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The association of diabetic microvascular and macrovascular disease with cutaneous circulation in patients with type 2 diabetes mellitus

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ABSTRACT

Aims: To study the impact of diabetic neuropathy, both peripheral sensorimotor (DPN) and cardiac autonomic neuropathy (CAN), on transcutaneous oxygen tension ($TcPO_2$) in patients with type 2 diabetes mellitus (T2DM).
Methods: A total of 163 participants were recruited; 100 with T2DM and 63 healthy individuals. Peripheral arterial disease (PAD) was defined as ankle-brachial index (ABI) values ≤0.90. Diagnosis of DPN was based on neuropathy symptom score and neuropathy disability score (NDS), while diagnosis of CAN on the battery of the cardiovascular autonomic function tests. $TcPO_2$ was measured using a TCM30 system.
Results: Patients with T2DM had lower $TcPO_2$ levels when compared with healthy individuals. Among the diabetic cohort, those who had either PAD, DPN or CAN had significantly lower $TcPO_2$ values than participants without these complications. Multivariate linear regression analysis, after controlling for diabetes duration, diastolic blood pressure, HbA1c, albumin to creatinine ratio and CAN score, demonstrated that $TcPO_2$ levels were significantly and independently associated with current smoking ($p = 0.013$), ABI ($p = 0.003$), and NDS ($p = 0.013$).
Conclusion: Presence of DPN is independently associated with impaired cutaneous perfusion. Low $TcPO_2$ in subjects with DPN may contribute to delay in healing of diabetic foot ulcers, irrespectively of PAD.

1. Introduction

Diabetic peripheral neuropathy (DPN) and cardiac autonomic neuropathy (CAN) are among the most common and studied forms of diabetic neuropathy.[1] DPN accounts for approximately 75% of the diabetic neuropathies and it occurs in about 30–50% of patients with type 2 diabetes mellitus (DM).[1,2] Peripheral arterial disease (PAD) is also common among patients with DM and its prevalence, when ankle-brachial index (ABI) is used as a screening method, is estimated between 20 and 30%.[3,4] Diabetic neuropathy and PAD, indicative of microvascular and macrovascular dysfunction in DM respectively, are major risk factors for the development of the diabetic foot ulcers that can further lead to lower limb amputations.[5]

Transcutaneous oxygen pressure ($TcPO_2$) is a non-invasive method that evaluates skin microcirculation and reflects tissue perfusion and oxygen delivery.[6–8] $TcPO_2$ has been traditionally used for the assessment of critical lower limb ischaemia, while recent studies suggest that it can be used for the prediction of cardiovascular mortality among patients with high cardiovascular risk.[9,10] However, the most important role of $TcPO_2$ seems to be the prediction of foot ulcers healing and lower limb amputations in patients with DM.[11,12]

Although intact macrocirculation and adequate blood flow are important determinants of $TcPO_2$, diabetic neuropathy has been shown to also significantly affect $TcPO_2$ values in the lower limbs of patients with DM.[8,13–15] Previous studies have shown that patients with DPN have lower $TcPO_2$ values than patients without DPN, while microvascular complications, including DPN, have been found to be independently associated with reduced $TcPO_2$.[13,14] Data, however, regarding the association of autonomic neuropathy with cutaneous circulation are obscure.[15,16]

Since $TcPO_2$ seems to reflect both microvascular and macrovascular circulation adequacy in patients with DM, we investigated the role of diabetic PAD and of diabetic neuropathy, both DPN and CAN, on $TcPO_2$ and examined potential determinants of $TcPO_2$ in patients with DM.

2. Subjects, materials and methods

2.1. Study population

A total of 163 participants (326 feet) were recruited for this study: 100 patients with type 2 DM followed at the Diabetes Center of our

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Hospital and 63 healthy individuals that were patients' friends and relatives. Diabetes status was defined according to the ADA criteria.[17]

Exclusion criteria were atrial flutter, fibrillation or use of pacemaker, severe liver or kidney disease (estimated glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2), stage III and IV New York Heart Association (NYHA) heart failure, chronic obstructive pulmonary disease, other causes of peripheral neuropathy, acute limb ischaemia, prior bypass surgery or percutaneous angioplasty to the lower limb arteries, foot ulcer, venous insufficiency, lower limb edema and acute illness in the previous 48 h.

