


# Impaired Vascular Endothelial Function is Associated with Peripheral Neuropathy in Patients with Type 2 Diabetes

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**Purpose:** It is believed that vascular endothelial dysfunction is involved in the occurrence of cardiovascular disease (CVD), and diabetic peripheral neuropathy (DPN) is associated with flow-mediated dilation (FMD), however, the correlation is still unclear. Aims of the present study is to explore the relationship between DPN parameters and FMD, providing a new approach for the prevention of CVD.

**Patients and Methods:** A total of 272 patients with T2DM from the Department of Endocrinology of The First Hospital of Lanzhou University according to the grading criteria were selected. FMD was measured by a new vascular ultrasound system and patients were divided into FMD>7%, 4%≤FMD≤7%, and FMD<4% groups. The Toronto Clinical Scoring System (TCSS) was used to assess the severity of DPN. The nerve conduction studies (NCS) assessed large fibre neuropathy by nerve conduction velocity (CV), compound muscle action potential (CMAP) amplitude (Amp), and distal motor latency (DML). SPSS 25.0 was used for statistical analysis.

**Results:** TCSS evaluation revealed that the percentage of patients with severe nerve injury was significantly higher in FMD<4% (70%) compared to FMD>7% (2%). Among the TCSS indicators of all subjects, the proportion of temperature disturbance was the most (73%), and joint position disturbance was the least (0). TCSS scores were negatively correlated with FMD ( $r=-0.756$ ,  $p<0.001$ ). More interesting, in FMD<4% group, CV and Amp were positively correlated with FMD, while DML was negatively correlated ( $p<0.05$ ). Linear regression analysis model showed that different systolic blood pressure (SBP), triglyceride (TG), TCSS and CV had statistically different effects on FMD.

**Conclusion:** High TCSS score and decreased CV of common peroneal and tibial nerves are risk factors of FMD injury, which provide potential value for timely prevention and treatment of cardiovascular diseases.

**Keywords:** type 2 diabetes, diabetic peripheral neuropathy, vascular endothelial function, cardiovascular disease, Toronto clinical scoring system

## Introduction

Diabetic neuropathy is one of the common diabetic complications with serious consequences, with an incidence of more than 50%. The injury may involve central and peripheral nerves, with the latter being more common.<sup>1</sup> Diabetic peripheral neuropathy (DPN), including autonomic and somatic neuropathy, is a common microvascular complication of diabetes.<sup>2</sup> The diabetic distal symmetrical polyneuropathy (DSPN) is the most common one of diabetic somatic neuropathies.<sup>2</sup> Clinical manifestations of DSPN are broad, this neuropathic pattern tends to occur after 50 years of age, mostly in patients with longstanding diabetes mellitus.<sup>3</sup> There are many screening and diagnostic methods for DPN, among which neuroelectrophysiological examination is objective, sensitive and reliable, and is often regarded as the gold standard.<sup>4,5</sup> Nerve conduction studies (NCS) are an essential tool in the evaluation of the peripheral nervous system.<sup>6</sup> It determines the functional status of peripheral nerves by examining the muscular nerve conduction function of the affected limb.<sup>4</sup> The Toronto Clinical Scoring System (TCSS) was proposed by Perkins et al<sup>7</sup> in 2001 and suggested to be used for DPN

screening and severity evaluation. Therefore, although the onset of DPN is hidden and the mechanism is complex, early diagnosis and treatment can be achieved through timely screening and electrophysiological examination.<sup>8</sup>

The effect of diabetic peripheral nerve injury on cardiovascular disease has little been reported, and that the results are inconsistent.<sup>9,10</sup> It is well known that diabetes is one of the risk factors leading to the development of vascular dysfunction and atherosclerotic disease, and diabetes cardiovascular disease is the main cause of death in patients with diabetes.<sup>11</sup> Although blood glucose, blood pressure, and lipid are key factors in diabetic cardiovascular disease, the results of a cohort study suggest that peripheral neuropathy is a predictor of cardiovascular disease in diabetic patients without cardiovascular disease at baseline, even after controlling for standard cardiovascular risk factors.<sup>12</sup> In contrast, an epidemiological study shows that cardiovascular risk factors are predictors of the development of peripheral neuropathy in patients with type 2 diabetes.<sup>13</sup> These studies show inconsistent causality, but they could still confirm an association between peripheral neuropathy and the risk of cardiovascular disease in patients with type 2 diabetes, moreover, the mechanism of the association remains largely unknown.

Vascular endothelium is a dynamic cellular interface that is essential for normal vascular function and if endothelial cells are dysfunctional, this promotes vascular smooth muscle proliferation, inflammatory cell infiltration, and platelet aggregation, which are key events in cardiovascular disease.<sup>14,15</sup> Some studies have linked risk factors for cardiovascular disease to endothelial dysfunction, for example, endothelial dysfunction is considered to be an important factor in atherosclerosis, hypertension, and heart failure.<sup>16</sup> Endothelial function assessment can directly measure the factors produced by endothelial cells, but these factors have a short half-life, and most of them require specialized laboratories, detection instruments, detection reagents, etc. So we use a noninvasive ultrasonic technique, which can indirectly reflect the vascular endothelial function, and it is using cuff pressure caused reflex hyperemia, increased blood flow to add the shear stress and stimulate the endothelial vascular active factor NO release,<sup>17</sup> causing blood flow mediated endothelial dependent vasodilation (FMD), which can be used as a quantitative imaging indicator of vascular endothelial function. A recent study showed a negative association between FMD and the severity of coronary heart disease, regardless of age, sex, or cholesterol level.<sup>18</sup> Several studies have confirmed the repeatability of FMD, which is one of the predictors of cardiovascular disease progression,<sup>19,20</sup> so application of FMD serves as a bridge between diabetes peripheral neuropathy and cardiovascular disease. This present study explored the relation between type 2 diabetic neuropathy and vascular endothelial function, and provided a new idea for the prevention of diabetic cardiovascular disease.

