

# Electrically Stimulated Axon Reflexes Are Diminished in Diabetic Small Fiber Neuropathies

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**Axon reflex mediated flare depends on the density and the function of cutaneous C-fibers and may be impaired in diabetic neuropathy. We induced neurogenic axon reflex flare by intracutaneous electrical stimulation and analyzed size and intensity of the flare on the dorsum of the foot and ventral thigh with laser Doppler imaging (LDI). We investigated 12 diabetic subjects with small fiber neuropathies (SFNs), 5 diabetic subjects without neuropathy (NO-Ns), and 14 healthy control subjects. Five of the normal subjects were reassessed after 12 months. In comparing patients with SFN to control subjects, we found that SFN flare size but not the intensity of vasodilation (flux) was reduced on the feet ( $P < 0.001$ ) and thighs ( $P < 0.007$ ). Furthermore, electrical thresholds for flare induction were increased (thighs  $P < 0.001$  and feet  $P < 0.03$ ). In NO-Ns, flare size at the feet ( $P < 0.02$ ) and flux at both sites (thighs  $P < 0.001$  and feet  $P < 0.002$ ) were even increased. Test/retest evaluation of our method revealed a good correlation ( $r = 0.83$ ,  $P < 0.004$ ). Intracutaneous electrical stimulation of C-fibers and scanning the flare with LDI is a sensitive tool to reliably detect small fiber impairment in diabetic SFN subjects and even increased neuropeptide release in NO-Ns. *Diabetes* 53:769–774, 2004**

**S**mall fiber neuropathy (SFN) is a common complication in diabetes and other disorders. Clinical features of this disease often include pain and dysesthesia such as burning feet and neurovascular abnormalities (1). Despite its high incidence and clinical importance, there are only a few methods to quantify peripheral small fiber dysfunction. These include quantitative sensory testing (2), analysis of heart rate variability, assessment of sudomotor axon reflexes (3), or skin biopsies (4). However, availability of these procedures in clinical routine is limited and sensitivity of the psychophysical methods may be questioned.

Analysis of axon reflexes is a simple and specific test for peripheral C-fiber function. Activation of nociceptive C-fibers in the skin by mechanical (5) or chemical stimuli

(6,7) induces a spreading vasodilation, which is visible as a “flare response” surrounding the injured site (8). The flare depends on both the density and function of C-fiber nociceptors in the skin, in particular the subgroup of mechanoinensitive “silent” nociceptors (9). The neuronal pathway of neurogenic vasodilation is organized as an axon reflex. Activation of peripheral C-nociceptors provokes nerve impulses, which are conducted centrally. At some branching points of the axonal tree these action potentials may invade peripheral branches (10), causing the release of vasodilatory neuropeptides from skin nerve terminals, e.g., calcitonin gene-related peptide and substance P (11,12).

In previous studies, quantitative analysis of vasodilation in axon reflex flares (flare intensity) employing single channel laser Doppler flowmetry (5,13) was not sensitive enough to reliably distinguish between control subjects and patients with mild to moderate SFN. In contrast, in a pilot study we have been able to demonstrate that the extension, and not the intensity, of histamine-induced flare was significantly reduced in SFN patients (14). However, there are some drawbacks of histamine stimulation; epidermal thickness varies seasonally. This could have an impact on the amount of histamine reaching histamine-sensitive nerve endings (15). Furthermore, only a minor population of C-fibers are activated by histamine (16), and the number of action potentials induced by a chemical stimulus in C-fibers varies. This makes standardization impossible. Therefore, the neurogenic flare reaction following chemical stimulation appears to be too variable to be considered a diagnostic tool for SFN.

In contrast, previous experiments have shown that neurogenic flare in human skin can be reliably induced with low variation by electrical current (17). In this study we employed standardized electrical stimulation at a high current density. Microdialysis fibers equipped with internal stainless steel electrodes were inserted in the dorsum of the foot and the ventral thighs for intradermal electrical stimulation. The flare response was recorded by laser Doppler imaging (LDI). The aim was to assess stimulus-response curves of the axon reflex flare for increasing current intensity. These stimulus-response curves were compared between diabetic subjects with SFN or without neuropathy (NO-Ns) and healthy control subjects.

