Differences in Foot and Forearm Skin Microcirculation in Diabetic Patients With and Without Neuropathy

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OBJECTIVE — We have compared the hyperemic response to heat and the endothelium-dependent and endothelium-independent vasodilatation between the dorsum of the foot and the forearm in diabetic neuropathic and non-neuropathic patients and healthy control subjects.

RESEARCH DESIGN AND METHODS — We studied the cutaneous microcirculation in the forearm and foot in 15 diabetic patients with neuropathy, in 14 diabetic patients without neuropathy, and in 15 control subjects matched for age, sex, BMI, and in the case of diabetic patients, for the duration of diabetes. Patients with peripheral vascular disease and/or renal impairment were excluded. The cutaneous microcirculation of the dorsum of the foot and the flexor aspect of the forearm was tested in all subjects. Single-point laser Doppler was employed to measure the maximal hyperemic response to heating of the skin to 44°C and laser Doppler imaging scanner was used to evaluate the response to iontophoresis of 1% acetylcholine chloride (Ach) (endothelium-dependent response) and 1% sodium nitroprusside (NaNP) (endothelium-independent response).

RESULTS — The transcutaneous oxygen tension was lower in the neuropathic group at both foot and forearm level, while the maximal hyperemic response to heat was similar at the foot and forearm level in all three groups. The endothelium-dependent vasodilatation (percent increase over baseline) was lower in the foot compared to the forearm in the neuropathic group (23 ± 4 vs. 55 ± 10 [mean ± SEM]; $P < 0.01$)], the non-neuropathic group (33 ± 6 vs. 88 ± 14; $P < 0.01$), and the control subjects (43 ± 6 vs. 93 ± 13; $P < 0.001$). Similar results were observed during the iontophoresis of NaNP ($P < 0.05$). No differences were found among the three groups when the ratio of the forearm:foot response was calculated for both the endothelium-dependent (neuropathic group, 2.25 ± 0.24; non-neuropathic group, 2.55 ± 0.35; and control subjects, 2.11 ± 0.26; P = NS) and endothelium-independent vasodilatation (neuropathic group, 1.54 ± 0.27; non-neuropathic group, 2.08 ± 0.33; and control subjects, 2.77 ± 1.03; P = NS). The vasodilatory response, which is related to the C nociceptive fiber action, was reduced at the foot level during iontophoresis of Ach in the neuropathic group. In contrast, no difference was found during the iontophoresis of NaNP at the foot and forearm level and of Ach at the forearm level among all three groups.

CONCLUSIONS — In healthy subjects, the endothelial-dependent and endothelial-independent vasodilatation is lower at the foot level when compared to the forearm, and a generalized impairment of the microcirculation in diabetic patients with neuropathy preserves this forearm-foot gradient. These changes may be a contributing factor for the early involvement of the foot with neuropathy when compared to the forearm.

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Abbreviations: Ach, acetylcholine chloride; CCPT, cutaneous perception threshold; NaNP, sodium nitroprusside; $TcPO_2$, transcutaneous oxygen tension; VPT, vibration perception threshold.

Although the exact etiology of diabetic neuropathy is not known, there is considerable evidence suggesting that impairment of the microcirculation is associated with the development of this condition (1,2). Impaired vasodilatation caused by decreased production of nitric oxide has been proposed as one of the early mechanisms that lead to nerve dysfunction (3,4). On the other hand, the development of neuropathy impairs the vasodilatation related to normally functioning C nociceptive fibers (Lewis' triple flare response), and renders the diabetic neuropathic foot unable to mount a hyperemic response during conditions of inflammation(5,6).

The two most widely accepted mechanisms that lead to the development of diabetic neuropathy are the intraneurial accumulation of sorbitol and changes to the nerve microvasculature that lead to endoneurial hypoxia (1,3). Increased lower-extremity capillary pressure upon assuming the erect posture, caused by early loss of postural vasoconstriction, has recently been proposed as an additional contributing factor (7). We therefore hypothesized that the vasodilatory response of the cutaneous microcirculation of the foot is reduced when compared to the forearm in diabetic patients with peripheral neuropathy. Furthermore, we theorized that the development of neuropathy will further impair the microcirculation caused by the involvement of the C nociceptive fibers. To test our hypothesis, we compared the hyperemic response to heat and the endothelium-dependent and endothelium-independent vasodilatation between the dorsum of the foot and the forearm in diabetic neuropathic and non-neuropathic patients and healthy control subjects.