## Materials and Methods

### Subjects

A total of 272 patients (148 males and 124 females) with type 2 diabetes were included in the Department of Endocrinology, First Hospital of Lanzhou University from December 2019 to December 2021, according to the classification and diagnostic criteria of diabetes by the World Health Organization (WHO) in 1999. All investigations were conducted in accordance with the ethical principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the First Hospital of Lanzhou University. Exclusion: ①Neuropathy caused by other causes, such as cervical and lumbar lesions (nerve root compression, spinal stenosis), cerebral infarction, Guillain-Barre syndrome, etc.; Severe arteriovenous vascular lesions (venous embolism, lymphangitis, etc.); Neurotoxic effects caused by drugs especially chemotherapeutic drugs and nerve damage from metabolic toxins caused by renal dysfunction; ②Patients who took thiazolidinediones, DPP-IV inhibitors, SGLT-2 inhibitors, GLP-1 receptor agonists and neurotrophic drugs within 3 months; ③Have a history of coronary heart disease, severe secondary or malignant hypertension; ④Patients with significant bradycardia (heart rate <50bpm)<sup>21</sup> and hypotension (Blood Pressure <90/60mmHg); ⑤Pregnant or lactating women.

### Data Collection

#### Clinical Characteristics

The patient's age, gender, duration of diabetes, medication status, smoking and drinking history were collected from reported previously. The patient's height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) were measured and the mean levels and standard deviations were recorded.

## Laboratory Tests

Blood sample collections were performed in the morning after 12 hours of fasting. Using high-performance liquid chromatography, we determined glycated haemoglobin (HbA1c) levels. Fasting plasma glucose (FPG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), homocysteine (HCY) and 25-hydroxyvitamin D [25(OH) D] levels were performed by Siemens Advia 1800.

## Determination of Vascular Endothelial Function

Patients who had undergone biochemical assessment were given brachial artery flow-mediated endothelial dependent vasodilation (FMD) in next morning by a novel vascular ultrasound system (UNEXEF 18VG, Nagoya, Japan). A fixed ultrasound physician was assigned to measure the brachial artery blood flow and unaware of the grouping of patients. Patients were asked to abstain from vitamin C, a high-fat diet, coffee, and to fast for at least 8 hours prior to the examination, such as coffee has a injurious effect on the endothelium by stimulating sympathetic nerves. Similarly, patients were asked to avoid strenuous exercise and rest quietly for at least 20 minutes before checking. The specific experimental conditions are as follows: The ambient temperature is kept at 20–24°C, the ambient humidity is 30–85%, the subject was in quiet state, supine position, and the cuff was tied to the subject's forearm. The examination physician placed the electronic linear probe at the brachial artery of the subject's forearm, and obtained the basic diameter of brachial artery lumen D0 when it was quiet. After obtaining the consent of the subject, the cuff was inflated, pressurized for about 2 minutes and 30 seconds, and then rapidly deflated to induce the dilation of the brachial artery. The maximum dilated diameter D1 of flow-mediated vasodilation within 2 minutes was recorded. The left and right brachial arteries were measured by the above procedure, and the average value of the two brachial arteries was taken as the record value of FMD. Calculation formula of FMD:  $FMD (\%) = (D1 - D0) / D0 \times 100\%$ .

## Other Vascular Function Indicators

Endothelial dysfunction is an important cause of macrovascular and microvascular injury and is considered as the earliest change in the progression of arteriosclerosis.<sup>22,23</sup> The ankle-brachial index (ABI) and brachial-ankle pulse wave velocity (baPWV) were used to evaluate arteriosclerosis. The ABI measurements were performed with the Omron Colin BP-203RPE III device (Omron Health Care). The systolic blood pressure (SBP) was measured in the ankle of both lower limbs and the brachial artery of both upper limbs. The SBP of each limb was collected twice with an interval of more than 1 minute. The value of the ABI for each limb was calculated dividing the greater SBP obtained in lower limb by the SBP of whichever was the higher in the upper limbs. The lowest value obtained was considered the ABI for that individual; abnormal threshold value is  $\leq 0.9$ . baPWV measurement: After resting in supine position for 5 minutes, patients were asked to use an automatic arteriosclerosis meter (BP-203RPE II Colin Komaki, Japan) to measure atherosclerosis. Appropriate cuff was tied to the upper arm and lower limb, the mark of the cuff bag of the upper arm was aligned with the brachial artery, and the lower edge of the cuff was 2–3 cm away from the horizontal line of the fossa elbows. The mark of the cuff bag of the lower limb was located in the inner side of the lower limb, and the lower edge of the cuff was 1–2cm away from the medial malleolus. The sensor was placed in the precardiac area, and the arterial pulse waveform was recorded for 5 minutes, and the instrument automatically output baPWV value.

## TCSS

The TCSS was applied to all patients in the current study by a professional clinician blinded to the grouping of patients. TCSS consists of nerve symptoms (lower limb pain, numbness, paresthesia, fatigue, walking imbalance, and upper limb symptoms), Sensory examinations (acromesthesia, temperature sensation, light touch, vibration sensation, joint position sensation) and nerve reflexes (ankle reflex and knee reflex). The total score of nerve symptoms was 6, 1 for the presence and 0 for the absence of symptoms. The total sensory score was 5, 0 for normal and 1 for abnormal, the total score of neural reflexes was 8, 0 for normal, 1 for weakened and 2 for disappeared. According to Perkins<sup>7</sup> grading standards, a score of 0–5 indicates no nerve injury, 6–8 indicates mild nerve injury, 9–11 indicates moderate nerve injury, and 12–19 indicates severe nerve injury.