## RESEARCH DESIGN AND METHODS

Twelve patients (5 women and 7 men, mean age  $46 \pm 3.3$  years) suffering from diabetic neuropathy were included in this study. The inclusion criterion was a clinically suspected small fiber impairment (SFN) because all patients suffered from neuropathic pain symptoms, such as burning sensation, lancinating, or spontaneous pain in the feet but not in the thighs. Among these

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LDI, laser Doppler imaging; LFN, large fiber neuropathy; NGF, nerve growth factor; NO-N, diabetic subject without neuropathy; PU, perfusion units; SFN, small fiber neuropathy.

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TABLE 1  
Biographical and clinical data of included patients

	SFN 1	SFN 2	SFN 3	SFN 4	SFN 5	SFN 6
Sex (M/F)/age (years)	M/50	M/40	F/38	M/47	M/55	F/34
Diabetes type	2	1	1	2	2	1
Duration of diabetes (years)	6	12	22	4	10	14
Neuropathy impairment score	12	24	31	28	60	22
Type of pain	BF	BF	BF, L, and P	L and P	BF and P	L
Sensory disturbances	Feet	Feet	Feet	Toes	Feet and lower leg	Toes
Plantar reflex	+	+	—	+	—	+
Nerve conduction velocity, peroneal nerve (m/s)	40	40	36	36	34	42
Amplitude of compound muscle action potential, peroneal nerve	2.4	2.8	2.8	1.0	1.2	1.6
Nerve conduction velocity, sural nerve (m/s)	42	44	42	48	Absent	40
Amplitude of sensory nerve action potential, sural nerve ( $\mu$ V)	3.2	3.7	2.2	4.8	Absent	2.0

Plantar reflexes: +, normal; —, reduced; —, absent. BF, burning feet; L, lancinating pain; P, spontaneous prickling sensations.

patients were five patients with type 1 diabetes (two men and three women) and seven patients with type 2 diabetes (five men and two women). The type of neuropathic pain resulting from SFN was assessed by the medical history and neuropathy impairment score (18) and was recorded as burning, lancinating, or spontaneous pain.

Moreover, we recruited five diabetic subjects (four women and one man, mean age  $43.6 \pm 10.8$  years; three patients with type 1 and two patients with type 2 diabetes) without clinical and electrophysiological evidence of neuropathy (NO-N). All patients underwent standard electrophysiological studies, such as the measurement of nerve conduction velocity of the peroneal and sural nerve (Table 1).

As a control group, 14 healthy subjects (8 women and 6 men, mean age  $36.5 \pm 2.3$  years) without any neurological or dermatological disorders were included. In five subjects (two women and three men, mean age  $41.4 \pm 6$  years) of this control group, reproducibility of the investigation was evaluated by retests performed with an interval of 12 months. All healthy volunteers were drawn from staff and relatives of the Department of Physiology, University of Erlangen-Nuremberg. All investigations were carried out in a temperature ( $23^\circ\text{C}$ ) and humidity (50% relative humidity) controlled laboratory. The time for acclimatization for all subjects was at least 1 h before starting the experiment.

Informed consent according to the Declaration of Helsinki was obtained from all participants and the study was approved by the local ethics committee.

**Electrical stimulation.** Electrical stimulation was performed in the center of the right dorsum of the foot and in the center of the right ventral thighs 20 cm above knee level. Two microdialysis fibers (DermalDialysis, Erlangen, Germany) were inserted intradermally for a length of 1.5 cm by a 25-gauge cannula in every test area. This technique ensured that the fibers were located at a depth of  $\sim 0.6$  mm intradermally (16). The fibers were placed transversally to the axis of the limb for a distance of 3 mm. They were equipped with internal stainless steel wires (0.1 mm in diameter) for electrical stimulation. Each fiber was perfused with 0.9% NaCl solution by a microdialysis pump (Pump 22; Harvard Apparatus) at a constant flow rate of  $4 \mu\text{l}/\text{min}$ .

After a baseline period of 60 min, when insertion-related vasodilatation had subsided (19), electrical stimulation was started. Electrical pulses (1 Hz, 0.5 ms stimulus duration) were continuously delivered for 21 min via a constant current stimulator (DS7; Digitimer, Hertfordshire, U.K.). Electrical stimulation commenced with a current of 6 mA. Current was stepwise increased thereafter to 8, 10, 12.5, 15, 17.5, and 20 mA every 3 min.

In previous studies, this setting and the frequency of 1 Hz has proven to be most effective in provoking axon reflex vasodilation in human skin (17). This technique guarantees the induction of a flare being constant for even more than 1 h (20). With these parameters, electrical stimulation was well tolerated and no lasting side effects were observed.

