Comparative Roles of Microvascular and Nerve Function in Foot Ulceration in Type 2 Diabetes

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OBJECTIVE — To determine the relative roles of different modalities of sensory nerve function (large and small fiber) and the role of microvascular dysfunction in foot ulceration in type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS — A total of 20 control subjects and 18 type 2 diabetic subjects with foot ulceration and 20 without were studied. None of the subjects had clinical features of peripheral vascular disease. The Computer-Aided Sensory Evaluator IV (CASE IV) was used to determine vibration detection threshold (VDT), cold detection threshold (CDT), warm detection threshold (WDT), and heat pain onset threshold (HPO). Vibration perception threshold (VPT) was also assessed by a neurothesiometer. Microvascular function (maximum hyperemia to skin heating to 44°C) was assessed using laser Doppler flowmetry (mean maximum hyperemia using laser Doppler flowmeter [LDF_{max}]), laser Doppler imaging (mean maximum hyperemia using laser Doppler imager [LDI_{max}]), and skin oxygenation with transcutaneous oxygen tension ($TcpO_2$).

RESULTS — VPT, VDT, CDT, and HPO were all significantly higher in individuals with ulceration than in those without (VPT and VDT: $P < 0.0001$) (CDT and HPO: $P = 0.01$). LDF_{max}, LDI_{max}, and $TcpO_2$ were significantly lower in the two diabetic groups than in the control subjects, but there was no difference between individuals with and without ulceration. Univariate logistic regression analysis revealed similar odds ratios for foot ulceration for VDT, CDT, HPO, and VPT (OR 1.97 [95% CI 1.30–2.98], 1.58 [1.20–2.08], 2.30 [1.21–4.37], and 1.24 [1.08–1.42], respectively). None of the microvascular parameters yielded significant odds ratios for ulceration.

CONCLUSIONS — This study found that there was no additional value in measuring small-fiber function with the CASE IV over measuring vibration by either CASE IV or the inexpensive neurothesiometer in discriminating between individuals with and without ulceration. Furthermore, none of the tests of microvascular function including the $TcpO_2$ were able to discriminate between individuals with and without ulceration, suggesting that such tests may not be of benefit in identifying subjects at greater risk of foot ulceration.

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Abbreviations: C, healthy control subjects; CASE IV, Computer-Aided Sensory Evaluator IV; CDT, cold detection threshold; D, type 2 diabetic subjects without foot ulceration; DU, subjects with type 2 diabetes with a current or previous history of foot ulceration; HPO, heat pain onset threshold; JND, just noticeable difference; LDF, laser Doppler flowmeter; LDF_{max}, mean maximum hyperemia using laser Doppler flowmeter; LDI, laser Doppler imager; LDI_{max}, mean maximum hyperemia using laser Doppler imager; $TcpO_2$, transcutaneous oxygen tension; VDT, vibration detection threshold; VPT, vibration perception threshold; WDT, warmth detection threshold.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The incidence of diabetes-related lower-extremity amputations continues to increase in the developed and developing world; in the U.S., it has been estimated to cost nearly $2 billion and account for 2,600 patient-years of hospital stays per year (1). Up to 80% of these amputations are preceded by foot ulceration (2). Furthermore, between 1995 and 1996, the Medicare costs for patients with foot ulcers were on an average three times higher than those of Medicare patients in general (3). Thus, there is a pressing need for a better understanding of the etiopathogenic factors involved in foot ulceration so that robust screening and preventative measures can be developed.

In the absence of macrovascular disease, impaired nerve function and deranged microvascular function have been implicated as important etiological factors (4–10). With regard to neuropathy, clinical assessment using simple clinical tools such as the neuropathy disability score, neuropathy symptom score, pressure perception using Semmes-Weinstein monofilaments, and vibration sensation with the neurothesiometer (Horwell Scientific Laboratory Supplies, Nottingham, U.K.) have been commonly used to identify individuals at risk of foot ulceration (8,11–16). These tools assess mainly large-fiber function. More recently, there has been increasing interest in the involvement of small-fiber neuropathy in the development of foot ulceration (17,18). The Computer-Aided Sensory Evaluator IV (CASE IV) (WR Medical Electronics, Stillwater, MN) has been advocated as a sensitive tool for assessing both small-fiber (thermal thresholds) and large-fiber (vibration thresholds) function (19–21), but to date its use has been largely limited to evaluating new treatments in large multicenter clinical trials.