## Peripheral Nerve Electrophysiological Examination

Nerve conduction studies (NCS) involves eliciting nerve action potentials at sites along a peripheral nerve and recording the response from another site along the nerve or from a muscle innervated by that nerve.<sup>24</sup> Motor NCS are performed by stimulating a nerve trunk and recording the response from a muscle supplied by that nerve. The patients were placed in supine position and examined with KEYPOINT4 electromyogram-induced potentiometer by a clinician who specializes in electromyography. The ambient temperature was 25°C and the skin temperature was 33–35°C. Stimulation was initiated at 0 mA and slowly increased (usually up to 50mA) until a maximal response was obtained. The resultant biphasic waveform was the compound muscle action potential (CMAP). The upper extremity protocol involved stimulation of the median and ulnar nerves of both wrists and elbows, recorded from the musculi abductor pollicis brevis and abductor digiti minimi, respectively. In the lower extremity, peroneal and tibial stimulation was performed at both ankle and knee joints while recording from the extensor digitorum brevis (peroneal) and abductor hallucis (tibial) muscles. The main CMAP parameters that were used for analysis include the conduction velocity (CV), distal motor latency (DML), and amplitude (Amp).

## Statistical Analysis

SPSS 25.0 statistical software (IBMSPSS statistical software V25.0) was used for statistical analysis of data in this study. Normal distribution measurements are expressed as mean±standard deviation. Univariate ANOVA was used for inter-group comparison of quantitative data of multiple groups. If ANOVA found significant differences, s-N-K test was used for multiple comparisons to determine which group had differences. Chi-square test was used for qualitative data. Kruskal-Wallis *H*-test was used for the grade distribution data of multiple groups. Correlational and multiple linear regression analyses were used to analyze the influencing factors of vascular endothelial function in type 2 diabetes patients.  $P < 0.05$  was considered statistically significant.

## Results

### Clinical and Biochemical Features

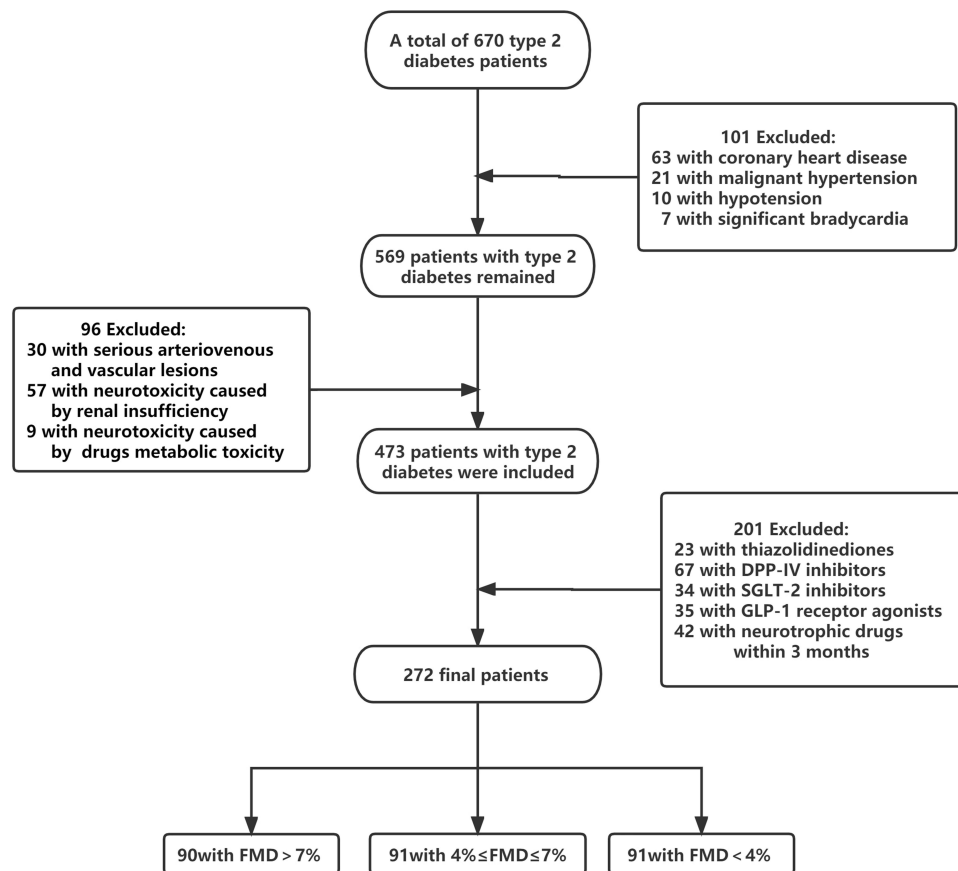
Figure 1 shows the flowchart. Patients were divided by FMD into three groups:  $FMD > 7\% = 90$  subjects ( $9.0 \pm 1.2\%$ ),  $4\% \leq FMD \leq 7\% = 91$  subjects ( $5.3 \pm 0.9\%$ ), and  $FMD < 4\% = 91$  subjects ( $2.4 \pm 0.9\%$ ). Table 1 lists the subjects' indicators. There were no significant differences in age, sex, BMI, duration of disease, FPG, HbA1c, TC, HDL, LDL, TG, SBP, DBP, HR, 25(OH)D, drinking rate and smoking rate among the three groups ( $p > 0.05$ ). It can be seen that duration of diabetes and SBP in  $FMD < 4\%$  group were higher than those in the first two groups, but there were no significant differences.

### Proportion of Peripheral Neuropathy Severity in Patients with T2DM

The proportion of severity of peripheral neuropathy in patients with T2DM obtained from TCSS is shown in Table 2. Percentage of patients with severe nerve injury was significantly higher in  $FMD < 4\%$  (70%) compared to  $FMD > 7\%$  (2%). Percentage of patients with severe nerve injury (70%) was significantly higher than those without (3%) in  $FMD < 4\%$  group, while in  $FMD > 7\%$  group, the proportion of patients with severe nerve injury (2%) was significantly lower than those without (62%). The severity of peripheral neuropathy can be considered different among the three groups ( $p < 0.05$ ). As shown in Figure 2 under section 3.4, FMD decreased gradually with the increase of TCSS, and the two were negatively correlated.