All participants gave written informed consent before participating in the study, which was conducted according to the principles of the Declaration of Helsinki and approved by the Hospital's Ethics Committee.[18]

2.2. Methods

Participants attended the Diabetes Laboratory once. All measurements were performed in the morning after 10–12 h fast and in a room temperature of 23 ± 1 °C. All individuals were asked to avoid smoking and caffeine intake for 8–10 h before the study; all medications were received after the end of the study. A structured questionnaire was used to assess the presence of previous or current diseases, use of medications and smoking habits. For the assessment of diabetic retinopathy an eye examination should have been performed no more than 3 months before the study.

In addition, height and weight were measured in light clothing; body mass index (BMI) was calculated by dividing the body mass (in kg) to the square of the height (in meters). Arterial blood pressure (BP) was measured using an appropriate cuff size two times at five-minute intervals with the participant in the sitting position. The mean value of the two measurements was used in the statistical analysis.

Blood was drawn to determine the glycated haemoglobin (HbA1c), serum lipids (total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides) and serum creatinine on an automated analyzer enzymatically. Low density lipoprotein (LDL-C) levels were calculated using Friedewald's equation.[19] The Modification of Diet in Renal Disease (MDRD) formula was used to calculate eGFR.[20] In addition, albumin to creatinine ratio (ACR) was measured in a first morning void urine sample using the same DCA analyzer.

All tests were performed after the participants were acclimatized in the examination room and rested for 20 min in a supine position.

2.2.1. Assessment of PAD

Bilateral brachial and ankle pressures (dorsalis pedis and posterior tibial artery) were measured using a hand-held Doppler device with a 5–10 mHz probe (dopplex II, Huntleigh Healthcare Ltd., Cardiff, UK) and ABI was calculated as a ratio of systolic blood pressures in the lower and upper extremities. The higher brachial systolic pressure was used as the denominator of the ABI. For each lower extremity the highest pressure in the dorsalis pedis or posterior tibial artery was used as the ABI numerator. PAD was defined according to the American Diabetes Association as ABI values ≤0.90, while ABI values 0.91–1.30 were considered normal and ABI values >1.30 as Mönckeberg sclerosis.[12] Since presence of PAD cannot be ruled out in patients with ABI values >1.30, those patients underwent further color duplex imaging and qualitative waveform analysis for the assessment of PAD.

2.2.2. Assessment of DPN

The assessment of DPN was based on participants' history and physical examination. Symptoms were assessed using the Neuropathy Symptom Score (NSS) which has been described previously and examines the presence of pain, cramps or aching in the feet.[21–23]

Signs of DPN were assessed using the Neuropathy Disability Score (NDS) which is based on the examination of the ankle reflexes, the temperature sensation, the vibration perception using a 128-Hz tuning fork and the pin-prick.[22] Furthermore, vibration perception threshold (VPT) at the great toe of both feet was assessed with a biothesiometer (Bio-Medical Instrument Company, Cleveland, OH, USA).

Diagnosis of DPN was based on the following criteria: presence of mild neuropathic signs (NDS = 3–5) with moderate neuropathic symptoms (NSS ≥ 5) or moderate neuropathic signs (NDS ≥ 6) irrespective of neuropathic symptoms.[23] The VPT was used as a semiquantitative assessment of vibration[24] and a more objective method to estimate neuropathy signs it but was not included in the final diagnosis of DPN.

2.2.3. Assessment of cardiac autonomic neuropathy

Cardiac autonomic function was assessed using the battery of the four standardized tests proposed by Ewing et al.[25–27] ECG recordings of RR intervals were measured automatically, using the computer-aided examination and evaluation system VariaCardio TF5 (Medical Research Limited, Leeds, UK).

In brief, heart rate response to deep breathing was evaluated by estimating the ratio of the maximum and minimum heart rates during six cycles of paced deep breathing (E/I index). Heart rate response to standing was assessed by calculating the ratio of the longest R-R interval (found at around beat 30) to the shortest R-R interval (found at around beat 15) after standing up (30:15 ratio). Heart rate response to the Valsalva maneuver was evaluated by estimating the ratio of the longest R-R interval after the maneuver to the shortest R-R interval during or shortly after the maneuver (Valsalva ratio). The heart rate-based tests were analyzed according to published age-related tables.[26,28,29] For patients older than 69 years of age we used the normal values available for the age above 65. Orthostatic hypotension was diagnosed when a fall in systolic BP >20 mm Hg was observed; a fall of 11–20 mm Hg was considered borderline and a fall of <10 mm Hg as normal.[28] Each normal autonomic function test was graded as 0, each borderline test as 1 and each abnormal test as 2. On the basis of the sum of this score, we calculated the total CAN score, which is the sum of the partial scores (minimum 0, maximum 8). CAN was diagnosed when at least two out of the four tests performed were abnormal.[28,30,31] Patients that were diagnosed with proliferative retinopathy did not perform the Valsalva examination.