RESEARCH DESIGN AND METHODS

Subjects

Three groups of subjects were studied. The first group included 15 diabetic patients with neuropathy, the second group included 14 diabetic patients without neuropathy, and the third group included 15 healthy nondi-

Table 1—*Characteristics of studied groups*

	Neuropathic group	Non-neuropathic group	Control subjects
n	15	14	15
M/F	9/6	8/6	8/7
Age (years)	54.9 (40–73)	48.9 (30–71)	47.6 (29–66)
BMI (kg/m²)	27.2 ± 1.3	27.4 ± 1.6	26.1 ± 0.9
Diabetes			
Type 1	5	4	—
Type 2	10	10	—
Diabetes duration (years)	18.9 (2–35)	18.3 (1–44)	—
Microalbuminuria	0	0	—
Macroalbuminuria	2	1	—
Smokers (past or current)	5	1	4

Data are means ± SEM, means (range), or *n*.

abetic subjects. As shown in Table 1, all groups were matched for age, sex, and BMI, and the two diabetic groups were matched for type and duration of diabetes and the existence of diabetes complications.

Exclusion criteria were as follows: *1*) patients with serious long-term complications of diabetes such as renal impairments (creatinine >180 μmol/l), 2) peripheral vascular disease (absent foot pulses and/or history of claudication), and *3*) any other chronic illness. The study was approved by the institutional review board of our institution, and all participants gave written informed consent.

Assessment of diabetic neuropathy

All participants underwent a thorough clinical examination, with particular emphasis on the existence of long-term complications of diabetes, such as retinopathy and nephropathy. Peripheral neuropathy was evaluated according to the guidelines of the San Antonio Consensus Statement (8). Neuropathic symptoms were evaluated by using a Neuropathy Symptom Score (NSS) and the clinical signs were assessed by using a Neuropathy Disability Score (NDS), as previously described (9).

Quantitative sensory testing included the assessment of vibration perception threshold (VPT) and cutaneous perception threshold (CCPT). The VPT was measured at the great toe on the dominant side of each patient using a Biothesiometer (Biomedical Instruments, Newbury, OH) (10). The age-related upper normal limits were derived from previously published data (10). A set of eight Semmes-Weinstein monofilaments was employed to evaluate the CCPT. The nylon monofilament was applied to the plantar surface of the great toe of both feet, and the smallest monofilament felt by the patient with his or her eyes closed was recorded (11). The peroneal motor nerve conduction velocity was measured using surface electrodes.

Assessment of microcirculation

All the following measurements were performed on the dorsum of the foot and the flexor aspect of the upper forearm. All participants were first acclimatized for 30 min in the examining room, which had a stable temperature of 21–22°C. The tests were performed with the subjects sitting in a reclining chair with the foot and forearm areas at the same level.

Transcutaneous oxygen tension. The transcutaneous oxygen tension ($TcPO_2$) measurements on the dorsum of the foot and forearm were made using a Microspan $TcPO_2$ meter (BCI International, Waukesha, WI). The electrode was left in place for 20 min, and a stable reading of >1 min was used for analysis (12).

Maximal hyperemic response to heat. The hyperemic response to heat was evaluated by using a single-point laser probe and a DRT4 Laser Doppler Blood Flow Monitor (Moor Instruments, Millwey, Devon, U.K.). After baseline blood flow measurements were made, the skin was heated to 44°C for 20 min using a small brass heater (Moor Instruments), following which the maximum blood flow was measured. The results were expressed in volts.