**LDI.** Superficial blood flow was quantified using an LDI (Moor, London, U.K.). LDI scans ( $256 \times 256$  pixels, scan resolution 4 pixels/s, distance 50 cm to site of electrical stimulation) were recorded at baseline and at intervals every 3 min during the electrical stimulation (total of eight scans). The LDI scans were started 1 min after changing the current and took  $\sim 115$  s. The size of the scanned area was  $144 \text{ cm}^2$ , with the stimulation site in the center. Blood flux was calculated for each pixel by means of intensity of the Doppler shift of the

backscattered laser light (arbitrary perfusion units [PUs]). Thereby, a two-dimensional map of the superficial blood flow is generated. Area and intensity of the neurogenic flare reaction were analyzed offline by dedicated software (MLDI 3.0; Moor, London, U.K.). Flare area was determined by total number of pixels ( $0.22 \text{ mm}^2/\text{pixel}$ ) in which flux values exceeded the mean flux by two SDs from the baseline picture. Total flare areas  $>0.5 \text{ cm}^2$  on the feet and  $>1 \text{ cm}^2$  on the thighs were regarded as significant. The minimum current needed to induce a flare reaction according to this definition was noted as electrical threshold.

**Pain rating.** Patients and subjects were asked to quantify pain sensation during electrical stimulation by a numeric rating scale from 0 to 10, in which the value of 0 indicated “no pain” and 10 “maximum pain.” Pain ratings were obtained every minute during stimulation.

**Statistics.** Statistics were calculated using an SPSS (SPSS 10.1 for Windows, Chicago, IL) software package. To identify significant differences between patient groups and control subjects, ANCOVA for repeated measures was performed. The ANCOVA incorporated seven consecutive measurements (during stimulation), one main factor (SFN subject or NO-N and control subjects), and age and sex as covariates. Since sphericity could not be assumed in all datasets, Greenhouse-Geisser correction was employed. Post hoc analysis was performed in order to allocate differences at the different stimulus intensities between SFNs or NO-Ns and control subjects. We used planned comparisons with *t* tests using Bonferroni’s correction.

Analyzing the test/retest reliability, we calculated the mean flare size and mean flux values from all stimulation intensities at thighs and feet and thereafter the Pearson correlation coefficient. Additionally, an ANOVA (seven consecutive measures and one main factor [test/retest]) was calculated. Covariate adjustment was not necessary in this analysis because both groups were identical.

Pain ratings in different subgroups were analyzed using Student’s *t* test for independent samples. To compare the current thresholds necessary to induce a flare, the Mann-Whitney *U* test was calculated. This nonparametric test was used because current intensities were ordinal scaled. Accordingly, the median current intensities and the ranges are given.

All further values are means  $\pm$  SE. Statistical significance was considered at  $P < 0.05$  or was corrected for multiple (i.e., seven) post hoc comparisons at  $P < 0.007$ .

## RESULTS

**Clinical symptoms of diabetic SFN patients.** As expected, the type 1 diabetic subjects included in our study were younger than type 2 diabetic subjects (mean age  $35.2 \pm 2.5$  vs.  $53.7 \pm 2.7$  years). The average duration of the disease was longer in type 1 diabetic subjects ( $15 \pm 2.1$  vs.  $8 \pm 1.4$  years). Diabetes was well controlled in all patients at the time of the experiment. SFN patients showed neurophysiologic abnormalities proving large fiber neuropathy (LFN), but the extent of neuropathy showed significant variability. Patients with advanced LFN

TABLE 1  
Continued

SFN 7	SFN 8	SFN 9	SFN 10	SFN 11	SFN 12	NO-N 1	NO-N 2	NO-N 3	NO-N 4	NO-N 5
F/65	M/61	M/26	F/46	F/38	M/52	F/26	F/27	F/25	M/67	F/73
2	2	1	2	1	2	1	1	1	2	2
12	10	17	3	10	11	8	13	3	2	2
56	42	12	10	46	11	0	0	0	0	0
BF and L	BF and L	BF and P	P	P	L	—	—	—	—	—
Feet	Feet and lower leg	Toes	Feet	Feet and lower leg	Feet	—	—	—	—	—
—	—	+	+	—	+	+	+	+	+	+
34	38	46	40	38	41	49	46	49	43	47
1.0	4.3	5.8	3.8	3.4	2.6	7.1	7	6.5	8.2	5.5
Absent	38	42	47	40	44	49	49	48	41	45
Absent	1.2	3.9	4.5	1.0	2.7	6.5	6.3	6	4.2	6.3

(absent plantar reflexes, highly pathological nerve conduction studies, and high neuropathy impairment score), patients with rather discrete LFN (normal plantar reflex, almost normal nerve conduction studies, and low neuropathy impairment score), and patients with intermediate symptoms were found in type 1 and 2 diabetic subjects. All patients showed neuropathic pain symptoms such as burning, lancinating, or spontaneous pain restricted in the feet, but not in the thighs, suggesting small fiber damage (Table 1).

