Abnormal LDIflare but Normal Quantitative Sensory Testing and Dermal Nerve Fiber Density in Patients with Painful Diabetic Neuropathy

SINGHAN T.M. KRISHNAN, MD[1]
CRISTIAN QUATTRINI, MD[2]
MARIA JEZIORSKA, PHD[2]
RAYAZ A. MALIK, PHD[2]
GERRY RAYMAN, MD[1]

OBJECTIVE — Abnormal small nerve fiber function may be an early feature of diabetic neuropathy and may also underlie painful symptoms. Methods for assessing small-fiber damage include quantitative sensory testing (QST) and determining intraepidermal nerve fiber density. We recently described a reproducible physiological technique, the LDIflare, which assesses small-fiber function and thus may reflect early dysfunction before structural damage. The value of this technique in painful neuropathy was assessed by comparing it with QST and dermal nerve fiber density (NFD).

RESEARCH DESIGN AND METHODS — Fifteen healthy control subjects, 10 subjects with type 2 diabetes and painful neuropathy (PFN), and 12 subjects with type 2 diabetes and painless neuropathy (PLN) were studied. LDIflare and QST were performed on the dorsum of the foot, and dermal NFD was determined.

RESULTS — Results of both large- and small-fiber quantitative sensory tests were abnormal in patients with PLN but not those with PFN compared with control subjects. Dermal NFD was also significantly reduced in the PLN group compared with control subjects (205.8 ± 165.3 vs. 424.9 ± 176.3 [mean ± SD]; $P = 0.003$) but not in the PFN group (307.6 ± 164.5). In contrast, the LDIflare (square centimeters) was reduced in both PFN (1.59 ± 0.41) and PLN (1.51 ± 0.56) groups compared with control subjects (4.38 ± 1.4) ($P < 0.001$ for both). NFD correlated significantly with the LDIflare ($r = 0.57$, $P < 0.0001$).

CONCLUSIONS — The LDIflare demonstrated impaired small-fiber function in patients with PFN when other assessments revealed no abnormality. We believe that this method has potential diagnostic value, particularly because it is noninvasive, has excellent reproducibility, and correlates with NFD. Furthermore, it may have an important role in assessing preventative therapies in early neuropathy.

Diabetes Care 32:451–455, 2009

Peripheral neuropathy affects between 40 and 60% of individuals with diabetes and is commonly diagnosed by assessing large-fiber sensory modalities. However, detection of small-fiber neuropathy may be of equal or more importance for several reasons. Structural and functional changes in small fibers precede large-fiber pathological changes and have been implicated in foot ulceration and delayed wound healing (1–3). Furthermore, C-fiber dysfunction may be involved in the genesis of neuropathic pain (4).

Until recently, few objective methods have been available to quantify small-fiber function. Quantitative sensory tests to define thermal and pain thresholds using the Computer Aided Sensory Evaluator–IV (CASE IV; WR Medical Electronics, Stillwater, MN) or the TSA-II NeuroSensory Analyzer (Medoc Advanced Medical Systems, Ramat Ysihai, Israel) have been used primarily in clinical research (5,6). However, they are dependent on subjective responses and therefore have a high interobserver variability and poor reproducibility (7,8). We recently described a novel and reproducible (coefficient of variation <15%) technique to assess small-fiber dysfunction, the "LDIflare," which measures axon reflex–mediated vasodilatation in response to skin heating (9). We have also demonstrated that LDIflare detects early C-fiber dysfunction in type 2 diabetes before small-fiber neuropathy can be detected by other currently available noninvasive methods (10). However, the structural basis for an abnormal LDIflare response has not been established.

Although intraepidermal nerve fiber density (IENFD), with good intraobserver reproducibility, has been increasingly used to diagnose small-fiber neuropathies, it is an invasive procedure (11,12). In the present study we assessed small-fiber function using quantitative sensory testing (QST) and the LDIflare and compared these results with the results of dermal NFD in foot skin biopsy specimens from the same area. Dermal NFD as opposed to IENFD was quantified to define the underlying structural basis of the LDIflare, as this depends on an abnormality in dermal blood flow. In addition, as there is no current consensus as to whether an abnormality in small-fiber dysfunction and damage underlie painful diabetic neuropathy, we compared diabetic patients with painful neuropathy (PFN) and painless neuropathy (PLN).