Laser Doppler iontophoresis. Endothelium-dependent vasodilatation in the cutaneous microcirculation was measured by the iontophoresis of acetylcholine chloride (Ach) and sodium nitroprusside (NaNP) was used to measure endothelium-independent vasodilatation (13). The iontophoresis instrument (MIC1 iontophoresis system [Moor Instruments]) consists of an iontophoresis delivery vehicle device that is attached firmly to the skin with a double-sided adhesive tape. The device contains two chambers that accommodate two single-point laser probes. One probe was used in the present study, the one that is placed outside but in close proximity (5 mm) to the iontophoresis solution chamber and measures the indirect response. The indirect vasodilatory response is due to stimulation of the C nociceptor fibers and measures the axon reflex–mediated vasodilatation. A small quantity (<1 ml) of 1% acetylcholine chloride solution or 1% of NaNP was placed in the iontophoresis chamber and a constant current of 200 μA was applied for 60 s, achieving a dose of 6 mC/cm^{-2} between the iontophoresis chamber and a second non-

Table 2—*Results of clinical examination*

	Neuropathic group	Non-neuropathic group	Control subjects	*P* value
Neuropathy Symptom Score	5.5 ± 1.1	0.4 ± 0.4	0.0 ± 0.0	<0.001*
Neuropathy Disability Score	20.1 ± 1.9	0.7 ± 0.5	0.0 ± 0.0	<0.001*
VPT (V)	48 ± 2	14 ± 2	14 ± 2	<0.001*
Semmes-Weinstein filaments	6.24 ± 0.27	3.6 ± 0.17	3.83 ± 0.16	<0.001*
Peroneal motor nerve conduction velocity (m/s)	31.7 ± 2.8	46.4 ± 2.6	47.4 ± 1.26	<0.001*
Foot transcutaneous oxygen tension (mmHg)	57 ± 3	70 ± 3	69 ± 4	<0.05*
Forearm transcutaneous oxygen tension (mmHg)	62 ± 3	71 ± 3	76 ± 4	<0.05†

Data are means ± SEM. **P* value for neuropathic group versus non-neuropathic group and control subjects. †*P* value for neuropathic group versus control subjects.

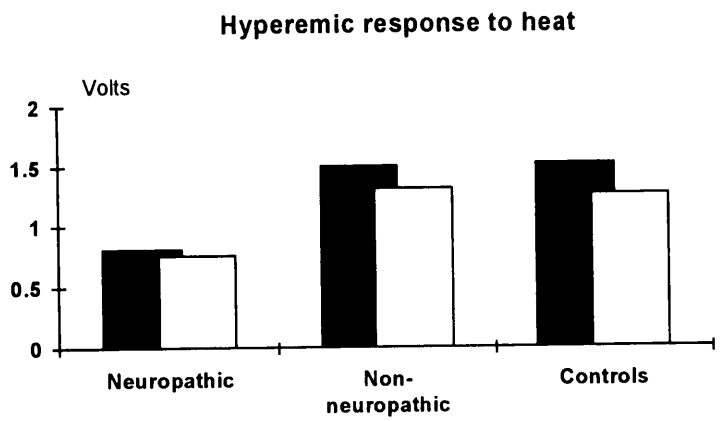

Figure 1—*The maximal hyperemic response to heat at the forearm (■) and foot (□). No difference was observed between the forearm and foot in any of the groups. The response in the neuropathic group was significantly lower compared with the other two groups at both the forearm and foot level ($P < 0.001$).*

active electrode placed 10–15 cm proximal to the chamber around the ankle or the wrist. This current caused a movement of the solution to be iontophoresed toward the skin and resulted in vasodilatation, which was recorded by the two laser probes.

We also measured the response to the iontophoresis of Ach and NaNP using a Laser Doppler Perfusion Imager (Lisca PIM 1.0, Lisca Development, Linkoping, Sweden) (14). This apparatus employs a 1-mW helium-neon laser beam of 633-nm wavelength, which sequentially scans an area of skin. The maximum number of measured spots is 4,096, and the apparatus produces a color-coded image of skin erythrocyte flux on a computer monitor. The scanner was set up to scan 32 × 32 measurement points over an area approximately 4 × 4 cm. Previous studies in our laboratory have shown satisfactory reproducibility rates with the coefficient of variation for the laser Doppler scanner evaluations being between 14 and 25%, while for the maximal response to heat it was 27.9% (13).

