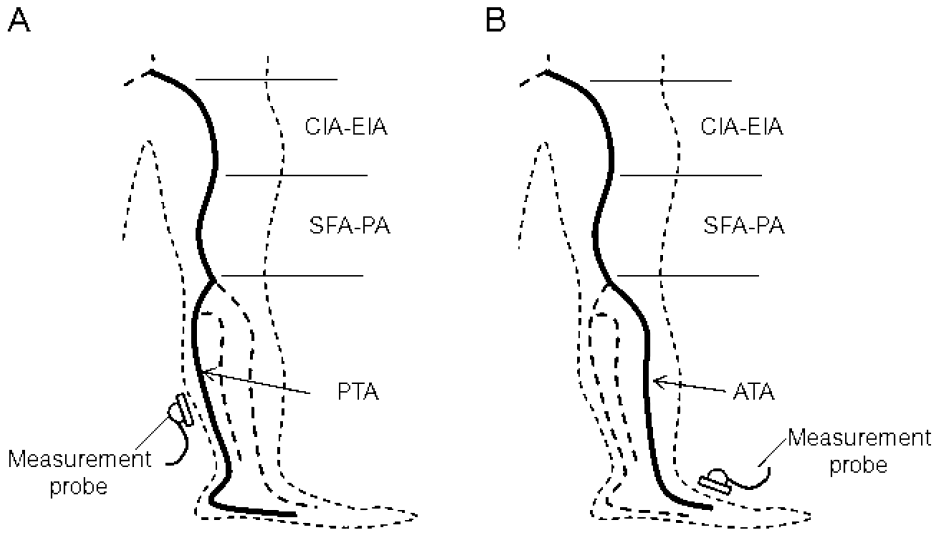

Figure 1. Arteries perfusing the calf and pedis dorsalis regions. Strings of CIA-EIA-CFA-PA-PTA and CIA-EIA-CFA-PA-ATA were defined as arteries perfusing the calf region (A) and pedis dorsalis region (B), respectively. Segments exhibiting 75% or greater stenosis on leg arteriography were rated as having significant stenosis. Abbreviations: CIA, common iliac artery; EIA, external iliac artery; CFA, common femoral artery; PA, popliteal artery; ATA, anterior tibial artery; and PTA, posterior tibial artery.

Hematology and biochemistry

Blood was sampled early in the morning after a fast of at least 12 h. The collected sample was subjected to routine hematological and biochemical tests. The estimated glomerular filtration rate (eGFR) was calculated by using the formula reported by Matsuo et al[23]. This equation originated from the modification of diet in renal disease (MDRD) study group arranged for Japanese individuals, and it is recommended by the Japanese Society of Nephrology and is given as follows: eGFR (mL/min/1.73 m^2) = 194 × $Scr^{-1.094}$ × $Age^{-0.287}$ × 0.739 (if female). The diagnosis of diabetes mellitus was based on the criteria of the American Diabetes Association[24]. Hemoglobin A1c (HbA1c) level was measured by high-performance liquid chromatography, and all HbA1c data are shown in Japan Diabetes Society (JDS) values.

Statistical analysis

Values are expressed as the means±standard deviation (SD). Frequency analysis among multiple groups was performed using the chi-square test. Statistical significance of parametric comparison was evaluated by analysis of variance (ANOVA) and Scheffé's test between multiple groups. Factors affecting $TcPO_2$ were determined by multiple regression analysis. Findings of $p<0.05$ were considered significant. Statistical analyses were performed using SPSS for Windows release 11.0 J (SPSS Inc., Chicago, IL) and STATVIEW 5.0 software (SAS Institute, Cary, NC).