RESEARCH DESIGN AND METHODS

— Type 2 diabetic patients with PFN ($n = 10$) and PLN ($n = 12$) and 15 healthy control subjects were studied. Patients with diabetes were recruited from the outpatient clinics of the Ipswich Hospital Diabetes Centre (Ipswich, U.K.). Subjects with absent pedal pulses or evidence of peripheral

From [1]The Diabetes Centre, Ipswich Hospital, Ipswich, U.K.; and [2]The University of Manchester, Manchester, U.K.
Corresponding author: Dr. G. Rayman, gerry.rayman@ipswichhospital.nhs.uk.
Received 7 August 2008 and accepted 4 December 2008.
Published ahead of print at http://care.diabetesjournals.org on 15 December 2008. DOI: 10.2337/dc08-1453.

vascular disease were excluded, and all subjects had an ankle brachial pressure index (ABPI) of >0.8. The study was approved by the local ethics committee, and all the subjects gave informed written consent.

Assessment of LDIflare

Subjects were allowed to acclimatize for 30 min in a temperature-controlled room in which the temperature was maintained at 25 ± 1°C. The foot temperature was measured proximal to the first and second metatarsal heads using an infrared thermometer (Linear Laboratories, Fremont, CA). The axon reflex–mediated LDIflare was examined using a laser Doppler imager (LDI) (Moor Instruments, Devon U.K.) and our established methodology (10). Skin proximal to the first and second metatarsal heads on the dorsum was heated with a circular skin heater (diameter 1.0 cm; Moor Instruments) to 44°C for 20 min. An area of 3.5 × 3.5 cm surrounding the heated skin was scanned with the LDI immediately after careful removal of the heater probe. We have shown previously that removal of the heater along with the holder does not have an impact on the size of the flare (10). On the flux image, the region of interest demarcated by the edge of the flare was drawn, and the area of the LDIflare was calculated using Moor LDI software (version 3.11) and expressed in square centimeters.

Clinical neuropathy assessment

Vibration perception threshold (VPT) was assessed using the Neurothesiometer (Horwell Scientific Laboratory Supplies, Nottingham, U.K.) at the pulp of the great toe using the ascending method of limits. The results were expressed in volts, and a value of 51 was assigned if the subjects could not feel the maximum vibration. The right foot was assessed using the Neuropen (Owen Mumford, Oxford, U.K.), which contains a 10-g monofilament to assess pressure perception and a Neurotip (Owen Mumford) for pinprick sensation (13,14). Ten-gram monofilaments were applied for 2 s on the plantar aspect of the first, third, and fifth metatarsal heads, and the Neurotip was applied at the eponychium of the first toe. Subjects with an abnormal response using the Neuropen assessment and/or impaired VPT (≥15 V, i.e., >95th percentile for this age-group) were classified as having neuropathy. Subjects with typical painful neuropathic symptoms with a visual analog scale score >4 for >6 months were classified as having PFN (15). Quantitative sensory tests using the CASE IV, including vibration detection threshold (VDT), cold detection threshold (CDT), warmth detection threshold (WDT), and heat pain onset (HPO) were performed with software CASE IV (version 4.27.1; WR Medical Electronics). VDT, CDT, and WDT were measured using the 4, 2, 1 stepping algorithm with null stimuli (5). The VDT was obtained on the dorsal aspect of the hallux, and CDT and WDT were examined on the dorsum of the midfoot. For each test, the computer calculated the "just noticeable difference" (JND) from the subject's responses, with a higher JND reflecting a larger amplitude of the stimulus (vibration) or larger change in temperature (thermal). A value of 26 was given if the JND was greater than the maximum of 25.

Skin biopsy

On a different day, skin biopsies were performed using a sterile 3-mm biopsy punch (Stiefel Laboratories, Bucks, U.K.) in the same area where the LDIflare had been assessed previously. All subjects tolerated the biopsy, and there was no infection or other adverse event.