The single probe measurements were used for the assessment of the hyperemic response to heat and the axon reflex–mediated vasodilatation while the laser scanner was employed for the total iontophoresis. All laser measurements were expressed as volts and depended on the voltage difference created by the returned light to the computer. Thus, higher blood flow at the skin level resulted in a higher amount of light picked by the single probe or the scanner and, therefore, higher voltage recorded by the computer. In this way, volt measurements represent flux, which is a combination of the number and the speed or red cells traveling through the area of examination. All blood flow measurements in the present study, as in all other studies using laser technology, are expressed in volts, despite the fact that voltage units are not measurements of blood flow.

Statistical analysis

The Minitab statistical package (Minitab, State College, PA) for personal computers was used for the statistical analysis. The Kruskal-Wallis test was used for nonparametric and the analysis of variance (ANOVA) test was used for the parametric comparisons among the three groups. In case of statistical significance, the Fisher's analysis was

Table 3—*Results of iontophoresis*

	Neuropathic group	Non-neuropathic group	Control subjects	*P* value
Ach				
Baseline blood flow (V)				
Forearm	0.63 ± 0.03	0.75 ± 0.07	0.61 ± 0.04	NS
Foot	0.53 ± 0.03	0.68 ± 0.05	0.56 ± 0.03	<0.05*
After the iontophoresis (V)				
Forearm	0.98 ± 0.05	1.34 ± 0.11	1.14 ± 0.11	<0.05†
Foot	0.67 ± 0.04	0.90 ± 0.07	0.79 ± 0.04	<0.05†
Increase over baseline (%)				
Forearm	55 ± 10	88 ± 14	93 ± 13	<0.05*
Foot	23 ± 4	33 ± 6	43 ± 6	<0.05‡
NaNP				
Baseline blood flow (V)				
Forearm	0.65 ± 0.03	0.75 ± 0.6	0.62 ± 0.03	NS
Foot	0.56 ± 0.03	0.70 ± 0.04	0.60 ± 0.03	<0.05†
After the iontophoresis (V)				
Forearm	0.86 ± 0.03	1.2 ± 0.09	1.06 ± 0.06	<0.05§
Foot	0.69 ± 0.03	0.97 ± 0.08	0.85 ± 0.04	<0.05§
Increase over baseline (%)				
Forearm	37 ± 7	64 ± 7	75 ± 11	<0.05§
Foot	25 ± 4	39 ± 4	42 ± 5	<0.05§

Data are means ± SEM. *P value for non-neuropathic group versus neuropathic group and control subjects; †P value for non-neuropathic group versus neuropathic group; ‡P value for non-neuropathic group versus control subjects; §P value for neuropathic group versus non-neuropathic group and control subjects.