2.2.4. Assessment of $TcPO_2$

The assessment of $TcPO_2$ was performed using the TCM30 system (Radiometer, Copenhagen, Denmark). With the patient in a supine position a special sticker was placed on the dorsal surface of the foot between the first and second metatarsal and 3–5 drops of a specific solution was instilled in the notch. After calibrating the devise and heating the Clarke-type electrode at 44 °C, the electrode was placed in the adhesive groove. After 30 min, when the indicated value on the devise screen was stable, $TcPO_2$ value was recorded. $TcPO_2$ was measured in both feet in all participants.

2.3. Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS software version 24.0 for Windows, Armonk, NY, USA) was used for analyses. Except for the study population description, we performed a limb-specific analysis.

The Kolmogorov-Smirnov test was used to test the variables for normal distribution of the data. Student's *t*-test and the Mann-Whitney test were used to assess differences in parametric and non-parametric continuous variables between the studied groups, while the chi-square test was used for categorical variables. Since there were significant differences between patients with type 2 DM in terms of age and gender, we used one-way analysis of variance (ANCOVA) to determine if significant differences in $TcPO_2$ exist between the diabetic cohort and the control group after adjusting for age and gender.

Bivariate correlations for continuous variable were tested using Pearson's or Spearman's correlation coefficients according to the specific indications in the total number of feet examined. Partial correlation was used to control for the effect of other possible confounding variables.

Linear regression analysis was performed to examine for associations between $TcPO_2$ and the studied parameters. In this analysis, we used the average values of $TcPO_2$, VPT, and ABI of the two feet. The parameters that were found to be significantly associated with $TcPO_2$ in the univariate analysis were entered in the multivariate regression analysis model (stepwise backward method). p values <0.05 (two-tailed) were considered statistically significant.

3. Results

3.1. Baseline characteristics

The demographic and clinical characteristics of the study participants are classified according to diabetes status in Table 1. Patients with DM ($n = 100$) were older, had higher BMI and BP values, worse lipid profile, more often PAD and lower $TcPO_2$ values than participants without DM ($n = 63$). When participants with PAD were excluded from the analysis, patients with DM ($n = 58$) had lower $TcPO_2$ levels when compared with participants without DM ($n = 55$) (51.3 ± 10.2 vs. 55.5 ± 9.3 mm Hg, respectively, $p = 0.001$). When participants with DPN were further excluded for the analysis, $TcPO_2$ levels remained lower in patients with DM ($n = 41$) when compared with individuals without DN ($n = 55$) (52.3 ± 10.3 vs. 55.5 ± 9.3 mm Hg, respectively, $p = 0.025$). After adjusting for age and gender, the difference in $TcPO_2$ values remained significant between patients with DM and healthy participants ($p = 0.011$).

Baseline characteristics of the diabetic cohort are presented in Table 2 according to the presence of DPN and CAN. Participants with DPN ($n = 48$) were older, had longer diabetes duration, higher systolic BP and ACR values, more often retinopathy and PAD and lower $TcPO_2$ values when compared with individuals without DPN ($n = 52$). Patients with CAN ($n = 20$) were younger, had higher ACR values, more often retinopathy and PAD and lower $TcPO_2$ values than participants without CAN ($n = 80$). Sixteen patients had both DPN and CAN, 32 had only DPN, 4 patients had only CAN and 48 participants had neither DPN nor CAN.

Table 1
Demographic, clinical and biochemical characteristics of the study participants.

