Relationships Between Type 2 Diabetes, Neuropathy, and Microvascular Dysfunction: Evidence From Patients With Cryptogenic Axonal Polyneuropathy

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OBJECTIVE

This study investigated whether the relationship between neuropathy and microvascular dysfunction in patients with type 2 diabetes is independent of diabetes-related factors. For this purpose, we compared skin microvascular function in patients with type 2 diabetes with that of patients with cryptogenic axonal polyneuropathy (CAP), a polyneuropathy of unknown etiology.

RESEARCH DESIGN AND METHODS

Cross-sectional information was collected from 16 healthy controls (HCs), 16 patients with CAP, 15 patients with type 2 diabetes with polyneuropathy (DPN), and 11 patients with type 2 diabetes without polyneuropathy. Axonal degeneration was assessed with skin biopsy and nerve conduction studies. Microvascular skin vasodilation was measured using laser Doppler fluxmetry combined with iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP).

RESULTS

Patients with CAP and DPN demonstrated a similar decrease in intraepidermal nerve fiber density and sural sensory nerve action potential compared with HCs. The vasodilator response to ACh was similar among patients with CAP (relative mean difference based on log values 13.3%; 95%CI −35.0–97.7%; *P* = 0.652) but was lower in the patients with diabetes with neuropathy (157.5%; 42.0–366.7%; *P* = 0.003) and without neuropathy (174.2%; 44.2–421.3%; *P* = 0.003) compared with HCs. No significant differences were found between the groups of patients with diabetes (*P* = 0.845). The vasodilator response to SNP was not significantly different among the groups (*P* = 0.082).

CONCLUSIONS

In this study, endothelium-dependent vasodilation was reduced in patients with type 2 diabetes regardless of the presence of polyneuropathy, whereas microvascular vasodilation was normal in patients with CAP. These data suggest that in type 2 diabetes, neuropathy does not contribute to impaired microvascular endothelium-dependent vasodilation and vice versa. In addition, this study suggests that impaired microvascular vasodilation does not contribute to CAP.

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Type 2 diabetes is associated with both generalized microvascular dysfunction (1) and peripheral nerve damage throughout the body (2). Several studies have shown that the presence of polyneuropathy in patients with diabetes is related to deterioration of microvascular endothelium-dependent and -independent vasodilation in the skin (3–5). Although the cross-sectional nature of these observations makes it impossible to prove causality, a bidirectional causal relationship between polyneuropathy and skin microvascular dysfunction has often been suggested. Specifically, a vicious cycle has been proposed in which microvascular disturbances lead to peripheral nerve ischemia, which in turn aggravates microvascular dysfunction (6).

However, baseline differences in hyperglycemic parameters—for example, more advanced or less controlled type 2 diabetes—could also explain the association between neuropathy and microvascular dysfunction. Indeed, studies examining this association are often hampered by group differences in baseline characteristics, such as duration of diabetes and HbA_{1c} (4,5), as well as age (3,4) and BMI (4). Importantly, these parameters are all individually associated with the incidence of neuropathy (7,8) and the severity of microvascular dysfunction (9,10).

At present, therefore, the nature of the association between polyneuropathy and skin microvascular dysfunction, specifically endothelium-dependent and -independent vasodilatation, in type 2 diabetes is not clear. We hypothesize that there is no direct causal relationship between neuropathy and microvascular endothelium-dependent vasodilation, but that this relationship can be explained by the confounding effects of diabetes-related factors such as hyperglycemia or metabolic syndrome characteristics. To this end, we assessed endothelium-dependent and -independent vasodilatation in the skin of patients with cryptogenic axonal polyneuropathy (CAP) and compared it with vasodilator responses in patients with type 2 diabetes with and without neuropathy. CAP is a common, length-dependent axonal polyneuropathy (11) that is clinically comparable to diabetic polyneuropathy but without an identifiable cause. Interestingly, peripheral microvascular function has not been assessed before in patients with CAP, even though CAP is associated with risk factors of (micro)vascular diseases such as the metabolic syndrome. This study therefore also explores the presence of skin microvascular dysfunction in CAP.