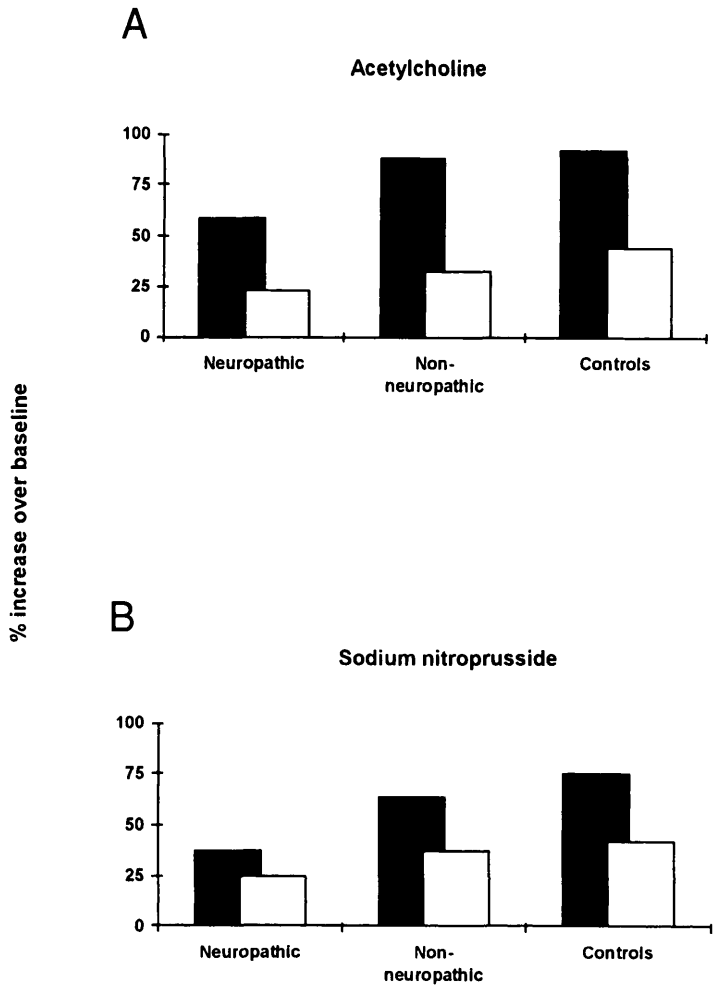

Figure 2—*The results of iontophoresis of Ach (endothelium-dependent) (A) and NaNP (endothelium-independent) (B) at the forearm (■) and foot (□). The response at the foot was significantly lower than that of the forearm in all three groups (P < 0.01). In addition, the responses at the foot and forearm level were lower in the neuropathic group when compared with the control subjects (P < 0.05).*

employed to identify the difference between individual groups. Results are mean ± SEM.

RESULTS — The results of the clinical examination are shown in Table 2. In summary, significant differences were observed in all neuropathy measurements between the neuropathic patients and the other two groups, while no differences were observed between the non-neuropathic patients and the control subjects. There was no evidence of clinical sensory neuropathy in the forearm skin area where the microcirculation was tested in any participant. Finally, the transcutaneous oxygen tension was lower in the neuropathic group at both foot and forearm level.

The maximal hyperemic response to heat was similar at the foot and forearm level in all three groups (Fig. 1). When comparisons were made among the three groups, the response in the neuropathic group was significantly reduced when compared with the non-neuropathic and control groups at both foot (neuropathic group 0.76 ± 0.09 V, non-neuropathic group 1.32 ± 0.20 V, and control subjects 1.26 ± 0.10 V; $P < 0.001$) and forearm level (neuropathic group 0.82 ± 0.08 V, non-neuropathic group 1.50 ± 0.17 V, and control subjects 1.52 ± 0.13; $P < 0.001$).

The baseline laser flux measurement at the foot level was lower than that of the forearm in the neuropathic group, but was similar in the non-neuropathic group and the control subjects (Table 3). When comparisons between the three groups were made, the endothelium-dependent and endothelium-independent vasodilatation was reduced in the neuropathic group when compared with the control subjects at both the foot and forearm level.

In contrast to the hyperemic response to heat, significant differences were found between foot and forearm level in both endothelium-dependent and endothelium-independent vasodilatation (Fig. 2). Thus, the percent increase in vasodilatation over baseline during the iontophoresis of Ach was lower in the foot compared to the forearm in the neuropathic group, the non-neuropathic group, and the control subjects. Similar results were observed during the iontophoresis of NaNP in the neuropathic group, the non-neuropathic group, and the control subjects.

No differences were found among the three groups when the ratio of the forearm:foot response was calculated for both the endothelium-dependent (Ach response; neuropathic group 2.25 ± 0.24, non-neuropathic group 2.55 ± 0.35, and control subjects 2.11 ± 0.26; P = NS) and endothelium-independent vasodilatation (NaNP response; neuropathic group 1.54 ± 0.27, non-neuropathic group 2.08 ± 0.33, and control subjects 2.77 ± 1.03; P = NS).

Nerve axon reflex–mediated vasodilatation, related to C nociceptive fibers, was reduced at the foot level during the iontophoresis of Ach, which directly stimulates these fibers, in the neuropathic group (12% increase over baseline ± 3.46) compared with the non-neuropathic group (73 ± 19) and the control subjects (212 ± 54), while a significant difference also existed between the last two groups ($P < 0.001$) (Fig. 3). In contrast, no difference was found with NaNP, which does not directly stimulate the C fibers (neuropathic group 21 ± 72, non-neuropathic group 46 ± 19, and control subjects 27 ± 11; P = NS). No differences were found at the forearm level among the three groups with both Ach (neuropathic group 269 ± 63, non-neuropathic group 267 ± 49, and control subjects 335 ± 81; P = NS) and NaNP (neuropathic group 73 ± 25, non-neuropathic group 47 ± 10, and control subjects 60 ± 14; P = NS).