Fixation immunostaining protocol

The biopsy specimen was immersed in 5 ml of 4% buffered paraformaldehyde for 18–24 h, washed with Tris-buffered saline (TBS) buffer for 15 min, and transported to the laboratory to be embedded in paraffin wax. Wax blocks were cut on the microtome into thin sections (5 μm), which were mounted on positively charged slides (three per slide), dewaxed in xylene, and gradually rehydrated through decreasing ethanol dilutions. In all cases, epidermal melanin was bleached with 0.25% $KMnO_4$ and 5% oxalate before serum protein block. Before applying primary antibody, an enzymatic antigen retrieval pretreatment with trypsin was used for anti-CD31 and anti-CD34 for blood vessels. Tissue was washed with Tween 20 detergent/TBS buffer before the run was started; 3% hydrogen peroxide was used to block endogenous peroxidase and a TBS-buffered solution of 1:10 normal serum was used for protein block. Dilution of anti-protein gene product (PGP) 9.5 rabbit anti-human polyclonal antibody was 1:100, and it was applied for 60–72 h at 5°C. CD34 (dilution 1:300) and CD31 (dilution 1:50) polyclonal mouse antihuman antibodies were applied overnight at 5°C. After addition of secondary antibody (swine anti-rabbit for PGP), a streptavidin-horseradish–conjugated peroxidase and 3′,3′-diaminobenzidine chromogen substrate were used to detect binding of the primary antibodies. Negative controls comprised sections that underwent the same runs except that the primary antibody was omitted. Developing time was exactly the same for all sections in each separate run, and in each run the sections were processed synchronously.

Image analysis

Patterns of immunostaining were examined by light microscopy (Leitz DM RB microscope). Digital images were captured at ×400 magnification with a Nikon digital camera and analyzed with Leica QWin Standard V2.4 (Leica Microsystem Imaging, Cambridge, U.K.) set to detect color intensities in a fixed and constant range. Every image was evaluated using a standardized Leica program to quantify the amount of stained and total areas (Leica QWin Standard V2.4). The PGP 9.5 positively stained profiles and blood vessel cross-sections were counted manually and divided by the dermal area to obtain a density (number per square millimeter). Because PGP 9.5 ubiquitously stains all nerve fibers, both sensory and autonomic C fibers in the dermis were included. The blood vessels counted were predominantly capillaries, although some precapillary arterioles or postcapillary venules may have been included as cross-sections were studied. Large arterioles and venules were not counted. All observations were performed on coded slides to prevent observer bias.

Statistical analysis

Descriptive statistics were used to describe subject characteristics. Nonparametric analysis (Kruskal-Wallis test and Mann-Whitney *U* test) was used to determine differences between the groups. Pearson's correlation coefficient was used to correlate the variables. The results are expressed as means ± SD. $P < 0.05$ was considered to be significant. SPSS (version 11.0, SPSS, Chicago, IL) was used for the statistical analysis.

RESULTS — Clinical characteristics of the subjects with diabetes and control subjects are shown in Table 1. All subjects were Caucasian and were matched for

Table 1—*Clinical characteristics of subjects*

	Healthy control subjects	Type 2 diabetic subjects	
		PFN	PLN
Sex (male/female)	5/10	5/5	6/6
Age (years)	54.4 ± 9.7	61.0 ± 11.2	62.9 ± 10.2
Duration (years)	—	12.1 ± 4.2	13.3 ± 4.29
BMI (kg/m^2)	25.4 ± 2.4	30.7 ± 3.1	32.3 ± 2.8
A1C (%)	—	8.2 ± 3.8	8.6 ± 3.5
ABPI	1.1 ± 0.1	1.0 ± 0.2	1.2 ± 0.1
VPT	7.08 ± 2.8	8.6 ± 2.2	37.1 ± 12.9
VAS (0–10)	0	5.7 ± 1.1	0

Data are means ± SD. There were no significant differences in age between the healthy control, PFN, and PLN groups. BMI was lower in the control subjects than in the PFN and PLN groups ($P = 0.001$ and $P = 0.0001$, respectively). Duration of diabetes and A1C were not significantly different between the PFN and PLN groups. ABPI was not different among the three groups. VPT was not significantly different between the healthy control and PFN groups but high in the PLN group ($P < 0.0001$). Visual analog scale (VAS) was high in the PFN group.

age. The duration of diabetes in the diabetic groups was similar. As expected, the BMI was lower in the control group but was similar in the two diabetic groups. A1C was not significantly different in the two diabetic groups. ABPIs were similar in all three groups.