### TCSS

Table 3 shows TCSS evaluation. The proportion of temperature disturbance (73%) was the most and joint position disturbance (0) was the least in the entire subject population. In  $FMD > 7\%$  group, temperature disturbance accounted for 55%, lower limb fatigue accounted for 50%, lower limb pain accounted for 44%; In  $4\% \leq FMD \leq 7\%$  group, temperature disturbance accounted for 71%, followed by vibration disturbance accounted for 63%, and fatigue accounted for 59%; In  $FMD < 4\%$  group, temperature disturbance accounted for 91%, followed by reflex disorder (ankle reflex disorder accounted



**Figure 1** Flow diagram. The chart shows patient inclusion and exclusion in the study.

for 90%, knee reflex disorder accounted for 88%), and vibration disturbance accounted for 78%. Neurological symptoms, sensory examination, and neural reflexes were considered to be different among the three groups using chi-square analysis.

## Correlation Analysis Between TCSS and FMD

Pearson correlation analysis showed that TCSS was significantly negatively correlated with FMD ( $r=-0.756$ ,  $p<0.001$ ) in Table 4. Nerve symptoms ( $r=-0.328$ ,  $p<0.001$ ), sensory examinations ( $r=-0.081$ ,  $p<0.05$ ) and nerve reflexes ( $r=-0.714$ ,  $p<0.001$ ) were correlated with FMD, respectively.

## Comparison of CV, Amp and DML of Motor Nerve in Three Groups

NCS parameters include conduction velocity (CV), CMAP amplitude (Amp) and distal motor latency (DML) of median nerve, ulnar nerve, common peroneal nerve and tibial nerve as shown in Table 5. It can be seen that the CV and Amp of FMD<4% group were lower than those of the first two groups ( $p<0.05$ ), and DML, on the other hand, was higher than the first two groups ( $p<0.05$ ). However, there were no significant differences in CV, Amp and DML between FMD>7% and  $4\%\leq\text{FMD}\leq 7\%$  groups. According to nerve CV, the damage degree of common peroneal nerve and tibial nerve was more serious than that of median nerve and ulnar nerve at the same level of FMD. That is, when FMD>7%, CV of four nerves was within the normal range (upper extremity>50m/s, lower extremity>38m/s), when  $4\%\leq\text{FMD}\leq 7\%$ , median nerve and ulnar nerve CV remained within the normal range, while the common peroneal nerve and tibial nerve CV slowed down ( $37.38\pm 7.50$ ,  $36.98\pm 5.47$ ).

**Table 1** Comparison of Clinical and Biochemical Features of Patients with T2DM

|                          | FMD>7% (n, 90) | 4%≤FMD≤7% (n, 91) | FMD<4% (n, 91) | p value |
|--------------------------|----------------|-------------------|----------------|---------|
| Age (y)                  | 53.4±9.6       | 53.4±10.7         | 53.4±8.9       | 0.91    |
| Gender (male/female)     | 51/39          | 43/48             | 54/37          | 0.37    |
| BMI (kg/m <sup>2</sup> ) | 22.9±1.8       | 24.8±4.0          | 24.3±3.1       | 0.17    |
| Duration (y)             | 9.1±6.5        | 9.3±7.1           | 10.3±7.6       | 0.64    |
| FPG (mmol/L)             | 9.5±3.6        | 9.9±4.0           | 9.9±4.2        | 0.76    |
| HbA1c (%)                | 8.4±2.1        | 8.5±1.8           | 8.4±1.8        | 0.99    |
| TC (mmol/L)              | 3.8±1.0        | 4.2±1.3           | 3.8±1.0        | 0.48    |
| TG (mmol/L)              | 1.6±0.8        | 1.6±2.1           | 1.6±1.1        | 0.10    |
| HDL (mmol/L)             | 1.0±0.2        | 1.0±0.3           | 0.9±0.2        | 0.18    |
| LDL (mmol/L)             | 2.5±0.8        | 2.7±0.9           | 2.5±0.8        | 0.70    |
| SBP (mmHg)               | 121.5±24.1     | 129.5±17.9        | 131.7±18.7     | 0.36    |
| DBP (mmHg)               | 76.2±8.7       | 80.0±11.3         | 76.7±8.3       | 0.43    |
| HR (bpm)                 | 83.6±11.6      | 88.6±11.4         | 83.1±12.5      | 0.22    |
| Smoking (%)              | 20(22.2%)      | 26(28.6%)         | 23(25.3%)      | 0.16    |
| Drinking (%)             | 14(15.6%)      | 18(19.8%)         | 12(13.2%)      | 0.16    |
| 25(OH)D(ng/mL)           | 24.6±9.1       | 17.9±6.1          | 17.5±6.8       | 0.11    |
| HCY (μmol/L)             | 13.1±3.4       | 13.4±3.6          | 16.8±13.5      | 0.23    |
| baPWV (cm/s)             | 1627.1±377.3   | 1623.1±263.7      | 1630.1±595.2   | 0.57    |
| ABI                      | 1.2±0.1        | 1.2±0.1           | 1.2±0.1        | 0.87    |

**Note:** Univariate ANOVA and Chi-square test are used in this table.

**Abbreviations:** FMD, flow-mediated dilation; F, female; M, male; BMI, body mass index; FPG, fasting blood glucose; HbA1C, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; HDL, High density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HCY, homocysteine; baPWV, brachial ankle pulse wave velocity; ABI, ankle-brachial index.