	Participants with DM $n = 100$	Participants without DM $n = 63$	p
Age (years)	66.5 ± 8.8	61.6 ± 8.5	<0.001**
Male gender n (%)	62 (62.0)	23 (36.5)	0.002*
Duration of diabetes (years)	12.0 [4.0, 21.8]	–	–
Body mass index (kg/m^2)	29.2 ± 4.8	26.5 ± 4.3	<0.001**
Systolic blood pressure (mm Hg)	146.6 ± 21.3	129.7 ± 17.9	<0.001**
Diastolic blood pressure (mm Hg)	78.0 ± 7.3	75.9 ± 6.8	0.009**
Current smokers, n (%)	15 (15.0)	18 (28.6)	0.036*
Fasting serum glucose (mg/dl)	158.1 ± 54.4	97.3 ± 9.8	<0.001**
HbA1c (%)	7.7 ± 1.5	5.6 ± 0.3	<0.001**
Total cholesterol (mg/dl)	166.8 ± 31.5	229.8 ± 49.3	<0.001**
HDL cholesterol (mg/dl)	45.6 ± 10.1	54.7 ± 9.8	<0.001**
LDL cholesterol (mg/dl)	93.0 ± 27.8	154.9 ± 40.7	<0.001**
Triglycerides (mg/dl)	141.1 ± 84.3	112.4 ± 57.5	<0.001*
Retinopathy, n (%)	25 (25.0)	0	–
Glomerular filtration rate (ml/min/1.73 m^2)	77.0 ± 28.9	70.6 ± 16.4	0.011**
Albumin-to-creatinine ratio (mg/g)	29.5 [9.0, 89.1]	10.0 [6.0, 16.6]	<0.001***
Peripheral arterial disease, n (%)	42 (42.0)	8 (12.7)	<0.001*
Ankle-brachial index right foot	0.98 ± 0.24	1.07 ± 0.13	0.003**
Ankle-brachial index left foot	0.97 ± 0.25	1.05 ± 0.14	0.008**
Average ankle-brachial index of both feet	0.97 ± 0.25	1.06 ± 0.13	<0.001**
Peripheral neuropathy, n (%)	48 (48.0)	0	–
Neuropathy disability score	4.0 [2.0, 6.0]	1.0 [0, 2.0]	<0.001***
Vibration perception threshold right foot (Volts)	23.5 ± 12.4	13.1 ± 7.5	<0.001**
Vibration perception threshold left foot (Volts)	23.6 ± 12.6	13.4 ± 8.3	<0.001**
Average vibration perception threshold of both feet (Volts)	23.5 ± 12.5	13.3 ± 7.8	<0.001**
Cardiac autonomic neuropathy, n (%)	20 (20.0)	0	–
$TcPO_2$ right foot (mm Hg)	49.2 ± 12.2	57.0 ± 9.7	<0.001**
$TcPO_2$ left foot (mm Hg)	46.8 ± 12.0	53.9 ± 10.6	<0.001**
Average $TcPO_2$ (mm Hg) of both feet	48.0 ± 12.1	55.4 ± 10.3	<0.001**

Data are n (%), means ± SD (standard deviation), median value (25, 75 percentile).
HbA1c: glycated haemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, $TcPO_2$: transcutaneous oxygen tension.
* p values for comparisons between groups by Chi-squared test.
** p values for comparisons between groups by Independent samples t-test.
*** p values for comparison between groups by Mann-Whitney U test.

3.2. $TcPO_2$ and PAD

$TcPO_2$ was significantly and positively correlated with ABI values in the diabetes cohort (Spearman's $r = 0.269$, $p < 0.001$). The association remained significant after controlling for age ($r = 0.318$, $p < 0.001$), diabetes duration ($r = 0.301$, $p < 0.001$), current smoking ($r = 0.319$, $p < 0.001$), presence of DPN ($r = 0.262$, $p < 0.001$) and presence of CAN ($r = 0.301$, $p < 0.001$). After controlling for all the above variables, the association between $TcPO_2$ levels and ABI remained significant ($r = 0.220$, $p = 0.002$).

3.3. $TcPO_2$ and DPN

$TcPO_2$ levels were significantly and negatively correlated with NDS in the diabetes cohort (Spearman's $r = -359$, $p < 0.001$). The association remained significant after controlling for age ($r = -0.373$, $p < 0.001$), diabetes duration ($r = -0.357$, $p < 0.001$), current smoking ($r = -0.358$, $p < 0.001$), presence of CAN ($r = -0.346$, $p < 0.001$) and presence of PAD ($r = -0.282$, $p < 0.001$). After controlling for all the above variables, the association between $TcPO_2$ levels and NDS remained significant ($r = -0.239$, $p = 0.001$). Similar significant correlations were found when VPT values were used instead of NDS (data not shown).

3.4. $TcPO_2$ and CAN

$TcPO_2$ levels were significantly and negatively correlated with CAN score in the diabetes cohort (Spearman's $r = -0.181$, $p = 0.010$). The association remained significant after controlling for age ($r = -0.270$, $p < 0.001$), diabetes duration ($r = -0.244$, $p = 0.001$), current smoking ($r = -0.195$, $p = 0.006$), presence of DPN ($r = -0.169$, $p = 0.017$) and presence of PAD ($r = -0.159$, $p = 0.024$). Moreover, the association between $TcPO_2$ levels and CAN score remained significant after controlling for the use of angiotensin-converting-enzyme inhibitors or angiotensin II receptor blockers ($r = -0.257$, $p < 0.001$) and the use of b-blockers ($r = -0.279$, $p < 0.001$). However, after controlling for all the above variables, the association between $TcPO_2$ levels and CAN score rendered insignificant ($r = -0.083$, $p = 0.248$).