#### Current-induced neurogenic flare in normal subjects.

Electrical stimulation provoked an intensity-dependent neurogenic flare. Thresholds for induction of flares were 8 mA (6–12.5 mA) in the thighs and 10 mA (6–20 mA) in the feet. Flare sizes were larger in women (feet: women  $2.82 \pm 0.76 \text{ cm}^2$  and men  $1 \pm 0.13 \text{ cm}^2$ ,  $P < 0.03$ ; thighs: women  $7.8 \pm 1.25 \text{ cm}^2$  and men  $4.2 \pm 0.73 \text{ cm}^2$ ,  $P < 0.03$ ) and in the thighs ( $6.5 \pm 0.97 \text{ cm}^2$ ) compared with the feet ( $1.9 \pm$

$0.48 \text{ cm}^2$ ,  $P < 0.01$ ) in control subjects. Flux values (flare intensity) were neither sex dependent nor was there a significant difference between thighs and feet (feet: women  $291 \pm 26 \text{ PU}$  and men  $215 \pm 24 \text{ PU}$ ; thighs: women  $266 \pm 16 \text{ PU}$  and men  $239 \pm 27 \text{ PU}$ ). Flare sizes at feet and thighs were age dependent (ANCOVA,  $P < 0.03$ ). There was, however, no age dependence of flare intensity (flux). **Current-induced neurogenic flare in SFN subjects and NO-Ns.** Electrical stimulation elicited neurogenic flare in all patients (SFN subjects and NO-Ns). In SFN subjects, higher intensity of electrical stimulation was required to induce a flare reaction. Median threshold in the SFN group was 17.5 mA in both the thighs (8–20 mA) and feet (10–20 mA), and the differences compared with control subjects were significant ( $P < 0.001$ ). In the NO-Ns, flare commenced at 10 mA (6–15 mA) in the feet and 8 mA (6–12.5 mA) in the thighs. This was not significantly different from the control group.