**Table 2** Proportion of Peripheral Neuropathy Severity in Three Groups

|                              | FMD>7% (n, 90) | 4%≤FMD≤7% (n, 91) | FMD<4% (n, 91) |
|------------------------------|----------------|-------------------|----------------|
| No nerve injury (n; %)       | 56(62%)        | 30(33%)           | 3(3%)          |
| Mild nerve injury (n; %)     | 22(25%)        | 24(26%)           | 9(10%)         |
| Moderate nerve injury (n; %) | 10(11%)        | 19(21%)           | 16(17%)        |
| Severe nerve injury (n; %)   | 2(2%)          | 18(20%)           | 63(70%)        |

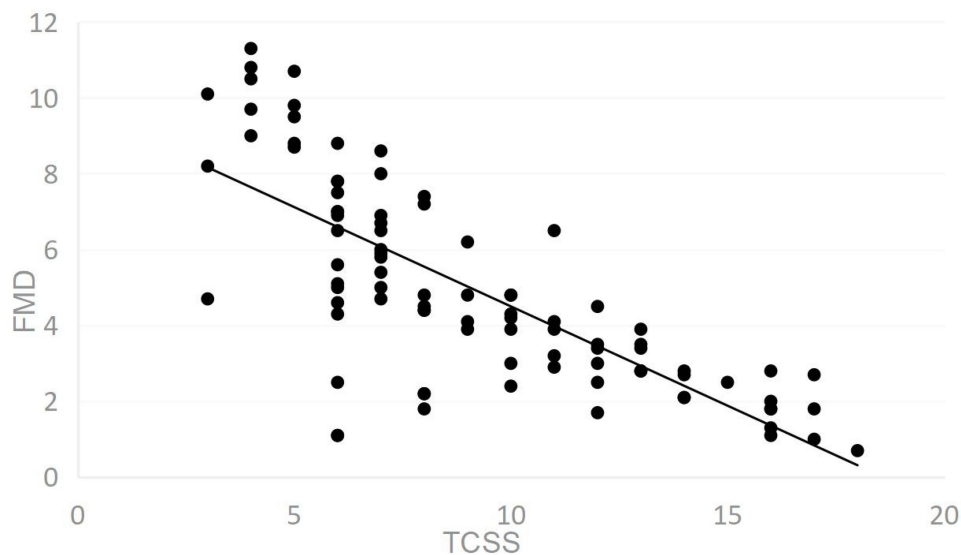
**Notes:** Kruskal-Wallis *H*-test is used in this table  $\chi^2$ , 11.114; *p*, 0.004; the severity of peripheral neuropathy can be considered different among the three groups.

## Correlation Analysis of Motor Nerve CV, Amp, DML and FMD

As shown in Table 6, correlation analysis showed that CV and Amp of median nerve, ulnar nerve, common peroneal nerve and tibial nerve were positively correlated with FMD, while DML was negatively correlated with FMD in FMD<4% group ( $p<0.05$ ), however, there was no correlation in 4%≤FMD≤7% and FMD>7% group, respectively.

## Regression Analysis for Endothelial Function Injury in Type 2 Diabetes

Linear regression analysis was conducted in Model 1 with each baseline indicator as independent variable and vascular endothelial function indicator FMD as dependent variable in Table 7. Age, Duration, FPG, HbA1c, SBP and TG were forced to enter the model, and other indicators were entered by stepwise ( $p<0.05$  entered,  $p>0.1$  removed). The results of Model 1 show that SBP and TG levels are statistically and independently associated with FMD ( $\beta=-0.60$   $p=0.002$ ,  $\beta=-0.57$   $p=0.017$ ). TCSS was added to model 2 on the basis of model 1, and the results showed that high TCSS score is a risk factor of FMD ( $\beta=-0.51$ ,  $p<0.001$ ). Model 3 added the CV, Amp and DML of each nerve on the basis of model 1, and the results showed that decreased CV of common peroneal nerve and tibial nerve was a risk factor of FMD injury ( $\beta=0.13$ ,  $p=0.014$ ;  $\beta=0.27$ ,  $p=0.003$ ).



**Figure 2** Scatter diagram of FMD and TCSS (0–19 points).

## Discussion

The endothelial is a complex structure that secretes special substances necessary to control blood vessel function and its dysfunction can lead to impaired vascular function, and then, impaired vascular function is also a risk factor for cardiovascular outcomes of diabetes, leading to the occurrence and development of atherosclerosis including coronary artery disease.<sup>25</sup> As a non-invasive test, FMD has become widely used technique for measuring endothelial function.<sup>26</sup> Peripheral endothelial function assessed by FMD is associated with coronary endothelial function,<sup>27</sup> which can be explained by the fact that endothelial dysfunction is a systemic disease.<sup>28</sup> Although FMD measures brachial arterial vascular function, the stimulation of FMD itself (reactive congestion) may be an important measure of peripheral microvascular function because reactive congestion is highly dependent on maximum forearm resistance.<sup>29,30</sup> It has

**Table 3** Abnormal Ratio of TCSS Indicators of Three Groups

|                                   | Total (n, 272) | FMD>7% (n, 90) | 4%≤FMD≤7% (n, 91) | FMD<4% (n, 91) | X <sup>2</sup> | p      |
|-----------------------------------|----------------|----------------|-------------------|----------------|----------------|--------|
| Nerve symptoms <sup>a</sup>       |                |                |                   |                | 44.249         | <0.001 |
| Lower limb pain                   | 141(52%)       | 40(44%)        | 50(55%)           | 51(56%)        |                |        |
| Numbness                          | 60(22%)        | 5(5%)          | 10(11%)           | 45(49%)        |                |        |
| Picotement                        | 44(16%)        | 5(5%)          | 3(3%)             | 36(40%)        |                |        |
| Fatigue                           | 137(50%)       | 45(50%)        | 54(59%)           | 68(75%)        |                |        |
| Walking imbalance                 | 33(12%)        | 5(5%)          | 9(10%)            | 19(21%)        |                |        |
| Upper-limb symptoms               | 35(13%)        | 0              | 9(10%)            | 26(29%)        |                |        |
| Sensory examinations <sup>b</sup> |                |                |                   |                | 26.758         | 0.003  |
| Acmeesthesia                      | 25(9%)         | 5(5%)          | 7(8%)             | 13(14%)        |                |        |
| Temperature sensation             | 198(73%)       | 50(55%)        | 65(71%)           | 83(91%)        |                |        |
| Light touch sensation             | 54(20%)        | 0              | 18(20%)           | 36(40%)        |                |        |
| Vibration sensation               | 138(51%)       | 10(11%)        | 57(63%)           | 71(78%)        |                |        |
| Joint-position sensation          | 0              | 0              | 0                 | 0              |                |        |
| Nerve reflexes <sup>c</sup>       |                |                |                   |                | 76.631         | <0.001 |
| Ankle reflex                      | 127(47%)       | 9(10%)         | 36(40%)           | 82(90%)        |                |        |
| Knee reflex                       | 119(44%)       | 9(10%)         | 30(33%)           | 80(88%)        |                |        |

**Notes:** Chi-square test is used in this table TCSS, Toronto clinical scoring system. a is for nerve symptoms; <sup>a</sup>X<sup>2</sup>, 44.249; p<0.001; b is for sensory examinations; <sup>b</sup>X<sup>2</sup>, 26.758; p, 0.003; c is for nerve reflexes; <sup>c</sup>X<sup>2</sup>, 76.631; p<0.001. Nerve symptoms; sensory examinations; and nerve reflexes can be considered different among the three groups.