Regarding the individual cardiac autonomic function tests, $TcPO_2$ levels were not significantly associated with E/I index ($r = 0.086$, $p = 0.228$), but they were significantly associated with 30:15 ratio ($r = 0.139$, $p = 0.050$) and the Valsalva ratio ($r = 0.226$, $p = 0.001$).

3.5. Factors associated with $TcPO_2$

Univariate linear regression analysis demonstrated that $TcPO_2$ was significantly and negatively associated with diabetes duration, current smoking, HbA1c levels, ACR, NDS, VPT, CAN score and presence of PAD, DPN and CAN, while a significant positive association was observed with diastolic BP and ABI (Table 3).

Table 2
Demographic, clinical and biochemical characteristics of participants with diabetes mellitus classified according to the presence of peripheral neuropathy and cardiac autonomic neuropathy.

	With DPN $n = 48$	Without DPN $n = 52$	p	With CAN $n = 20$	Without CAN $n = 80$	p
Age (years)	68.7 ± 8.0	64.6 ± 9.0	0.001**	63.7 ± 7.8	67.3 ± 8.9	0.019**
Male gender n (%)	35 (72.9)	27 (51.9)	0.031*	16 (80.0)	46 (57.5)	0.064*
Duration of diabetes (years)	18.0 [8.0, 26.5]	7.0 [2.3, 15.8]	<0.001***	15.0 [8.0, 23.8]	10.0 [3.3, 21.8]	0.096***
Body mass index (kg/m^2)	28.5 ± 4.4	29.8 ± 5.2	0.052**	28.9 ± 4.8	29.3 ± 4.9	0.656**
Systolic blood pressure (mm Hg)	151.6 ± 22.1	142.0 ± 19.5	0.001**	148.1 ± 20.0	146.2 ± 21.6	0.607**
Diastolic blood pressure (mm Hg)	77.0 ± 6.8	79.0 ± 7.6	0.050**	76.9 ± 5.2	78.3 ± 7.7	0.264**
Current smokers, n (%)	10 (20.8)	5 (9.6)	0.117*	5 (25.0)	10 (12.5)	0.161*
Fasting serum glucose (mg/dl)	159.8 ± 59.8	156.6 ± 49.1	0.680**	176.3 ± 61.9	153.6 ± 51.5	0.018**
HbA1c (%)	7.9 ± 1.4	7.5 ± 1.6	0.056**	8.0 ± 1.7	7.6 ± 1.5	0.163**
Total cholesterol (mg/dl)	170.1 ± 29.8	163.7 ± 32.8	0.151**	170.2 ± 34.8	165.9 ± 30.7	0.441**
HDL cholesterol (mg/dl)	44.0 ± 9.9	47.1 ± 10.1	0.032**	42.6 ± 12.2	46.4 ± 9.4	0.031**
LDL cholesterol (mg/dl)	95.7 ± 24.0	90.5 ± 30.8	0.191**	94.6 ± 32.0	92.6 ± 26.7	0.675**
Triglycerides (mg/dl)	152.3 ± 102.8	130.8 ± 61.3	0.072**	165.4 ± 110.2	135.0 ± 75.7	0.041**
Retinopathy, n (%)	19 (39.6)	6 (11.5)	0.001*	10 (50.0)	15 (18.8)	0.004*
Glomerular filtration rate (ml/min/1.73 m^2)	73.4 ± 27.5	80.4 ± 30.0	0.087**	78.6 ± 34.8	76.6 ± 27.4	0.703**
Albumin-to-creatinine ratio (mg/g)	46.5 [14.0, 148,7]	14.2 [7.0, 71.1]	<0.001***	65.7 [11.8, 230.1]	24.5 [9.0, 80.9]	0.038***
Peripheral arterial disease, n (%)	31 (64.6)	11 (21.2)	<0.001*	15 (75.0)	27 (33.8)	0.001*
Ankle-brachial index right foot	0.89 ± 0.26	1.05 ± 0.21	0.002**	0.84 ± 0.23	1.01 ± 0.24	0.007**
Ankle-brachial index left foot	0.87 ± 0.28	1.06 ± 0.19	<0.001**	0.80 ± 0.30	1.02 ± 0.22	0.005**
Average ankle-brachial index of both feet	0.88 ± 0.27	1.05 ± 0.20	<0.001**	0.82 ± 0.27	1.01 ± 0.23	<0.001**
Neuropathy disability score	6.0 [4.0, 8.0]	2.0 [1.0, 4.0]	<0.001***	6.5 [4.0, 9.8]	4.0 [2.0, 6.0]	<0.001***
Vibration perception threshold right foot (Volts)	31.4 ± 12.0	16.2 ± 7.4	<0.001**	30.1 ± 14.8	21.8 ± 11.3	0.028**
Vibration perception threshold left foot (Volts)	31.1 ± 12.3	16.6 ± 8.1	<0.001**	32.0 ± 15.8	21.5 ± 10.8	0.010**
Average vibration perception threshold of both feet (Volts)	31.2 ± 12.1	16.4 ± 7.8	<0.001**	31.03 ± 15.1	21.7 ± 11.0	0.001**
$TcPO_2$ right foot (mm Hg)	45.3 ± 12.4	52.8 ± 10.8	0.002**	45.4 ± 12.8	50.1 ± 11.9	0.120**
$TcPO_2$ left foot (mm Hg)	43.4 ± 12.8	49.9 ± 10.4	0.007**	41.7 ± 17.0	48.0 ± 10.1	0.123**
Average $TcPO_2$ (mm Hg) of both feet	44.4 ± 12.6	51.3 ± 10.6	<0.001**	43.6 ± 15.0	49.1 ± 11.1	0.033**