With regard to microvascular function, this has been mainly assessed using laser Doppler flowmetry. A variety of abnormalities have been described, including impairment in vasodilatory responses to skin heating, needle injury, postocclusive hyperemia, and response to ion-

Table 1—*Clinical characteristics of subjects*

	C group	D group	DU group
n	20	20	18
Age (years)	52.0 (44.7–60.0)	60.5 (51.5–68.0)	67.0 (60.7–73.2)
Duration (years)	—	6.0 (3.0–9.7)	6.0 (2.7–16.0)
BMI (kg/m^2)	24.4 (22.4–27.2)	29.9 (25.9–33.1)	28.5 (26.1–32.9)
HbA_{1c} (%)	—	7.95 (7.50–8.50)	8.30 (7.90–9.15)
Ankle-brachial pressure index	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.2 (1.0–1.3)

Data are medians (interquartile range). There were no significant differences in the age between groups C and D, but the groups with foot ulcer (DU) were older ($P = 0.01$). The BMI was lower in the control subjects (C) compared to groups D and DU ($P < 0.0001$ and $P = 0.001$, respectively). Duration and HbA_{1c} were not significantly different between groups D and DU. Ankle-brachial pressure index was not different in the three groups.

tophoresis of vasoactive substances (22–29). Of particular interest is the finding of reduced maximal vasodilation to injury, including thermal (heating 44°C) and lacerating (needle prick) injury in people with diabetes (30,31). It has been suggested that the impaired injury response explains the propensity to foot ulceration in patients with diabetes and development of foot ulceration in the absence of large-vessel disease. Finally, it has been demonstrated that in diabetic subjects, transcutaneous oxygen tension ($TcpO_2$) of <30 mmHg in the foot when the skin is heated to 44°C is an independent predictor for foot ulceration (32) and that, in the absence of macrovascular disease, $TcpO_2$ reflects microvascular dysfunction (33). Although, readily demonstrable and increasingly suggested to be involved in the development of diabetic foot ulceration, there have been no studies specifically examining the role of microvascular dysfunction in foot ulceration and the value of such tests in identifying those at risk.

Thus, the aim of this study was to evaluate in subjects with type 2 diabetes *1*) the comparative roles of different modalities of sensory function in foot ulceration using the CASE IV and *2*) to determine in the same individuals whether abnormalities of microvascular function assessed using laser Doppler methods and $TcpO_2$ distinguish between individuals with and without ulceration and are thus implicated in foot ulceration. This study may thus identify valuable screening tools for individuals at risk of foot ulceration.

RESEARCH DESIGN AND METHODS

— Three groups of subjects were studied (Table 1). The first group included 18 subjects with type 2 diabetes with a current or previous history of foot ulceration (DU), the second group included 20 type 2 diabetic subjects without foot ulceration (D), and the third group included 20 healthy control subjects (C). Subjects were recruited on a consecutive basis from the diabetes outpatient clinics of the Ipswich Diabetes Centre. None of the subjects had clinical features of peripheral vascular disease, and all had an ankle-brachial pressure index of >0.8. Because of the need for cardiovascular protection, some of the subjects with diabetes were on aspirin (10 in D and 12 in DU) and antihypertensive (10 in D and 9 in DU) and lipid-lowering medications (5 in D and 6 in DU). The study was approved by the local ethical committee, and all of the subjects gave informed consent to take part in the study.

Assessment of neurological function

Subjects were allowed to acclimatize for 30 min in a temperature-controlled room with the temperature maintained at 25 ± 1°C. Quantitative sensory testing was carried out using the CASE IV with software version 4.27.1 (WR Medical Electronics). Vibration detection threshold (VDT), cold detection threshold (CDT), and warmth detection threshold (WDT) were measured using the 4, 2, and 1 stepping algorithm with null stimuli as described by Dyck et al. (19). The VDT was performed on the dorsal aspect of the hallux. The subjects pressed "yes" or "no" buttons depending on whether they felt the stimuli. Heat pain onset threshold (HPO) was assessed using the nonrepeating algorithm with null stimuli and a visual analog scale (19). CDT, WDT, and (HPO) were examined using the standard CASE IV thermode applied on the dorsum of the mid-foot. For each test, the computer calculated the "just noticeable difference" (JND) from the subject's responses. The concept of JND is based on the ability to discriminate two levels of stimuli. The CASE IV system uses a set of 25 standardized vibratory and thermal stimulation levels. Each level of stimulation corresponds to 1 JND unit. Thus, a higher JND reflects a larger amplitude of the stimulus (vibration) or larger change in temperature (thermal) (34). A value of 26 was given if the JND was above the maximum of 25.