**Table 4** Correlation Between Components of TCSS and FMD

|                           | FMD (%) |        |
|---------------------------|---------|--------|
|                           | r       | p      |
| TCSS total points (0–19)  | –0.756  | <0.001 |
| Nerve symptoms (0–6)      | –0.328  | <0.001 |
| Sensory examination (0–5) | –0.081  | <0.05  |
| Nerve reflexes (0–8)      | –0.714  | <0.001 |

**Note:** Pearson correlation analysis is used in this table.

**Abbreviations:** TCSS, Toronto clinical scoring system; FMD, flow-mediated dilation.

**Table 5** Comparison of Peripheral Motor Nerve Measurements in Three Groups

|                       | FMD>7% (n, 90) | 4%≤FMD≤7% (n, 91) | FMD<4% (n, 91)           |
|-----------------------|----------------|-------------------|--------------------------|
| CV (m/s)              |                |                   |                          |
| Median nerve          | 51.18±6.56     | 50.23±5.32        | 48.75±5.43 <sup>ab</sup> |
| Ulnar nerve           | 50.61±4.85     | 50.16±5.23        | 45.05±5.05 <sup>ab</sup> |
| Common peroneal nerve | 38.43±4.98     | 37.38±7.50        | 35.45±7.90 <sup>ab</sup> |
| Tibial nerve          | 38.04±3.51     | 36.98±5.47        | 34.3±5.55 <sup>ab</sup>  |
| Amp (mV)              |                |                   |                          |
| Median nerve          | 6.27±2.30      | 6.16±3.51         | 4.82±1.99 <sup>ab</sup>  |
| Ulnar nerve           | 7.45±2.49      | 6.93±2.31         | 6.84±2.80 <sup>ab</sup>  |
| Common peroneal nerve | 1.19±1.39      | 0.91±1.17         | 0.80±0.87 <sup>a</sup>   |
| Tibial nerve          | 5.25±2.84      | 5.16±2.50         | 4.40±3.45 <sup>ab</sup>  |
| DML (ms)              |                |                   |                          |
| Median nerve          | 3.08±0.72      | 3.50±0.72         | 4.29±3.07 <sup>ab</sup>  |
| Ulnar nerve           | 2.53±0.97      | 2.56±0.81         | 2.67±0.52 <sup>ab</sup>  |
| Common peroneal nerve | 6.02±3.41      | 6.23±1.97         | 6.62±1.92 <sup>ab</sup>  |
| Tibial nerve          | 4.43±1.05      | 4.89±2.08         | 5.64±2.27 <sup>ab</sup>  |

**Notes:** Univariate ANOVA and s-N-K test are used in this table. Compared with FMD > 7% group; <sup>a</sup>p<0.05; Compared with 4%≤FMD≤7% group; <sup>b</sup>p<0.05.

**Abbreviations:** CV, conduction velocity; Amp, amplitude; DML, distal motor latency.

been widely documented that endothelial dysfunction has been associated with almost every risk factor for atherosclerosis and cardiovascular disease, such as hypertension, normal blood pressure with a family history of hypertension, smoking, dyslipidemia, diabetes and obesity.<sup>31</sup>

In addition to above causes of endothelial dysfunction, neuropathy can also cause vasodilation responses disorder. Previous studies have shown that the normal neurovascular response is conducted through the C-nociceptive nerve fibers. These fibers would stimulate adjacent C fibers, which secrete substance P, calcitonin gene-related peptide, and histamine, causing vasodilatation and increased blood flow to the injured tissues, thereby promoting wound healing, however, this impaired neurovascular reflex leads to a significant reduction in blood flow in diabetic neuropathy.<sup>32,33</sup> A Caselli et al explored the role of C-nociceptive fibers in nerve axon reflex-related vasodilation through anesthesia block C-nociceptive fibers in diabetes divided the patients into diabetic neuropathy and non-neuropathy, and the results showed that the function of C-nociceptive fibers was the main factor affecting the vasodilation related to neuron-axon reflex, which provided evidence for the vascular dilation and injury caused by diabetic neuropathy.<sup>9</sup> Nitric oxide (NO) is a potent vasodilator, which interacts with neural inputs and other local vasoactive mediators such as prostaglandin, endothelin, and endodermal hyperpolarizing factors co-determine overall vascular tone and blood flow.<sup>34</sup> NO is synthesized by nitric oxide synthase, whose isoenzymes include neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS), and endothelial nitric oxide synthase (eNOS).<sup>35</sup> nNOS is mainly expressed in specific neurons of the central nervous system, and nNOS-derived NO is also involved in vasodilation in the peripheral nervous system as an atypical neurotransmitter. eNOS is mainly expressed in vascular endothelial cells and also peripheral