Data are n (%), means ± SD (standard deviation), median value (25, 75 percentile).
DPN: diabetic peripheral neuropathy, CAN: cardiac autonomic neuropathy, HbA1c: glycated haemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, $TcPO_2$: transcutaneous oxygen tension.
* p values for comparisons between groups by Chi-squared test.
** p values for comparisons between groups by Independent samples t-test.
*** p values for comparison between groups by Mann-Whitney U test.

Multivariate linear regression analysis, after controlling for diabetes duration, diastolic BP, HbA1c, ACR and CAN score, demonstrated that $TcPO_2$ levels were significantly and independently associated only with current smoking, ABI and NDS.

4. Discussion

In this cross-sectional study, we demonstrated that patients with type 2 DM have lower $TcPO_2$ levels than individuals without DM. In addition, we found that in subjects with type 2 DM, $TcPO_2$ levels at the lower extremities are associated significantly and independently with the presence of both PAD and DPN.

Individuals with DM have been reported to have reduced $TcPO_2$ levels when compared with subjects without DM and more importantly this difference persisted for equivalent degrees of PAD and at the absence of diabetic neuropathy.[8,16,32] Our study demonstrated similar results and although the pathogenic mechanism of this finding needs further investigation, we cannot exclude the possibility that early atherosclerotic macrovascular and neuropathic microcirculation defects could not be detected by the methods used in our laboratory.

Defects of microcirculation in diabetes are characterized by both structural and functional changes such as impaired autoregulation of vascular tone and blood flow, unlike defects of macrocirculation that are characterized by occlusive disease of the large vessels. $TcPO_2$ is a non-invasive method for evaluating cutaneous microcirculation. It assesses skin oxygenation through measuring the oxygen that is diffusing from the tissues.[7] Since oxygen delivered to the tissue is affected by blood flow, $TcPO_2$ measurement can also provide valuable information about macrocirculation.[32] Indeed in our study, $TcPO_2$ levels in the diabetic cohort were significantly and independently associated with the presence of PAD.

Although $TcPO_2$ has been used for the assessment of PAD in some studies, the results regarding its performance for the diagnosis of PAD are not unanimous.[6,33] However, $TcPO_2$ measurement is extremely useful for the non-invasive diagnosis of critical limb ischaemia.[9] Moreover, $TcPO_2$ assessment is recommended in the presence of medial arterial calcification and/or ABI values >1.3.[24] Nevertheless, $TcPO_2$ most essential utility is the prediction of diabetic foot ulcer healing and lower limb amputations in patients with DM.[11,12]

DPN, together with PAD and trauma, are the main factors that lead to the development of diabetic foot ulcers.[5] However, impaired wound healing due to defects in foot microcirculation is the primary cause of chronic diabetic wounds that can further result in amputation.[34] Over the last years, several studies have shown that DPN is associated with defects in microcirculation.[13,14,35]

Arora et al.[13] compared $TcPO_2$ values and the hyperemic response to heat by a laser Doppler blood flow monitor between the forearm and the dorsum of the foot in three different groups of participants: 15 patients with diabetes and DPN, 14 patients with diabetes but without DPN and 15 healthy individuals. Patients with PAD were excluded. Forearm and foot $TcPO_2$ values were lower in the neuropathic group when compared with patients without DPN. Maximal hyperemic response to heat was significantly reduced at both forearm and foot in the neuropathic group when compared with the two other groups. However, nerve axon reflex-mediated vasodilatation that is mostly related to the stimulation of the C nociceptor fibers was reduced only at the foot and not at the forearm of the neuropathic patients when compared with patients without DPN and healthy participants. These findings suggest that impaired foot microcirculation in diabetes could be attributed to hyperglycemia induced functional changes of the microvasculature, increased lower extremity capillary pressure upon assuming the upright position and reduced vasodilatory response due DPN.[13] Although our study cannot confirm this hypothesis, presence of DPN was

Table 3
Association between the studied parameters and $TcPO_2$ in participants with type 2 diabetes mellitus.