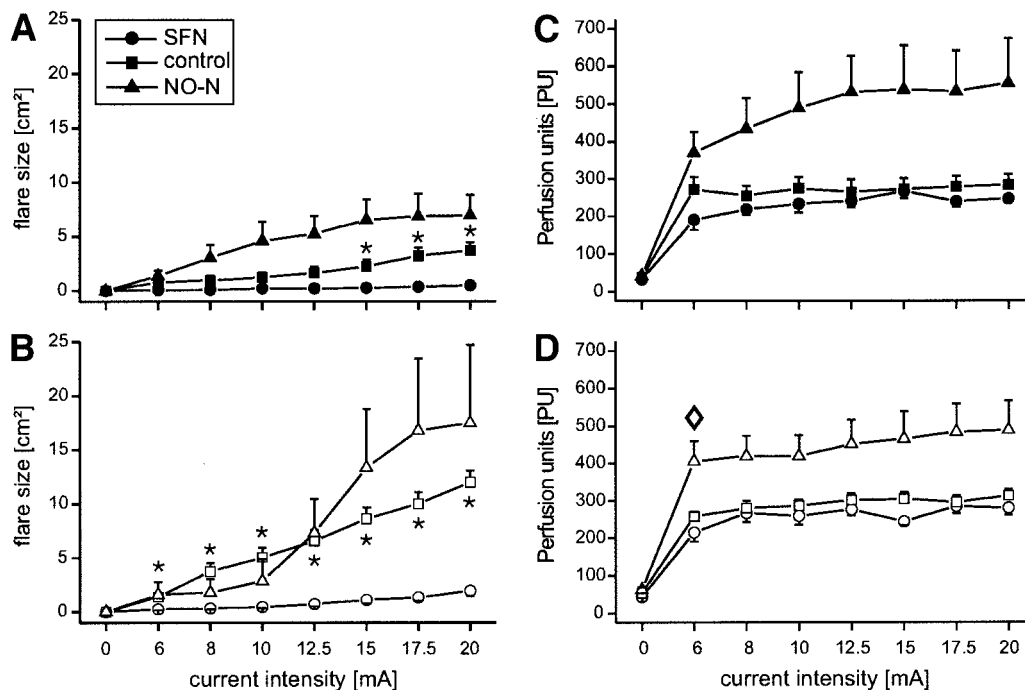


FIG. 1. Plotted are stimulus-response curves for the flare size at feet (A) (filled symbols) and thighs (B) (open symbols) and the respective flux values (feet, C, filled symbols; thighs, D, open symbols). A and B: Flare size was significantly reduced in SFN subjects (● and ○) and significantly enlarged in NO-Ns (▲ and △). Post hoc analysis of NO-N and control subjects revealed no significant differences. \*Significant differences as revealed by post hoc analysis between SFN and control subjects (■ and □) at each current intensity. C and D: Intensity of the flux within the flare did not differ between SFN and control subjects. There was, however, an increased flux in NO-Ns compared with control subjects. Post hoc analysis revealed a significant difference for 6-mA current intensity at the thigh (◇).



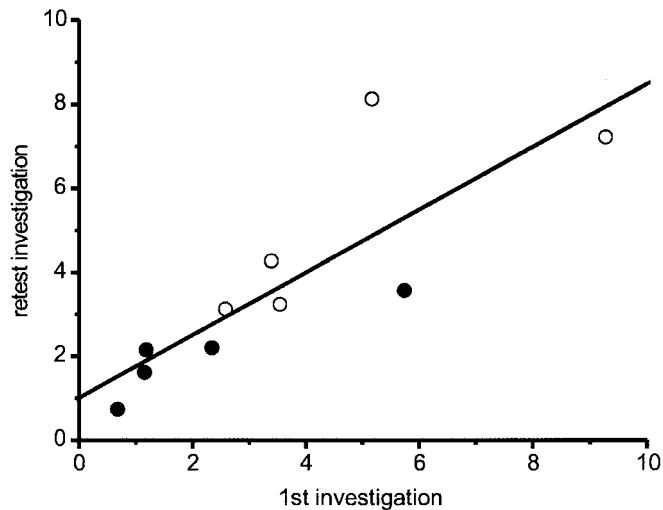


FIG. 2. Plotted are mean values of flare size of test (x-axis) and retest (y-axis) of five normal subjects in the feet (●) and thighs (○). The correlation between first and second investigation was significant ( $r = 0.83$ ,  $P < 0.004$ ). A separate analysis of the different test areas revealed a significant correlation in the feet ( $r = 0.91$ ,  $P < 0.05$ ). Correlation was weaker in the thighs ( $r = 0.74$ ), in which significance was not reached ( $P = 0.15$ ).

**Comparison between diabetic patients (SFN subjects and NO-Ns) and control subjects.** In the dorsal side of the feet, flare size was significantly reduced in SFN subjects (ANCOVA,  $P < 0.01$ ). Post hoc analysis revealed significant differences with current intensities  $\geq 15$  mA (Fig. 1A). In the thighs, flare size was also significantly smaller in SFN patients (ANCOVA,  $P < 0.001$ ). Post hoc analysis revealed significant differences at all stimulation intensities (Fig. 1B). For both stimulation sites, the differences between patients and control subjects became more obvious with increasing current. The flare intensity (flux) was not different between SFN subjects and control subjects in the feet or in the thighs.

Comparison between NO-Ns and control subjects revealed that flare size in the thighs was not different between patients and control subjects (ANCOVA) (Fig. 2B). However, flare size in the feet was even enlarged in NO-Ns (ANCOVA,  $P < 0.02$ ). Post hoc analysis was not able to allocate significant differences (Fig. 2A). The flare intensity (flux) in the thighs (ANCOVA,  $P < 0.001$ ) and in the feet (ANCOVA,  $P < 0.003$ ) was increased in the NO-N group compared with the control group. Post hoc analysis was again not able to allocate significant differences with one exception, flux in the thighs at 6 mA (Fig. 1C and D). **Current-induced pain.** Electrical stimulation was well tolerated and moderately painful. The pain rating raised with increasing current intensity. The pain ratings were slightly lower in the thighs (control subjects numeric rating scale  $3.2 \pm 0.5$ , SFN subjects  $3.3 \pm 0.5$ , and NO-Ns  $2.4 \pm 0.9$ ) than in the feet (control subjects  $3.5 \pm 0.7$ , SFN subjects  $3.9 \pm 0.5$ , and NO-N  $3.2 \pm 0.8$ ) but did not differ significantly between the different groups.