**Table 6** Correlation Analysis of Motor Nerve CV; Amp; DML and FMD

|                       | FMD>7% (n, 90) |       | 4%≤FMD≤7% (n, 91) |       | FMD<4% (n, 91) |        |
|-----------------------|----------------|-------|-------------------|-------|----------------|--------|
|                       | r              | p     | r                 | p     | r              | p      |
| CV (m/s)              |                |       |                   |       |                |        |
| Median nerve          | 0.002          | 0.987 | 0.010             | 0.677 | 0.357          | 0.017  |
| Ulnar nerve           | 0.194          | 0.297 | 0.190             | 0.267 | 0.472          | <0.001 |
| Common peroneal nerve | 0.040          | 0.779 | 0.121             | 0.665 | 0.650          | <0.001 |
| Tibial nerve          | 0.059          | 0.681 | 0.098             | 0.543 | 0.798          | <0.001 |
| Amp (mV)              |                |       |                   |       |                |        |
| Median nerve          | 0.005          | 0.972 | 0.111             | 0.767 | 0.340          | 0.028  |
| Ulnar nerve           | 0.182          | 0.201 | 0.220             | 0.134 | 0.431          | 0.010  |
| Common peroneal nerve | 0.222          | 0.118 | 0.321             | 0.101 | 0.791          | <0.001 |
| Tibial nerve          | 0.121          | 0.397 | 0.226             | 0.267 | 0.790          | <0.001 |
| DML (ms)              |                |       |                   |       |                |        |
| Median nerve          | -0.274         | 0.072 | -0.254            | 0.102 | -0.359         | 0.017  |
| Ulnar nerve           | -0.095         | 0.539 | -0.143            | 0.213 | -0.576         | 0.009  |
| Common peroneal nerve | -0.158         | 0.304 | -0.145            | 0.144 | -0.801         | <0.001 |
| Tibial nerve          | -0.220         | 0.151 | -0.221            | 0.151 | -0.767         | <0.001 |

**Note:** Pearson correlation analysis is used in this table.

**Abbreviations:** CV, conduction velocity; Amp, amplitude; DML, distal motor latency.

**Table 7** Multiple Linear Regression Analysis of Risk Factors for Endothelial Function Injury in T2DM

|                   | Model1 (R2, 0.262) |         | Model2 (R2, 0.588) |          | Model3 (R2, 0.535) |         |
|-------------------|--------------------|---------|--------------------|----------|--------------------|---------|
|                   | $\beta$            | p       | $\beta$            | p        | $\beta$            | p       |
| Age               | -0.71              | 0.395   | -0.70              | 0.395    | -0.71              | 0.395   |
| Duration          | -0.68              | 0.121   | -0.67              | 0.122    | -0.69              | 0.125   |
| FPG               | -0.54              | 0.111   | -0.53              | 0.110    | -0.53              | 0.110   |
| HbA1c             | -0.55              | 0.159   | -0.55              | 0.156    | -0.56              | 0.159   |
| SBP               | -0.60              | 0.002** | -0.60              | 0.002**  | -0.60              | 0.002** |
| TG                | -0.57              | 0.017*  | -0.57              | 0.015*   | -0.57              | 0.017*  |
| Smoking           | -0.015             | 0.056   | -0.015             | 0.056    | -0.014             | 0.055   |
| 25 (OH)D          | 0.13               | 0.067   | 0.13               | 0.056    | 0.12               | 0.056   |
| TCSS <sup>a</sup> |                    |         | -0.51              | <0.001** |                    |         |
| CV <sup>1b</sup>  |                    |         |                    |          | 0.19               | 0.052   |
| CV <sup>2b</sup>  |                    |         |                    |          | 0.24               | 0.067   |
| CV <sup>3b</sup>  |                    |         |                    |          | 0.13               | 0.014*  |
| CV <sup>4b</sup>  |                    |         |                    |          | 0.27               | 0.003** |
| Amp <sup>3b</sup> |                    |         |                    |          | 0.37               | 0.070   |
| Amp <sup>4b</sup> |                    |         |                    |          | 0.04               | 0.070   |
| DML <sup>3b</sup> |                    |         |                    |          | -0.04              | 0.098   |
| DML <sup>4b</sup> |                    |         |                    |          | -0.08              | 0.088   |

**Notes:** \* $p<0.05$ ; \*\* $p<0.01$ . Model1: Age; Duration; FPG; HbA1c; SBP and TG are forced to enter the model; and other indicators are entered by stepwise ( $p<0.05$  entered;  $p>0.1$  removed). Model2: Model1 +TCSS score (Age; Duration; FPG; HbA1c; SBP and TG are forced to enter the model; other indicators are entered stepwise;  $p<0.05$  entered;  $p>0.1$  removed) (a is removed from Model3). Model3: CV; Amp and DML of Model1 + median nerve<sup>1</sup>; ulnar nerve<sup>2</sup>; common peroneal nerve<sup>3</sup> and tibial nerve<sup>4</sup> (Age; Duration; FPG; HbA1c; SBP and TG were forced into the model; and other indicators were entered by stepwise.  $p<0.05$  entered;  $p>0.1$  removed) (b is removed from Model2).

nerve, and its derived NO is a physiological vasodilator.<sup>35</sup> Veves et al observed immunohistochemical staining of eNOS in skin biopsies of the dorsal feet of diabetic patients with peripheral nerve and vascular disease, diabetic patients with peripheral neuropathy and healthy subjects, and found that the intensity of eNOS staining decreased in both diabetic patients, and therefore is a possible mechanism of endothelial dysfunction in diabetic neuropathy.<sup>36</sup> Candice Chapouly et al has confirmed that the loss of Desert Hedgehog (Dhh) signaling expression in the nerve was

associated with increased endoneurial capillary permeability and decreased Claudin5 expression, which lead to the development of diabetic neuropathy.<sup>10</sup> Thus, Dhh signaling plays a key role in maintaining blood nerve barrier integrity. Ando et al found that type 2 diabetic microfibrillar neuropathy may be caused by impaired endothelial dysfunction.<sup>23</sup> Above studies indicate that vascular dysfunction and peripheral neuropathy have a complex causal relationship.