Results

The characteristics of study subjects

The characteristics of the study population are shown in Table 1. Study subjects included 11 non-diabetes patients (non-DM) and 41 type 2 diabetes patients, among whom 7 had no diabetic neuropathy (no-DN), 11 had DPN alone without DAN (DPN), and 23 had both DPN and DAN

(DPN+DAN). There were no diabetes patients who had DAN alone without DPN. The non-DM group did not exhibit neuropathic signs and symptoms. The frequency of females in the DPN+DAN group was highest among the 4 groups. Naturally, hemoglobin A1c (HbA1c) was significantly lower in the non-DM group compared with the no-DN, DPN alone, and DPN+DAN groups, while the DPN+DAN group had lower HbA1c levels than the no-DN group. Hb levels in the DPN and DPN+DAN groups were significantly lower than that in the non-DM group. Regarding the other clinical factors, there was no statistical difference among the groups.

Diabetes patients with autonomic neuropathy may have decreased $TcPO_2$ levels in their lower extremities

To assess the association between DN and skin microcirculation, we compared REST-$TcPO_2$ and Ex-$TcPO_2$ levels among the 3 diabetic groups of no-DN, DPN, and DPN+DAN. In the DPN+DAN group, Ex-$TcPO_2$ levels in both the calf and dorsalis pedis regions were significantly lower compared with those in the no-DN group (the calf region, $p<0.01$; the dorsalis pedis region, $p<0.05$ vs no-DN group) (Fig. 2). REST-$TcPO_2$ levels in the DPN+DAN group showed a tendency of reduction in both the calf and dorsalis pedis regions, although there were no statistically significant changes.