The neurological assessments are shown in Table 2. VPT, VDT, WDT, CDT, and HPO were significantly higher in the PLN group but not in the PFN group compared with healthy control subjects (Table 2). However, the LDIflare was significantly reduced in both diabetic groups compared with the healthy control subjects (healthy control subjects 4.38 ± 1.4 cm^2, PLN group 1.59 ± 0.41 cm^2, and PFN group 1.51 ± 0.56 cm^2; $P < 0.0001$). In contrast, the NFD was significantly reduced in the PLN group compared with that in healthy control subjects (205.8 ± 165.3 vs. 424 ± 176.3 mm^2; $P = 0.003$) but not in the PFN group (307.6 ± 164.5 vs. 424 ± 176.3 mm^2; $P = 0.13$). There was no significant difference between the PFN and PLN groups for either LDIflare or NFD (LDIflare: PLN 1.59 ± 0.41 cm^3 vs. PFN 1.51 ± 0.56 cm^3; $P = 0.49$; NFD: PLN 205.8 ± 165.3 mm^2 vs. PFN 307.6 ± 164.5 mm^2; $P = 0.12$). There was also no significant difference in dermal vascular density among any groups (Table 2). The LDIflare correlated significantly with dermal NFD (Fig. 1) ($r = 0.57$; $P < 0.0001$) in all subjects combined and also within control subjects ($r = 0.53$; $P < 0.05$) and in the PFN group ($r = 0.71$; $P < 0.05$) but not in the PLN group ($r = 0.38$, $P = 0.22$).

CONCLUSIONS — A significant number of patients with diabetic neuropathy present with pain as their first neuropathic symptom. Many of these patients have no objective clinical signs. It has been suggested that this is because conventional bedside tests such as reflexes, pressure sensation, and vibration relate to large-fiber function, whereas pain sensation conveyed by small unmyelinated C-fibers and A-δ fibers is not revealed by these tests (15). In this study we compared the ability to define an underlying abnormality in small-fiber dysfunction, using the novel technique of LDIflare with established QST using CASE IV and dermal skin NFD in patients with diabetic neuropathy.

In diabetic patients with PLN, as expected, results of all neurological tests including VPT, QST, and LDIflare and NFD were abnormal, consistent with the extensive nerve damage in this group (10). However, in patients with PFN, there was no bedside evidence of neuropathy; i.e., they had intact reflexes and 10-g monofilament sensation. Furthermore, large-fiber (VPT and VDT) and, more surprisingly, small-fiber function (WDT, CDT, and HPO) assessed by QST did not differ significantly from those in the control subjects.

In contrast, in the PFN group, the LDIflare was the only test that showed abnormal results, and, indeed, this showed impairment as severe as that in the PLN group. The NFD in the PFN group, although lower, was not significantly different from that of the control subjects and lay between that of patients with PLN and the control group.

A reduced LDIflare response may occur because of impaired C-fiber function, loss of C-fibers, reduced microvascular vasodilatation (including in response to vasoactive peptides), or reduced blood vessel density. The latter is unlikely, as there was no significant difference in the dermal blood vessel density between the three groups, and, indeed, in previous studies we have found no reduction in blood vessel density in individuals with diabetes (9). Although it is widely recognized that hyperemic responses are reduced in individuals with diabetes, this reduction only relates to maximal hyperemia; we have previously shown that the flare response in terms of the area over which the flare spreads after skin heating is clearly demonstrable even in subjects with severely impaired maximal hyperemia (10). Thus, the smaller flares are not a result of either reduced blood vessel density or reduced maximal vasodilatation response. We specifically assessed dermal NFD as opposed to IENFD, as dermal NFD provides a direct measure of the structural integrity of the innervation of the dermal blood vessels and, hence, the