For comparison with CASE IV measurements, vibration sensation was also measured using the neurothesiometer (Horwell Scientific Laboratory Supplies) at the pulp of the great toe. Vibration perception threshold (VPT) was measured using the ascending method of limits. A mean of three values was taken for analysis. The results were expressed in volts, and a value of 51 was assigned if the subjects could not feel the maximum vibration.

Assessment of microcirculation

Microvascular blood flow was examined using a laser Doppler imager (LDI) (Moor Instruments, Devon, U.K.) and laser Doppler flowmeter (LDF) (Perimed PF-2b; Perimed, Bury St. Edmunds, U.K.). In addition, $TcpO_2$, which is influenced by microvascular function, was measured using a $TcpO_2$ monitor (Novometrix Medical Systems, Wallingford, CT).

The LDF and $TcpO_2$ are well known and widely used in the assessment of skin microvascular blood flow and skin oxygenation. Unlike the LDF, the LDI is a recent technique that uses a faster scan pattern, with a 632.8-nm red laser beam eliminating direct tissue contact of a laser probe. A flux image is produced using a palette of 16 equally spaced colors in which dark blue represents lowest perfusion and red highest perfusion. We are familiar with the LDF technique but chose to also use the LDI because it assesses larger areas with better spatial resolution without skin contact and would theoretically provide more precise measurement of the hyperemic response. This was confirmed in 10 subjects with the coefficient of variation for the mean maximal hyperemia repeated on three different occasions for LDF being 11 and 7% for LDI. This study would also allow us to compare the results from the two laser Doppler techniques, which record blood flow using different units of perfusion.

Table 2—*Comparison of neurological tests in the three groups*

	C group	D group	DU group
	20	20	18
VPT (V)	5.5 (4.3–6.9)	12.0 (7.9–18.6)	51.0 (40.0–51.0)*
VDT (JND)	17.4 (16.3–19.9)	21.0 (19.3–22.3)	26.0 (22.6–26.0)*
CDT (JND)	10.4 (7.5–14.4)	16.5 (13.0–19.4)	26.0 (21.2–26.0)†
WDT (JND)	17.8 (15.8–19.8)	26.0 (19.5–26.0)	26.0 (26.0–26.0)
HPO (JND)	22.00 (20.2–23.0)	23.4 (21.0–26.0)	26.0 (26.0–26.0)†

Data are medians (interquartile range). Except for WDT, all of the measurements were significantly lower in subjects with foot ulcers than in those without (D vs. DU; *$P < 0.0001$, †$P = 0.001$).

The data were recorded to a computer using the software recommended by the manufacturer (MoorLDI version 3.11 for LDI and Perisoft for Windows version 2 for LDF).

Basal and maximal hyperemic response to heat

At the end of the nerve function testing, the subjects rested comfortably on a couch in a semi-recumbent position. The right leg was supported with a trough to prevent movement. The foot temperature was measured proximal to the first and second metatarsal heads with an infrared thermometer (Linear Laboratories, Fremont, CA). Room temperature and relative humidity were also recorded. The room temperature was maintained at 25 ± 1°C. This temperature was chosen specifically to comply with the recommended room temperature for the nerve function tests described above.

LDI

The laser head of the LDI was aligned to be perpendicular to the dorsum of the foot at a fixed distance of 30 cm. An area of 7.5 cm × 4 cm proximal to the metatarsal heads was scanned using the LDI to assess the basal flow. To determine biological zero, the scan was repeated after 2 min of arterial occlusion at ankle level. The biological zero is the residual signal recorded by the LDI when the blood flow has ceased. The hyperemic response was recorded by scanning the same area after 20 min of heating as described in the following section.

Offline analysis allowed calculation of the hyperemic response from the scanned images by defining the area on the flux image corresponding exactly to the skin area covered by the heater, and, as previously described, the biological zero image was subtracted from the mean maximum hyperemia of the postheating image (mean maximum hyperemia using LDI [LDI_{max}]). The results are expressed in arbitrary perfusion units.