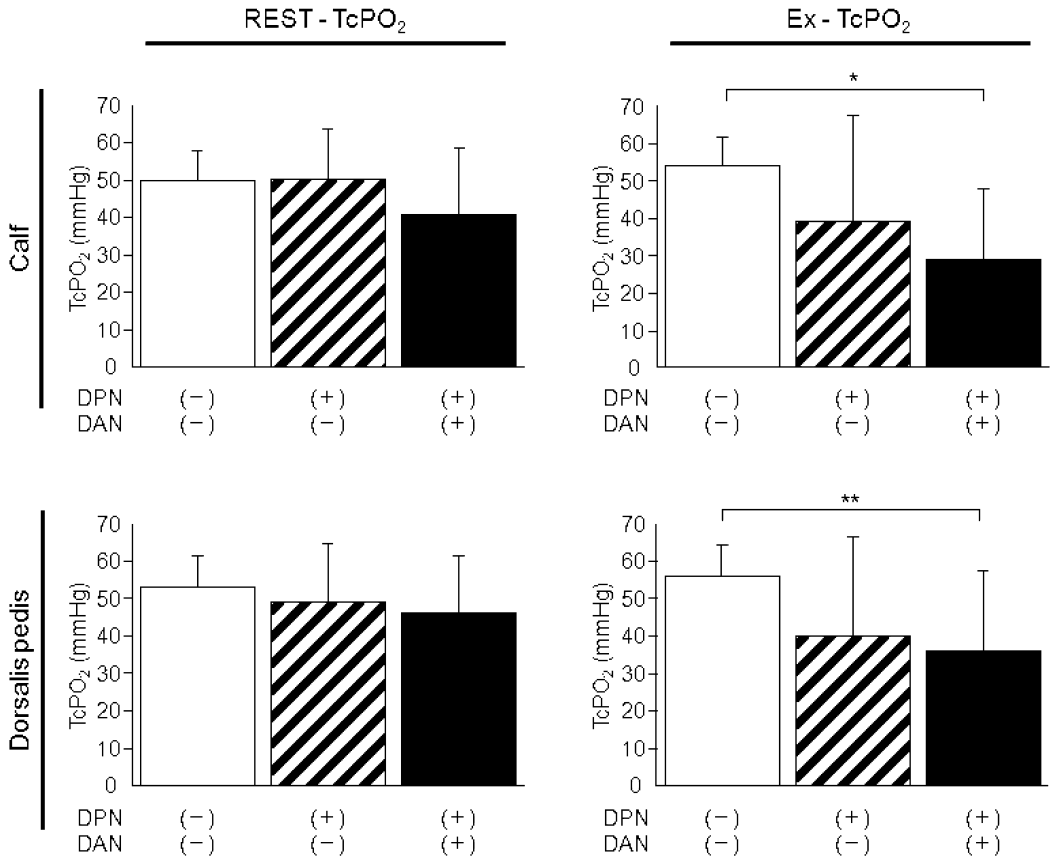

Figure 2. REST- and Ex-$TcPO_2$ levels of the calf and pedis dorsalis regions among diabetes subjects with or without diabetic neuropathy. Data are shown as means (SD). *$p<0.01$ and **$p<0.05$ for the DPN+DAN group compared to the no-DN groups. The diabetic groups without any neuropathy (open column), with DPN alone (hatched column), and with both DPN and DAN (closed column) included 14, 22, and 46 limbs, respectively. Abbreviations: DN, diabetic neuropathy; DPN, diabetic peripheral sensory neuropathy; DAN, diabetic autonomic neuropathy; REST-$TcPO_2$, the resting $TcPO_2$ value; and Ex-$TcPO_2$, the post-exercise minimum $TcPO_2$ value.

Multiple regression analysis of clinical factors affecting $TcPO_2$ in the calf or dorsalis pedis region

Figure 2 shows the possible association between DAN and reduced $TcPO_2$ in diabetes patients. However, the association was still unclear because diabetes patients with DN are very likely to have macroangiopathy as another complication, and there were several differences in clinical characteristics among the diabetic groups (Table 1). Thus, we performed multiple regression analysis using the data of 104 limbs to adjust for the influences of possible affecting factors including stenosis of the perfusing arteries, according to our previous report[15].

Table 1. Clinical characteristics of study subjects

		DM			
		non-DM	no-DN	DPN	DPN+DAN
Number	(patients)	11	7	11	23
	(limbs)	22	14	22	46
Age	(years)	56.5±14.3	63.9±18.4	61.9±9.1	63.7±8.5
Sex	(Female %)	18.2	14.3	36.4	63.0*
Smoker	(%)	40.9	14.3	36.4	50.0
DM duration	(years)	–	16.7±26.2	13.2±7.7	20.1±8.4
BMI	(kg/m^2)	24.0±4.2	23.6±0.50	22.3±5.2	23.7±5.0
sBP	(mm Hg)	128.8±22.5	131.9±19.4	139.9±21.3	142.0±16.1
dBP	(mm Hg)	80.3±8.0	78.4±11.5	77.5±10.7	75.4±9.3
Hemoglobin	(g/dL)	13.8±1.6**	13.3±1.8	12.4±1.4	12.3±1.6
HbA_{1c}	(%)	5.5±0.7†	9.3±2.4	8.4±1.6	7.3±1.5††
eGFR	(mL/min/1.73 m^2)	73.6±34.8	72.5±49.4	67.1±19.7	61.3±26.7
LDL	(mg/dL)	106.2±35.6	125.8±21.5	101.7±29.0	117.0±23.9
HDL	(mg/dL)	44.0±19.3	38.8±10.7	43.5±15.4	39.7±8.1
TG	(mg/dL)	157.9±98.9	121.2±40.8	131.7±34.9	126.4±61.0

Data are denoted as means±SD. *$p<0.05$, significant difference among the groups; **$p<0.05$, compared with the DPN and the DPN+DAN groups; †$p<0.05$, compared with the other groups; and ††$p<0.05$, compared with the no-DN group. Abbreviations: DM, diabetes mellitus; DN, diabetic neuropathy; DPN, diabetic peripheral sensory neuropathy; DAN, diabetic autonomic neuropathy; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; HDL, high density lipoprotein; and TG, triglyceride.

In the calf region, after adjustment for sex, age, smoking, Hb, eGFR, and arterial stenoses, DM was shown as an independent determinant of REST-$TcPO_2$ (Table 2A, model 1). When the factor of DN was added to the regression model, DAN, but not DPN, was shown to be an independent determinant instead of DM (Table 2A, models 2 and 3), revealing that the large part of the influence of DM on REST-$TcPO_2$ in the calf region was mediated by DAN. Furthermore, the influence of DAN was independent of DPN and macroangiopathy (Table 2A, model 4). In accordance with our previous report[15], arterial stenosis was the strongest independent determinant of Ex-$TcPO_2$ (Table 2A, model 5). DAN, but not DPN, was also shown as a significant determinant of Ex-$TcPO_2$ independent of arterial stenosis (Table 2A, models 6-8).