	Beta coefficient	*p*
Univariate analysis		
Age (years)	−0.100	0.157
Gender (men)	−0.023	0.750
Duration of diabetes (years)	−0.153	0.031
Body mass index (kg/m^2)	0.052	0.467
Systolic blood pressure (mm Hg)	−0.061	0.390
Diastolic blood pressure (mm Hg)	0.144	0.042
Current smokers	−0.249	<0.001
Fasting serum glucose (mg/dl)	−0.008	0.911
HbA1c (%)	−0.148	0.037
Total cholesterol (mg/dl)	0.077	0.278
HDL cholesterol (mg/dl)	0.106	0.136
LDL cholesterol (mg/dl)	0.045	0.530
Triglycerides (mg/dl)	0.007	0.921
Retinopathy	−0.001	0.985
Glomerular filtration rate (ml/min/1.73 m^2)	0.081	0.225
Albumin-to-creatinine ratio (mg/g)	−0.143	0.046
Ankle-brachial index	0.335	<0.001
Peripheral artery disease	−0.323	<0.001
Neuropathy disability score	−0.383	<0.001
Vibration perception threshold (Volts)	−0.302	<0.001
Peripheral neuropathy	−0.289	<0.001
Cardiac autonomic neuropathy score	−0.257	<0.001
Cardiac autonomic neuropathy	−0.184	0.009
Multivariate analysis[a]		
Current smoking	−0.167	0.013
Ankle-brachial index	0.212	0.003
Neuropathy disability score	−0.167	0.013

Gender, smoking status, retinopathy, peripheral arterial disease, peripheral neuropathy and cardiac autonomic neuropathy were analyzed as categorical variable; all the other variables were analyzed as continuous variables in the univariate and multivariate analyses. Data of $TcPO_2$ and ankle-brachial index are the average values of the two feet.
Beta coefficient: standardized regression coefficient, HbA1c: glycated haemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, $TcPO_2$: transcutaneous oxygen tension.
[a] After adjustment for diabetes duration, diastolic blood pressure, Hba1c, albumin-to-creatinine ratio and cardiac autonomic neuropathy score. When presence of peripheral arterial disease, peripheral neuropathy and cardiac autonomic neuropathy as categorical variables were used instead of ankle-brachial index, neuropathy disability score and cardiac autonomic neuropathy score, respectively, the results of the multivariate model did not differ. When vibration perception threshold was used instead of neuropathy disability score in the model, the results of multivariate analysis model did not differ.

associated significantly with impaired cutaneous circulation assessed with $TcPO_2$ independent from the presence of PAD, glycemic control or diabetes duration.

Deng et al.[35] examined 381 hospitalized patients with diabetes and divided them as having clinical DPN, subclinical DPN, confirmed DPN or non-DPN based on the diagnostic criteria recommended by ADA. $TcPO_2$ was measured at the dorsum of the foot with the patient initially in the supine and then in the sitting position and the difference was calculated. Sitting-supine position $TcPO_2$ difference was significantly associated with the presence of DPN, while a cut-off value of 19.5 mm Hg had a sensitivity of about 61% and a specificity of about 74% for the identification of DPN. Again, this finding was attributed to the impaired sympathetic axon reflex response that results in a relative hyperperfusion in the sitting position.[35]

Although it is reported that diabetic neuropathy may lead to the opening of arterio-venous shunts due to sympathetic dysfunction,[36,37] studies examining the association of autonomic neuropathy with cutaneous microcirculation are limited and have small number of participants. Uccioli et al.[16] examined a total of 56 participants (20 healthy controls, 16 patients with type 2 DM without CAN and 20 patients with type 2 DM with CAN) without PAD and reported that although both groups with diabetes had lower $TcPO_2$ values when compared with healthy individuals, no difference was observed between patients with and without CAN. In our study, participants with CAN had lower $TcPO_2$ levels when compared with patients without CAN. However, when patients with PAD were excluded from the analysis ($n = 42$), $TcPO_2$ levels did not differ significantly between patients with CAN ($n = 5$) and individuals without CAN ($n = 53$) (48.7 ± 12.6 vs. 51.6 ± 10.0 mm Hg, respectively, $p = 0.403$). Nevertheless, it should be considered that the number of participants with CAN but without PAD was rather small to draw definite conclusions.