Since the studied diabetic groups included both type 1 and 2 diabetic patients, we looked for possible differences between type 1 and 2 diabetes. No differences or trends were noticed in the iontophoresis or maximal response to heat results between type 1 and 2 diabetes. Finally, a strong correlation was found between baseline blood flow measurements before the iontophoresis of Ach and NaNP at both forearm ($r = 0.85$, $P < 0.0001$) and foot level ($r = 0.84$, $P < 0.0001$), indicative of the very satisfactory reproducibility of

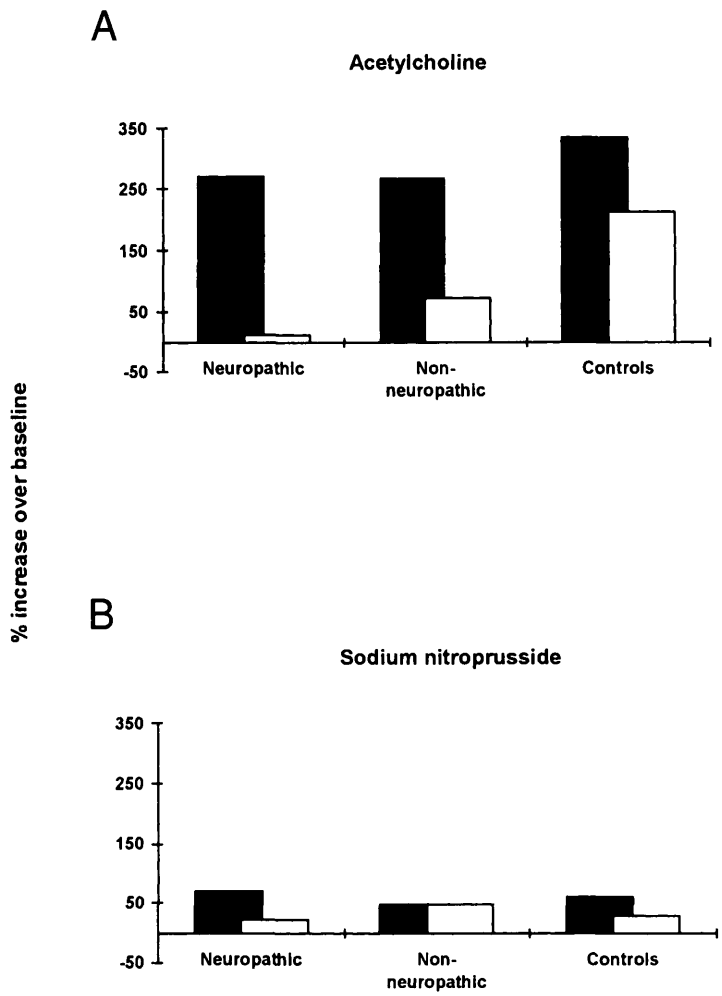

Figure 3—*The axon reflex–mediated vasodilatation related to the C nociceptive fiber. At the foot level (□), the response to Ach (A), which directly stimulates the C fibers, was lowest in the neuropathic group but was also reduced in the non-neuropathic group (P < 0.001), while no differences were found at the forearm level (■). A much smaller response was observed during the iontophoresis of NaNP (B) (a non-specific stimulus) at both foot and forearm level and was similar in all three groups.*

the laser scanner imaging technique.

CONCLUSIONS — The main finding of the present study is that endothelium-dependent and endothelium-independent cutaneous vasodilatation is lower in the foot when compared to the forearm in healthy subjects, and this difference persists in non-neuropathic and neuropathic diabetic patients. This difference existed despite a similar baseline blood flow at the foot and forearm level in control subjects and non-neuropathic diabetic patients, and a similar maximal hyperemic response at the foot and forearm level in all three groups. In addition, when compared with the control group, the microcirculation in the neuropathic diabetic patients was impaired at both the foot and forearm levels.