LDF

After recording the baseline and biological zero scans with the LDI, a specially constructed heater holding the laser probe from the LDF was affixed to the dorsum of the foot proximal to the first and second metatarsal heads, using double-sided adhesive discs. Basal flow and biological zero were recorded for 1 min each. The heater was then switched on, and the skin was heated and maintained at 44°C for 20 min. At the end of heating period, blood flow measurements were taken from nine sites within the heated area by rotating the heating block containing the laser probe. The mean of the nine measurements minus the biological zero was used for analysis (mean maximum hyperemia using LDF [LDF_{max}]). After these measurements with the LDF, the heating probe was removed and the area was immediately scanned with the LDI as described above. The results were expressed in arbitrary perfusion units.

$TcpO_2$

$TcpO_2$ measurements were made on the lateral aspect of the mid-foot. A modified Clarke-type electrode was affixed to the lateral aspect of the dorsal mid-foot. The electrode was heated to 44°C. The partial pressure of oxygen expressed in mmHg was recorded for 25 min, and the mean of the last 5 min was used for analysis ($TcpO_2$).

Statistical analysis

Kruskal-Wallis and Mann-Whitney *U* tests were used to compare the different variables from the three groups. Binary univariate logistic regression analysis was used to determine the factors significantly associated with diabetic foot ulceration. The control data were not included in this analysis. Descriptive statistics and the odds ratios with 95% CIs were calculated for the significant variables. The SPSS version 10.0 software package was used for the statistical analysis of the data.

RESULTS — Clinical characteristics of the subjects with diabetes and control subjects are shown in Table 1. All the subjects were Caucasian. There was no significant difference in the ages between groups C and D, but subjects in the group with foot ulcers (DU) were older than groups C and D ($P = 0.01$). The ankle-brachial pressure index was greater in the DU group than the D group, but these differences did not reach statistical significance. As expected, the BMI was lower in the control group but was similar in the two diabetic groups. The neurological assessments are shown in Table 2. With the exception of WDT, there were significant differences in all the neurological parameters between individuals who had and had not ulcerated. To determine the important neurological variables associated with foot ulceration in the two groups with diabetes, univariate logistic regression analysis was performed. This revealed statistically significant odds ratios for foot ulceration for VDT, CDT, HPO, and VPT (Table 3).

The results of microvascular function are shown in Table 4. LDF_{max}, LDI_{max}, and $TcpO_2$ were significantly lower in the diabetic subjects (groups D and DU) than in group C. However, there was no significant difference in any of the measurements of microvascular function between the D and DU groups. None of the microvascular tests (LDF_{max}, $P = 0.36$; LDI_{max}, $P = 0.99$; and $TcpO_2$, $P = 0.7$) had a significant odds ratio.

Table 3—*Binary logistic regression results*

	Univariate odds ratio (95% CI)	Univariate *P*
VDT (JND)	1.97 (1.30–2.98)	0.001
CDT (JND)	1.58 (1.20–2.08)	0.001
HPO (JND)	2.30 (1.21–4.37)	0.01
VPT (V)	1.24 (1.08–1.42)	0.002

Univariate odds ratios with 95% confidence intervals for developing foot ulceration and their significance are shown.

Table 4—*Microvascular assessment*

	C group	D group	DU group
n	20	20	18
LDF_{max} (PU)	148.8 (139.1–183.7)	77.6 (62.5–121.8)	104.3 (66.3–137.0)
LDI_{max} (PU)	584.7 (505.7–678.2)	339.4 (255.4–485.2)	380.6 (270.1–513.2)
$TcpO_2$ (mmHg)	65.5 (58.0–69.2)	52.0 (44.7–57.0)	52.5 (43.7–58.2)

Data are medians (interquartile range). All of the microvascular measurements were significantly ($P < 0.0001$) lower in both the diabetic groups (D and DU) compared with control subjects. There was no significant difference in any of the measurements between the diabetic groups (D and DU). PU, perfusion units.

In this study, we were able to compare the VPT and VDT methods, which both assess vibration sensation but in different parts of the hallux and using different vibratory stimuli. There was good correlation between the methods ($r = 0.7$). Similarly, the $TcpO_2$ correlated well with the maximum hyperemia to heating assessed by both LDI and LDF ($r = 0.56$ and $r = 0.47$), confirming the influence of microvascular hyperemia in this measurement. Finally, this study allowed us to compare the two laser Doppler methods in the determination of the hyperemic response. There was excellent correlation between the methods ($r = 0.82$).