So far, the mechanism of interaction between peripheral nerve injury and vascular endothelial disorder is still unclear.<sup>37</sup> The relationship between DPN and FMD has been studied in recent years. By using laser Doppler imaging scanner to evaluate the response to iontophoresis of 1% acetylcholine chloride (ACh) and 1% sodium nitroprusside (NaNP), Arora et al compared these two responses in forearm and dorsolateral feet of healthy subjects, patients without and with diabetic peripheral neuropathy, and the results showed that endothelium-dependent and independent vasodilation was reduced in the group with peripheral neuropathy compared with the other two groups, and the reduction was more pronounced in feet than in forearm.<sup>38</sup> In present study, TCSS was used to measure accurately the indicators of peripheral neuropathy, which has the advantage of being simple, comprehensive (including symptoms and signs), and can preliminarily evaluate the severity of DPN. Our study revealed incidence of defining variables in TCSS: lower limb pain developed in 52% of patients, lower limb fatigue in 50%, temperature disturbance in 73%, hypoesthesia of acupuncture pain in 25%, vibration disturbance in 51%, ankle reflexes reduced or absent in 47%, and knee reflexes reduced or absent results in 44%. In other words, hyposensitivity to temperature is the most common sign of peripheral neuropathy in patients with type 2 diabetes. Furthermore, we observed the incidence of parameters in TCSS for different FMD levels. It was found that small nerve fibers were mainly involved in FMD>7%; however, for FMD<4% group, the involvement of large nerve fibers increased. The correlation analysis showed that TCSS score was negatively correlated with FMD ( $r=-0.756, p<0.001$ ), and neurological symptoms ( $r=-0.328, p<0.001$ ), sensory examinations ( $r=-0.081, p<0.05$ ), nerve reflexes ( $r=-0.714, p<0.001$ ) were also negatively correlated with FMD, respectively. Arora et al's research is consistent with us. More interesting, our paper applies TCSS that can evaluate the function of myelinated small or unmyelinated nerve fibers and small nerve fibers are the first to be damaged in the onset of diabetes, that is, A $\delta$  myelinated small nerve fibers are first involved, and then C unmyelinated nerve fibers are gradually involved, and then A $\beta$  myelinated large nerve fibers are developed. In the early stage of diabetes, sensory nerve damage is a significant clinical feature, which mainly caused by nerve demyelination and axonal degeneration. Therefore, TCSS can be used to detect DPN earlier and accurately.

Electrophysiology can be thought as an extension of the clinical neurologic examination.<sup>4</sup> Because the measures are objective and the results do not depend on the patient's subjective response, these are currently the most reliable methods of measuring the peripheral neuromuscular system.<sup>4</sup> In the current study, NCS was used to objectively assess peripheral neuromuscular function. It detects large myelinated A $\beta$  nerve fibers and records the presence, nature, distribution, and severity of peripheral nerve injury. In demyelinating polyneuropathies, there is a reduction of nerve conduction velocities with a relative preservation of nerve potential amplitudes, contrasted with the situation in axonal neuropathies where losing of amplitudes and relative preservation of conduction velocities are observed.<sup>4</sup> In this study, we observed CV, Amp and DML of median nerve, ulnar nerve, common peroneal nerve, tibial nerve and their relationship with FMD. It can be seen that the CV and Amp of FMD<4% group were lower than those of the first two groups ( $p<0.05$ ), and DML, on the other hand, was higher than the first two groups ( $p<0.05$ ). However, there were no significant differences in CV, Amp and DML between FMD>7% and 4% $\le$ FMD $\le$ 7% groups. Correlation analysis showed that CV and Amp of median nerve, ulnar nerve, common peroneal nerve and tibial nerve were positively correlated with FMD, while DML was negatively correlated with FMD in FMD<4% group ( $p<0.05$ ), suggesting that the damage of large nerve fibers may be associated with severe vascular endothelial dysfunction. A study showed that after adjusting for age, sex, smoking status and body mass index, patients with diabetes have higher mortality risk than those without diabetes, moreover, with concurrent peripheral neuropathy can lead to an increased hazard ratio, however, the results did not reveal the effect of specific indicators of peripheral neuropathy on mortality.<sup>39</sup> For the first time, we used multiple regression analysis to find that high TCSS score and decreased CV of common peroneal and tibial nerves are risk factors of FMD injury.

There is no doubt that the smaller the FMD, the more serious the endothelial function damage. In present study, 7% as the cut-off point value for endothelial dysfunction was based on previous studies and guidelines,<sup>31,40</sup> and we also confirmed that when FMD>7%, the majority of patients did not have neuropathy and nerve CV, Amp and DML were not correlated with FMD. The data were collected and analyzed and for the first time we found that when FMD<4%, the

majority of patients had neuropathy, and there was a significant correlation between nerve conduction indexes and FMD. When FMD was between 4 and 7%, patients have mild or severe peripheral nerve damage, but there was no significant correlation between nerve conduction indicators and FMD.

The advantage of this study is that TCSS is negatively correlated with FMD, and the simple and comprehensive advantages of TCSS can be used to quickly assess the vascular endothelial function of patients. Furthermore, the relationship between motor NCS and FMD was further studied, which provided a more detailed guidance for cardiovascular disease prevention in patients with peripheral neuropathy in type 2 diabetes. There are still deficiencies that this study is a retrospective study with a limited number of cases, requiring further large-scale prospective studies and the causal relationship between the two is difficult to explain clearly, and basic research is needed to further clarify its internal mechanism.

## Conclusion

In conclusions, peripheral neuropathy of type 2 diabetes may be correlated with FMD, and damage to large nerve fibers may be associated with more severe vascular endothelial disorders. High TCSS score and decreased CV of common peroneal and tibial nerves are risk factors of FMD injury, which provide potential value for timely prevention and treatment of cardiovascular diseases.

## Abbreviations

FMD, flow-mediated dilation; F, female; M, male; BMI, body mass index; FPG, fasting blood glucose; HbA1C, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HCY, homocysteine; baPWV, brachial ankle pulse wave velocity; ABI, ankle-brachial index; TCSS, Toronto clinical scoring system; CMAP, compound muscle action potential; CV, conduction velocity; Amp, amplitude; DML, distal motor latency.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Disclosure

The authors report no conflicts of interest in this work.

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