Impairment of the microcirculation is believed to play a prominent role in the development of neuropathy (1–4,15). High capillary pressures at the foot level, related to erect posture, have been proposed as contributors to the development of microangiopathy, which may be one of the reasons for neuropathy affecting the lower extremity more commonly than the forearm (9,16). Our results indicate that the vasodilatory response of the cutaneous microcirculation of the foot is lower than that of the forearm in healthy subjects and that it progressively worsens in diabetic patients, being lower in patients with than in those without neuropathy. The finding of microvascular impairment in areas not usually affected by neuropathy, such as the forearm, also indicates that the vascular changes are independent of neuropathic changes. Rather, it can be assumed that synergistic impairment of the foot microcirculation by both the erect pressure and diabetes is responsible for the development of neuropathy. Further studies that directly examine vascular changes in the peripheral nerves, rather than in the skin will be required to confirm this hypothesis.

Recent histological observations have shown no difference in the cutaneous capillary density in the foot and forearm in both healthy control subjects and diabetic patients, and previous studies have shown no evidence of increased microvascular occlusion in the lower extremity of diabetic patients(17,18). Furthermore, noninvasive measurements of the skin capillary density in the upper extremity have shown no differences between diabetic patients and healthy subjects (19). Early reduction in capillary size and thickening of basement membrane of the foot capillaries are the only observed histological changes (20,21). Our finding that no difference existed between forearm and foot baseline blood flow measurements and maximal hyperemic response to heat, which causes vasodilatation independent of the Lewis' flare response (22), is in agreement with these studies and indicates that functional changes are responsible for the observed results.

We have previously shown that the microvascular function of the foot is impaired in diabetic neuropathic patients with or without peripheral vascular disease, while other investigators have shown a similar impairment in the forearm of type 2 diabetic patients (23,24). In the present study, despite the existence of a consistent trend, no significant differences were observed in the non-neuropathic patients when compared with healthy control subjects, either at the foot or the hand level. However, it should be remembered that the study was designed mainly to have adequate statistical power to asses the differences between the foot and forearm in the same group. Therefore, the possibility that a difference between non-neuropathic patients and healthy control subjects was missed because of the small number of subjects cannot be excluded, and this point should not be considered as one of the primary end points of the present study.

The baseline blood flow measurements were consistently higher in the non-neuropathic group and reached statistical significance at the foot level. This was also an unexpected finding, and a statistical significance would have probably been reached for the forearm level had more subjects been

studied. Further studies are required to examine how this increase in blood flow is related to the absence of serious long-term diabetes complications in these patients despite their long diabetes duration.

Impairment of the C nociceptive fibers can lead to an impaired Lewis' reaction in the neuropathic foot in diabetes, rendering it unable to mount a hyperemic response under conditions of inflammation (6). Our results in the neuropathic group are in agreement with this hypothesis, while the finding of a less severe impairment in the non-neuropathic patients indicate that subclinical neuropathy may have been present in these patients. The fact that the response to Ach (which specifically stimulates the C fibers) was considerably higher than that of NaNP (a nonspecific stimulator of the C fibers that causes a response related to the constant current circulation) is in agreement with previous studies and validates this technique as a reliable method (25). It is also of interest that no differences were found at the forearm level, proving that neither clinical nor subclinical neuropathy was present at the examined area.

Epidermal thickness has been demonstrated to be higher at the foot level compared to the forearm in both diabetic and healthy subjects (17). This may be responsible for the small differences observed in the foot and forearm measurements. However, these differences were equally present in the baseline and post-iontophoresis measurements and were proportionally much lower when compared with the differences of the percent of increase over baseline. Therefore, we believe that epidermal thickness cannot be considered as the major factor for the observed differences between foot and forearm microcirculation.

In summary, our results suggest that in healthy subjects, the endothelium-dependent and endothelium-independent vasodilatation is lower in the foot when compared to the forearm and that a generalized impairment of the microcirculation in diabetic patients with neuropathy preserves this forearm-foot gradient. These changes may be one of the reasons for diabetic neuropathy affecting the lower extremity first, as compared with the upper extremity.

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