**Reproducibility of the current-induced neurogenic flare in normal subjects ( $n = 5$ ).** There was no difference between test and retest values regarding size and flux of the flare response (ANOVA, NS). Despite the limited number of subjects, we found a significant correlation between test and retest of the mean flare size (see RESEARCH

DESIGN AND METHODS) ( $r = 0.83$ ,  $P < 0.004$ ). Separate calculation revealed a significant correlation at the feet ( $r = 0.91$ ,  $P < 0.05$ ). With greater flare sizes at the thighs, values were also positively correlated but without reaching significance ( $r = 0.74$ ,  $P = 0.15$ ) (Fig. 2). Flux values at both feet and thighs were not significantly correlated.

## DISCUSSION

In the present study, we could demonstrate that the size but not the intensity of vasodilation of electrically induced axon reflex flare indicates C-fiber damage in patients with diabetic SFN. Reduction of flare size was not confined to the symptomatic feet, but was even more pronounced in the clinically unaffected thighs. Furthermore, our method not only detects C-fiber impairment but also detects increased flare size and intensity in NO-Ns, indicating increased release of neuropeptides, provided that peripheral C-fibers are intact.

**Which factors determine axon reflex flares in human skin.** Histamine flare, for example, is larger than an electrically-provoked flare (14), although histamine activates only a small proportion of afferent C-fibers. This is due to the huge receptive fields of histamine-sensitive C-fibers, which are a subgroup of mechanoinensitive C-fibers. These fibers determine the axon reflex flare and have high electrical thresholds (21,22). If we applied stimulus intensities of  $\sim 80$  mA (20), most of these mechanoinensitive fibers would be excited (23). In this case, the flare would become at least as large as after application of capsaicin (24). However, such high stimuli intensities cannot be used for routine clinical testing.

For clinical use, a standardized C-fiber stimulation protocol is essential. There are limitations of chemical stimulation (7). For instance, there is nonneurogenic vasodilation with acetylcholine or limited reproducibility with histamine due to seasonal variability (15). The electrical C-fiber stimulation has the advantage of intensity and frequency of current that can be well controlled. This standardized stimulation allows the detection of age dependence of axon reflexes, as it has been demonstrated for other neurophysiological parameters of peripheral nerve function.

The axon reflex flare further depends on the neurosecretory function of the terminals of activated primary afferent neurons (8). Our study confirms that flare sizes are larger in thighs compared with feet (14). As microneurographic data have shown, innervation territories of C-fiber neurons vary between body regions; mechanosensitive C-fibers show larger innervation territories in proximal body areas (25). The same may be the case for mechanoinensitive C-fibers, although a previous study was unable to identify differences between lower legs and feet (21). On the other hand, the number of epidermal C-fiber terminals in skin punch biopsies increases from distal to proximal body regions (26,27). This first glance contradiction may be explained by a higher number of nerve terminals branching off from a limited number of C-fiber neurons at proximal body regions (21,25). Alternatively, function of nerve fibers in distal body regions also might decline earlier under physiological conditions, as suggested by the age-related and length-dependent decrease of nerve conduction in healthy subjects (28).

### Which factors reduce axon reflex flares in diabetes.

Pain ratings did not differ between control subjects and SFN patients, suggesting that there was no major impairment of the central connections of afferent fibers. Different fiber classes contribute to electrically-induced pain because pain was described as short and stinging (A $\delta$ -fibers) with a delayed burning component (C-fibers) (29). In contrast to these proximally directed pain pathways, the distal efferent function of C-fibers was impaired not only in distal limbs but also in proximal skin areas. We plotted intensity-response relations and found that the thresholds for flare reaction were higher within the SFN group. A higher electrical threshold could be the result of different mechanisms; reduced epidermal nerve fiber density or increased electrical thresholds of single axons.

Nerve fiber reduction in diabetic skin leads to smaller axon reflex flares because fewer fibers are excited due to a statistically larger distance to the stimulation electrode and the reduced release of neuropeptides from the sparse axonal tree. If these neuropeptides (e.g., calcitonin gene-related peptide) remain below a critical level at the flare borders, the flare size shrinks (22). The overlap of innervation territories within the central flare makes flare intensity rather unchanged in SFN because there is a steep dose-response curve of calcitonin gene-related peptide-induced vasodilation.