Table 2—*Neurological assessments*

	Healthy control subjects	Type 2 diabetic subjects	
		PFN	PLN
LDIflare (cm^2)	4.38 ± 1.4	1.59 ± 0.4*	1.51 ± 0.56*
Dermal nerve density (mm^2)	424 ± 176.3	307.6 ± 164.5	205.8 ± 165.3†
Dermal vascular density (mm^2)	115.8 ± 23.7	129.9 ± 23.8	103.4 ± 27.1
VPT (V)	7.0 ± 2.8	8.7 ± 2.2	37.0 ± 12.9*
VDT (JND)	18.4 ± 3.2	19.5 ± 3.2	23.0 ± 3.7‡
CDT (JND)	10.4 ± 5.0	13.8 ± 5.1	19.5 ± 4.6*
WDT (JND)	17.5 ± 2.0	18.3 ± 6.1	25.2 ± 1.8*
HPO (JND)	21.6 ± 1.8	21.3 ± 3.0	25.3 ± 1.6*

Data are means ± SD. Except for LDIflare, none of the neurovascular parameters were significantly different in the PFN group compared with the healthy control subjects. *P* values compared with healthy control subjects: *$P < 0.0001$; †$P = 0.003$; ‡$P = 0.005$.

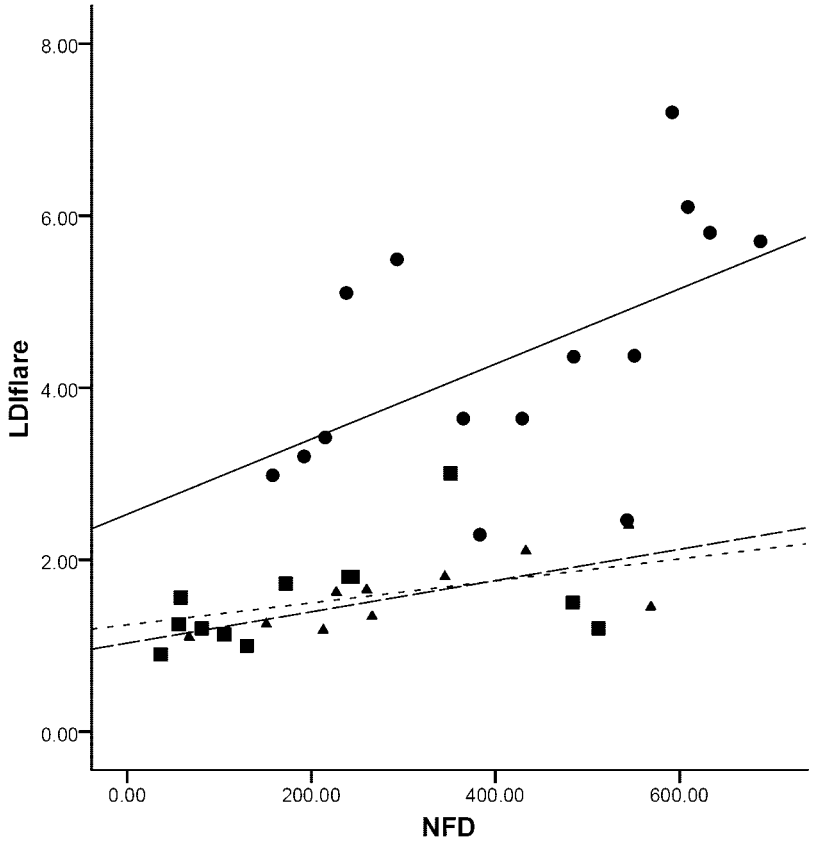

Figure 1—*Correlation of LDIflare and NFD. The LDIflare results correlated significantly with dermal NFD (r = 0.57; P < 0.0001) in all subjects combined and within control subjects (●) (r = 0.53; P < 0.05) and in the PFN group (▲) (r = 0.71; P < 0.05) but not in the PLN group (■) (r = 0.38; P = 0.22).*

LDIflare. We found no significant difference in dermal NFD between those with PFN and control subjects; this finding suggests that the reduced flare reflects functional as opposed to structural impairment in the axon reflex pathway. This observation further supports the paradigm that impaired small-fiber function occurs before the development of large-fiber neuropathy, but, in addition, we now demonstrate that this precedes structural defects to the small fibers.