In the dorsalis pedis region, sex, age, Hb, eGFR, and arterial stenosis were shown to be independent determinants of REST-$TcPO_2$ (Table 2B, model 1-4). Hb, eGFR, arterial stenosis, and DM were identified as independent determinants of Ex-$TcPO_2$ (Table 2B, models 5-8).

However, autonomic neuropathy was not shown to be an independent determinant of either REST-$TcPO_2$ or Ex-$TcPO_2$ in the dorsalis pedis region.

Table 2. Multiple regression analysis of clinical factors affecting $TcPO_2$ in the calf (A) or dorsalis pedis region (B)

A) Calf region

	REST-$TcPO_2$				Ex-$TcPO_2$			
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
Sex (female=0, male=1)	−0.260*	−0.266*	−0.329**	−0.329**	−0.083	−0.057	−0.065	−0.059
Age	−0.423**	−0.402**	−0.454***	−0.459***	−0.168	−0.255	−0.268	−0.275
Current smoker (no=0, yes=1)	−0.197	−0.144	−0.135	−0.148	−0.121	−0.180	−0.170	−0.201
sBP	0.093	0.119	0.088	0.082	0.107	0.107	0.129	0.117
Hemoglobin	0.109	0.084	0.132	0.141	−0.031	0.031	0.089	0.114
eGFR	0.521***	0.513***	0.514***	0.518***	0.233	0.301*	0.331*	0.339*
Arterial stenosis (no=0, yes=1)	−0.120	−0.090	0.002	−0.00017	−0.625***	−0.516***	−0.409***	−0.407***
DM (no=0, yes=1)	−0.242*	−0.122	−0.043	−0.069	−0.088	0.078	0.213	0.161
Peripheral neuropathy	–	−0.178	–	0.054	–	−0.113	–	0.129
Autonomic neuropathy	–	–	−0.389**	−0.406**	–	–	−0.329**	−0.383**
R^2	0.250**	0.264**	0.342***	0.343***	0.436***	0.437***	0.494***	0.498***

B) Dorsalis pedis

	REST-$TcPO_2$				Ex-$TcPO_2$			
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
Gender (female=0, male=1)	−0.255*	−0.270*	−0.277*	−0.277*	0.087	0.117	0.107	0.122
Age	−0.432***	−0.397**	−0.409**	−0.406**	−0.112	−0.169	−0.171	−0.180
Current smoker (no=0, yes=1)	−0.057	−0.015	−0.027	−0.020	−0.010	−0.105	−0.073	−0.134
sBP	−0.001	−0.004	−0.012	−0.008	0.058	0.020	0.043	0.023
Hemoglobin	0.363**	0.333*	0.347**	0.342*	0.130	0.191*	0.187*	0.231*
eGFR	0.371**	0.348**	0.352**	0.350**	0.153	0.201*	0.208*	0.222*
Arterial stenosis (no=0, yes=1)	−0.457***	−0.475***	−0.464***	−0.464***	−0.787***	−0.686***	−0.661***	−0.641***
DM (no=0, yes=1)	0.050	0.032	0.038	0.026	−0.173*	−0.219*	−0.315**	−0.250*
Peripheral neuropathy	–	−0.066	–	−0.026	–	0.070	–	0.199
Autonomic neuropathy	–	–	−0.071	−0.062	–	–	−0.086	−0.173
R^2	0.348***	0.361***	0.363***	0.363***	0.750***	0.761***	0.764***	0.774***

Multiple regression analyses were performed using the data of 104 limbs. This table provides standard regression coefficients (β) and levels of significance. R^2, multiple coefficient of determination. Sex, current smoker, arterial stenosis, and DM were represented by dummy variables as shown in the table. Arterial stenosis was diagnosed when there was significant stenosis (≧75%) in any region through CIA-EIA-CFA-PA-PTA or CIA-EIA-CFA-PA-ATA for the calf and dorsalis pedis region, respectively. *$p<0.05$, **$p<0.01$, and ***$p<0.001$. Abbreviations: CIA, common iliac artery; EIA, external iliac artery; CFA, common femoral artery; PA, popliteal artery; ATA, anterior tibial artery; PTA, posterior tibial artery; sBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; DM, diabetes mellitus; REST-$TcPO_2$, the resting $TcPO_2$ value; and Ex-$TcPO_2$, the post-exercise minimum $TcPO_2$ value.