In addition, diabetic neuropathy also directly increases electrical excitability by changes in ion channels distribution (calcium-activated potassium channels [30], and voltage-gated, Tetrodotoxin-resistant sodium channels [31]). Diabetic rat axons show a reduced inward rectification, hyperpolarization, and slower conduction velocity (32). In addition, there is evidence for smaller axonal diameters of unmyelinated fibers (33,34) in nerve biopsies from diabetic neuropathy patients. Smaller axonal diameters will further increase electrical thresholds. Interestingly, reduced C-fiber conduction velocities and increased electrical thresholds were recently demonstrated by microneurographic single fiber recordings in chronic pain states (35). Thus, our observation that electrical stimulation of higher intensity is required to provoke axon reflex flare in diabetic SFN patients might be a combined effect of axonal loss, changes of ion channel properties, and reduced axonal diameters. Irrespective of the relative contribution of single mechanisms, increased electrical thresholds of C-fibers will facilitate the differentiation between patients and control subjects.

Nonneuropathic impairment of skin perfusion, which can regularly be observed in diabetes, theoretically may also affect axon reflex vasodilation. However, reduction of skin perfusion will mainly reduce the intensity of the axon reflex flare, whereas flare size is based on the intact innervation causing the flare's low vulnerability to vascular impairment (36,37).

Our results were very different in NO-Ns. Figure 1 overestimates the differences, since the majority of our NO-Ns were women and young. However, statistical analysis was adjusted for sex and age and the differences remained. Enhanced neuropeptide release from C-fibers in diabetes has been demonstrated in recent rat experiments (38). Our results may not be caused by increased excitability of diabetic C-fibers because the current thresholds

for induction of the flare were unchanged and pain ratings were not different from control subjects. That is, the neuropeptide content of diabetic C-fibers must be increased. There is evidence that nerve growth factor (NGF) is upregulated in diabetes, possibly in response to minimal C-fiber damage (39). NGF, in turn, upregulates neuropeptides in intact axons, whereas depletion of NGF in damaged axons leads to neuropeptide loss (40,41). We found no clinical evidence for C-fiber impairment in NO-Ns, and therefore, we assume that NGF-induced increase of neuropeptides in intact axons overrides a possible decrease of neuropeptides in minimally lesioned axons. In SFN, however, axonal damage or depletion of neuropeptides predominates.

### Other methods to assess skin innervation in diabetes.

The noninvasive and painless quantitative sensory testing is a routine test for small fiber dysfunction. There is considerable variability of quantitative sensory testing results in neuropathy patients (42), and the data depend on active cooperation (43). Since the spatial extension of flare might be a surrogate of morphologic and functional changes of peripheral C-fibers and its independence of patients' cooperation, it should be less variable than quantitative sensory testing.

An alternative tool to assess skin fibers is skin biopsies. They provide histological data and can be regarded as the gold standard. However, skin biopsies are more invasive than electrical stimulation, and quantitative analysis is time consuming (44). In clinically advanced diabetic neuropathy, skin biopsies show dermal fiber loss. This fiber loss was not restricted to symptomatic skin area (45). That is, skin biopsies corroborate our functional results that clinically nonaffected skin areas already show structural and functional impairment of thin nerve fibers (27). On the other hand, in patients with very early neuropathy and impaired glucose tolerance (45), skin biopsies demonstrated slightly reduced nerve fiber density but there was simultaneously an increased distal fiber branching, which counteracts fiber loss (4,46). The trigger for distal sprouting of primary afferent fibers may be NGF, which might also contribute to increased neuropeptide release and flare intensity in our NO-Ns, as discussed above. These striking analogies of histological data and the results presented herein should be further investigated in a combined study comparing epidermal fiber density and axon reflex flare in the same patients.

In conclusion, our results indicate that analysis of electrically-induced flare size might be a valuable tool for assessment of small fiber dysfunction in peripheral neuropathies. It may even be helpful in other pain disorders with C-fiber pathology, such as erythromelalgia or postherpetic neuralgia. Our method is sensitive enough to detect impairment of efferent neurosecretory C-fiber function earlier than impairment of afferent sensory function. It remains to be evaluated whether the analysis of electrical thresholds provides an additional measure for possible neuropathic changes.

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