Boyko et al. investigated the determinants of $TcPO_2$ in 657 participants with DM.[38] Diagnosis of DPN was based on loss of the 10-g monofilament perception at least on one site of the foot, while diagnosis of CAN was based on the presence of 2 out of 3 abnormal cardiovascular autonomic neuropathy tests (deep breathing, orthostatic test, 30:15 ratio). Similar to our results, $TcPO_2$ at the dorsal of the foot did not differ between participants with and without CAN. Moreover, the investigators reported that CAN is not an important determinant of $TcPO_2$, since independent predictors of low $TcPO_2$ values were only low ABI, higher height, high HbA1c and presence of foot edema.

Zimny et al. investigated the association of $TcPO_2$ with parameters of DPN and CAN in 3 different group of participants (21 patients with type 2 DM and DPN, 20 patients with type 2 DM without DPN and 21 healthy individuals) without PAD.[15] Diagnosis of DPN was based on VPT, while of CAN on the presence of two out of three abnormal cardiovascular reflex tests. Supine $TcPO_2$ values were significantly lower in the neuropathic group when compared with the non-neuropathic and non-diabetic group. However, sitting $TcPO_2$ levels did not differ among the three groups of participants, while sitting-supine position $TcPO_2$ difference was significantly higher in the neuropathic group. In the group of patients with neuropathy the sitting/supine $TcPO_2$ ratio was negatively correlated with the heart rate variation coefficient at rest and during deep breathing. Thus, the exaggerated increase in sitting $TcPO_2$ levels in the neuropathic group was attributed to the impaired vessel autoregulation due to the presence of autonomic neuropathy. Although in our study we did not measure sitting $TcPO_2$ levels, supine $TcPO_2$ values correlated significantly and negatively with total CAN score. However, the significance was lost after controlling for the presence of DPN and PAD. This finding could suggest that DPN and PAD are more important determinants of foot cutaneous circulation assessed with $TcPO_2$ than CAN. Apart from the relationship between diabetic macrovascular and microvascular disease with $TcPO_2$, we found that the microvascular dysfunction was strongly associated with smoking, which is in accordance with previous studies.[39,40]

A strength of the study is that we evaluated at the same time diabetic macrovascular impairment and microvascular dysfunction in terms of DPN and CAN and their association with cutaneous perfusion in a large cohort of participants. However, this is a cross-sectional study that provides associations between $TcPO_2$ and the studied variables, but it cannot explain the underlying pathophysiology and cannot imply a cause and effect relationship between the associated variables. Moreover, assessment of autonomic neuropathy was based on the battery of cardiovascular reflex tests since peripheral autonomic function tests such as sympathetic skin response or quantitative sudomotor axon reflex test were not available at our Laboratory the time the study was conducted. Nevertheless, although the combination of cardiovascular reflex tests with sudomotor function tests may allow a more accurate diagnosis of diabetic autonomic neuropathy, for the time being the former is the gold standard method in clinical autonomic testing.[30] Another limitation is that our diabetic cohort and the control group were not matched for age and gender, due to the difficulties in recruiting healthy participants. However, differences in $TcPO_2$ between the two groups remained significant after adjusting for these variables. Moreover, the age-related normal values for the cardiovascular autonomic tests used in our study are available for people younger than 69 years of age. Thus, for patients older than 69 years of age we used the normal values available for the age above 65. Although false positive results among older individuals cannot be ruled out, given the small percentage of older patients having CAN (14% of the patients with DM older than 69 years of age) the fact that the combination of all four tests provides an accurate diagnosis of CAN, we consider our results reliable.

Our findings confirm the results of older studies reporting that patients with DM have lower $TcPO_2$ values when compared with healthy individuals. Moreover, our study emphasizes the fact that the observed impaired cutaneous foot perfusion in diabetes is evident irrespective of the presence of PAD and diabetic neuropathy. However, we were not able to further explore the underlying pathophysiological mechanisms of this phenomenon. In the diabetic cohort $TcPO_2$ values were significantly associated with the presence of PAD and DPN regardless of other risk factors. The clinical implication of our study is that diabetic neuropathy might cause further and beyond the presence of macrovascular disease decreased $TcPO_2$ values and this could independently contribute in delaying the healing of diabetic foot ulcers. Although the interaction among macrovascular disease, diabetic neuropathy and impairment of the foot microcirculation is complex, it seems that both PAD and DPN have profound effects on cutaneous circulation of the lower extremities.

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