Discussion

It has been generally believed that autonomic neuropathy contributes to the reduction of the flow in nutritional capillaries in the skin through dilated A-V shunts, because the failure of sympathetic control was reported to cause vasodilatation of A-V anastomoses[11,12]. However, the actual association between DAN and reduced nutritive capillary flow has not yet been clearly

revealed. We, for the first time, demonstrated that DAN, but not DPN, was associated with reduced nutritive capillary flow and tissue oxygenation in the calf region.

Previous reports failed to show a relationship between DAN and $TcPO_2$[25,26]. However, they did not exclude the influence of factors potentially affecting $TcPO_2$, such as sex, age, smoking, anemia, and peripheral arterial flow. In particular, diabetes patients with DN are very likely to have macroangiopathy, which is a strong determinant of $TcPO_2$. We previously reported the clinical factors affecting $TcPO_2$ and that their effects on $TcPO_2$ are altered by measurement sites and conditions[15]. By adjusting for the influence of the affecting factors, according to our previous finding, we successfully determined the effects of DAN on $TcPO_2$.

In this analysis, it was also observed that the effects of DAN on regional $TcPO_2$ were different between the calf region and the pedis dorsalis region. However, in this study, it was still unclear as to why DAN did not affect $TcPO_2$ in the pedis dorsalis region similar to its effect on $TcPO_2$ in the calf region. A possible explanation for this discrepancy is that $TcPO_2$ in the pedis dorsalis region might be more directly affected by the status of arterial blood flow but not by alteration of the microcirculation because of the thin tissue volume in the region relative to that in the calf region. Arterial stenosis and Hb were consistently shown as independent determinants for REST-$TcPO_2$ in the pedis dorsalis region, in contrast to that in the calf region (Tables 2A and 2B, models 1-4).

Interestingly, for Ex-$TcPO_2$ in the dorsal region, DM was a determinant factor independent of macroangiopathy and autonomic neuropathy, revealing other possible components that reduce peripheral circulation in diabetes. A possible component might be arterial calcification or arterial stiffness. Mönckeberg sclerosis and medial artery calcification are well-known phenomena associated with both diabetes and chronic kidney disease (CKD). While male gender, aging, DM, and eGFR (CKD) were determinants of Ex-$TcPO_2$ in the dorsal region, they are also known as risk factors for increased arterial wall stiffness[27-29]. Both arterial calcification and arterial stiffness have been reported to be associated with decreased peripheral circulation[30,31]. Therefore, arterial calcification and arterial stiffness might be additional factors contributing to reduced peripheral circulation in diabetes. Unfortunately, arterial calcification and arterial stiffness were not measured in this study.

A limitation of this study is that effects of reduction in systemic arterial blood oxygen tension due to the cardiopulmonary dysfunction could not be eliminated. Impaired cardiopulmonary function could be induced by autonomic neuropathy. Despite successfully establishing the association between DAN and $TcPO_2$, it was not known whether the association was due to only the impaired A-V shunt flow. However, because none of the examined patients exhibited clinical signs or had laboratory data suggestive of compromised cardiopulmonary function (e. g., chronic respiratory failure, heart failure), it can at least be said that the study included no patients with extremely compromised cardiopulmonary function.

Conclusion

DAN can reduce $TcPO_2$ in the calf region not only during exercise but also at rest. On the other hand, $TcPO_2$ in the pedis dorsalis region is strongly regulated by factors other than autonomic neuropathy. We, for the first time, showed the different effects of DAN on regional $TcPO_2$.

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