Several studies have shown that in diabetes the degree of intraepidermal nerve fiber loss correlates with the severity of the neuropathy (16–18). However, few studies have focused specifically on dermal NFD in painful diabetic neuropathy. Lauria et al. (19) found reduced IENFD in a study of six patients with painful diabetic neuropathy compared with that in normal control subjects. In another study of patients with neuropathic pain, Sorensen et al. (20) paradoxically found more severe IENFD loss compared with that in those with PLN. From this finding, they concluded that loss of IENFD cannot explain genesis of pain in all patients. Loseth et al. (21) also reported significantly lower IENFD and higher cold perception thresholds in patients with diabetes and normal nerve conduction studies whether they had painful symptoms or not.

There are several reasons why we did not find a significant difference in nerve fiber density in comparison with the above studies. Patient selection may be important, as our patients had no clinical signs of neuropathy and may thus represent an earlier phase in the pathological process. The majority of studies have examined IENFD as opposed to dermal NFD. From previous reports it would appear that IENFD may be a more useful diagnostic test for detecting early structural nerve damage, as intraepidermal nerves are more distal than dermal nerves. However, the assessment of dermal NFD may provide more mechanistic insights into the pathogenesis of painful diabetic neuropathy, as it provides a measure of dermal blood vessel innervation and, hence, any potential impact on dermal blood flow. Indeed, we have previously demonstrated an impairment of cutaneous endothelium-related vasodilatation and C-fiber–mediated vasoconstriction in painful diabetic neuropathy and suggested that inappropriate local blood flow regulation may have a role in the pathogenesis of pain in diabetic neuropathy (22). A recent study confirms the validity of assessing alterations in dermal NFD in thin sections and has specifically demonstrated a reduction in arteriolar innervation in patients with small-fiber neuropathy (23). Furthermore, the assessment of dermal NFD in addition to IENFD has been shown to improve the diagnostic sensitivity for detecting painful sensory neuropathy (24). Finally, functional defects in unmyelinated C-fibers may precede structural defects (25), which would be detected by an abnormal LDIflare but with no effect on NFD, as demonstrated in this study. It is of importance that the LDIflare results correlated with NFD in the groups combined as well as separately in the control and PFN groups. This result would be expected because the size of the flare response should relate not only to neural function but also to the actual number of functioning nerves. It was not unexpected that there would be no correlation between the flare response and NFD in the PLN group because whether or not dermal nerve fibers were identified, all modalities of nerve function were severely impaired or absent with little or no graduation in this group.

In summary, using the LDIflare technique, we have demonstrated abnormal C-fiber function in subjects with symptomatic PFN in whom results of conventional quantitative sensory tests were normal and in whom there was no significant reduction in NFD. Because of the small sample size in the current study, further studies with larger numbers of patients are required to confirm these findings and to determine the sensitivity and specificity of the LDIflare as a diagnostic modality in painful diabetic neuropathy. Because the LDIflare detects small-fiber dysfunction before the occurrence of potentially irreversible structural loss of nerve fibers, in addition to its potential diagnostic value, it may have an important role in assessing preventative therapies in early neuropathy.

Acknowledgments— No potential conflicts of interest relevant to this article were reported.