CONCLUSIONS

Nerve function

Quantitative sensory testing using CASE IV has been shown to be useful in detecting diabetic sensory polyneuropathy (19,21,34). Recent studies have used the CASE IV tests to examine the relative role of small- and large-fiber dysfunction in Charcot neuroarthropathy (35,36). This is the first study to examine the role of the several different modalities of nerve function detected by CASE IV in foot ulceration. As expected, abnormalities in CASE IV measurements were more pronounced in subjects with foot ulcers. Although expected, greater C-fiber dysfunction was not found. We did not find any particular modality to be more useful in distinguishing between individuals with and without foot ulcers. Thus, there appears to be no particular additional value in measuring small-fiber function with these tests in comparison to those that assess large-fiber function using vibration sensation. Although CASE IV is useful in detecting changes in nerve function and thus useful in therapeutic evaluation, the tests are time-consuming, the equipment is expensive, and there is the need for expertise in the measurement. Our results suggest that in clinical practice, the neurothesiometer may be preferred because it is less expensive, is easy to use, and is of similar value to the VDT used by the CASE IV when assessing vibration sensation. Furthermore, there is considerable data from previous studies relating VPT to ulcer risk; for example, a VPT of >25 V is associated with a 20% cumulative annual incidence of foot ulceration compared with 3% in individuals with a VPT of <15 V, i.e., a sevenfold increase in ulcer risk (8). We did not evaluate the 10-g Semmes-Weinstein monofilaments or clinical scores such as NDS, but previous studies have demonstrated their predictive value to be to be equally good (5,12,37).

Microvascular function

In 1986, Rayman et al. (23) were the first to demonstrate that maximum hyperemia to skin heating is impaired in type 1 diabetes and is related to the duration of diabetes and severity of complications. The same group demonstrated a relationship between the severity of the impaired hyperemic response and basement membrane thickening and suggested that the abnormality could in part be related to a structural limitation in vasodilatory capacity (31). Since then, several investigators have confirmed the findings in type 1 diabetes using a variety of different stimuli (38–43). In contrast, later studies in type 2 diabetes have demonstrated microvascular abnormalities at the time of diagnosis (25). It is accepted that people with type 2 diabetes have a pre-diabetic stage and may have had diabetes for many years before diagnosis. Also microvascular abnormalities have been shown to be present at the stage of impaired glucose tolerance (44) and may be partially reversed by good control in early type 2 diabetes (45). This suggests that the initial reversible vascular abnormality may relate to metabolic dysfunction, whereas later microvascular abnormalities may be due to irreparable structural damage. If microvascular abnormalities are important etiopathological factors in foot ulceration, the ability to reverse them by good metabolic control and pharmacological treatments may be extremely important. Furthermore, simplified microvascular function tests such as $TcpO_2$ may be valuable in screening individuals at risk of foot ulceration.

Disappointingly and unexpectedly, subjects with foot ulceration did not have more severe abnormalities in the microvascular hyperemic responses. In fact, with both LDF and LDI techniques, individuals without foot ulceration appeared to have the greater abnormality, although these differences were not significant ($P = 0.36$ and 0.65, respectively). Furthermore, none of the subjects in the DU group had a $TcpO_2$ of <30 mmHg, which has been previously shown to be an independent predictor of foot ulceration (32). This suggests that in the absence of macrovascular disease, skin oxygenation is not critically impaired in subjects with foot ulceration.

Thus, in conclusion, this study confirms that in the absence of macrovascular disease, impaired nerve function (large and small nerve fiber) is associated with foot ulceration in type 2 diabetic subjects; however, there appears to be no additional value in measuring small-fiber function over vibration sensation. This study also showed that neither $TcpO_2$ nor the more sophisticated measurements of microvascular hyperemic responses were able to discriminate between individuals with and without ulceration. This would suggest that such tests may not be of benefit in identifying individuals at greater risk of foot ulceration. However, longitudinal studies in similar groups would be necessary to confirm this. Finally, it is important to state that our findings do not exclude a potential role for microvascular abnormalities to impair wound healing in diabetes.

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