References

1. Levitt NS, Stansberry KB, Wynchank S, Vinik AI: The natural progression of autonomic neuropathy and autonomic function tests in a cohort of people with IDDM. *Diabetes Care* 19:751–754, 1996
2. Celiker R, Basgoze O, Bayraktar M: Early detection of neurological involvement in diabetes mellitus. *Electromyogr Clin Neurophysiol* 36:29–35, 1996
3. Vinik AI, Erbas T, Stansberry KB, Pittenger GL: Small fiber neuropathy and neurovascular disturbances in diabetes mellitus. *Exp Clin Endocrinol Diabetes* 109: 451–473, 2001
4. Galer BS, Gianas A, Jensen MP: Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life. *Diabetes Res Clin Pract* 47:123–128, 2000
5. Dyck PJ, O'Brien PC, Kosanke JL, Gillen DA, Karnes JL: A 4, 2, and 1 stepping algorithm for quick and accurate estimation of cutaneous sensation threshold. *Neurology* 43:1508–1512, 1993
6. Gruener G, Dyck PJ: Quantitative sensory testing: methodology, applications, and future directions. *J Clin Neurophysiol* 11: 568–583, 1994
7. Shy ME, Frohman EM, So YT, Arezzo JC, Cornblath DR, Giuliani MJ, Kincaid JC, Ochoa JL, Parry GJ, Weimer LH: Quantitative sensory testing: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 60:898–904, 2003
8. Gelber DA, Pfeifer MA, Broadstone VL, Munster EW, Peterson M, Arezzo JC, Shamoon H, Zeidler A, Clements R, Greene DA: Components of variance for vibratory and thermal threshold testing in normal and diabetic subjects. *J Diabetes Complications* 9:170–176, 1995
9. Rayman G, Malik RA, Sharma AK, Day JL: Microvascular response to tissue injury and capillary ultrastructure in the foot skin of type I diabetic patients. *Clin Sci (Lond)* 89:467–474, 1995
10. Krishnan ST, Rayman G: The LDIflare: a novel test of C-fiber function demonstrates early neuropathy in type 2 diabetes. *Diabetes Care* 27:2930–2935, 2004
11. Polydefkis M, Hauer P, Griffin JW, McArthur JC: Skin biopsy as a tool to assess distal small fiber innervation in diabetic neuropathy. *Diabetes Technol Ther* 3:23–28, 2001
12. Smith AG, Howard JR, Kroll R, Ramachandran P, Hauer P, Singleton JR, McArthur J: The reliability of skin biopsy with measurement of intraepidermal nerve fiber density. *J Neurol Sci* 228:65–69, 2005
13. Abbott CA, Carrington AL, Ashe H, Bath S, Every LC, Griffiths J, Hann AW, Hussein A, Jackson N, Johnson KE, Ryder CH, Torkington R, Van Ross ER, Whalley AM, Widdows P, Williamson S, Boulton AJ: The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. *Diabet Med* 19: 377–384, 2002
14. Paisley AN, Abbott C, van Schie C, Boulton AJM: A comparison of the Neuropen against standard quantitative sensory-threshold measures for assessing peripheral nerve function. *Diabet Med* 19:400–405, 2002
15. Tesfaye S, Kempler P: Painful diabetic neuropathy. 48:805–807, 2005
16. Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC: The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. *Brain* 127:1606–1615, 2004
17. Quattrini C, Tavakoli M, Jeziorska M, Kallinikos P, Tesfaye S, Finnigan J, Marshall A, Boulton AJ, Efron N, Malik RA: Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes* 56:2148–2154, 2007
18. Quattrini C, Jeziorska M, Boulton AJ, Malik RA: Reduced vascular endothelial growth factor expression and intra-epidermal nerve fiber loss in human diabetic neuropathy. *Diabetes Care* 31:140–145, 2008
19. Lauria G, Morbin M, Lombardi R, Borgna M, Mazzoleni G, Sghirlanzoni A, Pareyson D: Axonal swellings predict the degeneration of epidermal nerve fibers in painful neuropathies. *Neurology* 61:631–636, 2003
20. Sorensen L, Molyneaux L, Yue DK: The relationship among pain, sensory loss, and small nerve fibers in diabetes. *Diabetes Care* 29:883–887, 2006
21. Loseth S, Stalberg E, Jorde R, Mellgren SI: Early diabetic neuropathy: thermal thresholds and intraepidermal nerve fibre density in patients with normal nerve conduction studies. *J Neurol* 255:1197–1202, 2008
22. Quattrini C, Harris ND, Malik RA, Tesfaye S: Impaired skin microvascular reactivity in painful diabetic neuropathy. *Diabetes Care* 30:655–659, 2007
23. Dabby R, Vaknine H, Gilad R, Djaldetti R, Sadeh M: Evaluation of cutaneous autonomic innervation in idiopathic sensory small-fiber neuropathy. *J Peripher Nerv Syst* 12:98–101, 2007
24. Vlckova-Moravcova E, Bednarik J, Dusek L, Toyka KV, Sommer C: Diagnostic validity of epidermal nerve fiber densities in painful sensory neuropathies. *Muscle Nerve* 37:50–60, 2008
25. Lauria G, Devigili G: Skin biopsy as a diagnostic tool in peripheral neuropathy. *Nat Clin Pract Neurol* 3:546–557, 2007