RESEARCH DESIGN AND METHODS

This study was approved by the medical ethics committee of the Leiden University Medical Centre, Leiden, the Netherlands (NL46921.058.13) and was performed in accordance with the Declaration of Helsinki of 2013 (12). All participants signed a written informed consent form. The study was performed at the Centre for Human Drug Research in Leiden and at the VU University Medical Center in Amsterdam.

Subjects

A total of 64 subjects were included in the study, divided among 4 groups: 16 healthy controls (HCs), 16 patients with CAP (CAP group), 16 patients with diabetes without neuropathy (DM group), and 16 patients with diabetes and neuropathy (DPN group). We excluded all patients with type 1 diabetes ($n = 6$) after exploring their demographics; they were not equally divided among the two groups with diabetes and had a large influence on baseline characteristics (especially age and BMI). In this article we present data from the 26 remaining patients with type 2 diabetes: 11 patients with type 2 diabetes without polyneuropathy, and 15 patients with type 2 diabetes and polyneuropathy.

We included adult subjects aged 18–80 years with a BMI between 18 and 32 kg/m^2. Patients in the DM group had a clinical diagnosis of diabetes without overt neuropathy, defined as a questionnaire score below 4 and a clinical exam score below 3 on the Michigan Neuropathy Screening Instrument (13). Patients in the DPN group had a clinical diagnosis of diabetes, and the diagnosis of diabetic polyneuropathy was made by a neurologist based on the medical history, signs and symptoms, and findings upon clinical examination. All patients with CAP had a diagnosis of CAP confirmed by a neurologist based on signs and symptoms, clinical examination, and an abnormal electromyograph suggestive of axonal polyneuropathy, in combination with the absence of an identifiable underlying etiology (e.g., negative family history, no diabetes, no vitamin deficiency). In both neuropathic groups, all patients were required to have neuropathic pain with an average pain score above 4 (on a scale from 0 to 10) to maximize the likelihood of involvement of small nerve fibers. This inclusion criterion served to increase homogeneity in and between the two neuropathic groups.

In these four groups we assessed axonal damage of small and large nerve fibers to ensure that the clinical signs and symptoms of polyneuropathy translated to objective measurements. In addition, we examined skin microvascular function using laser Doppler fluxmetry and iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). The skin microvascular vasodilatory response (i.e., the relative change from baseline) was designated as our primary outcome measurement. All research methods are described below in further detail.

Assessment of Neuropathy

Axonal degeneration was assessed with skin biopsy and nerve conduction studies for small-fiber and large-fiber involvement, respectively. Skin biopsies were taken 10 cm above the lateral malleolus under local anesthesia (2% lidocaine/adrenalin). A 3-mm disposable circular punch was used to obtain the skin biopsy, which contained epidermis and a small amount of underlying fatty tissue. The biopsy section was then fixed in paraformaldehyde-lysine-periodate at 4°C for 18–24 h. The biopsy sections were subsequently stained with PGP9.5, and the number of intraepidermal nerve fibers (IENFs; fiber density) was assessed. To evaluate large-fiber function, standardized nerve conduction studies were performed using a Synergy EMG system (Natus Medical Inc.). The sensory nerve action potential (SNAP) amplitude of the sural nerve was chosen as a single measurement because it is most specific for a diagnosis of axonal polyneuropathy (14–16). The Neuropathy Impairment Score–Lower Limbs (NIS-LL) was used as a clinical variable. This score is based on a combination of parameters derived from the physical examination: measurements of reflexes and motor and sensory nerve function (17).

Assessment of Microvascular Function

The microcirculatory bed of the skin is easily accessible and therefore commonly

used to measure microvascular function (18). A common noninvasive method to measure skin blood flow is laser Doppler fluxmetry, which measures the Doppler shift of moving red blood cells. In combination with iontophoresis of vasoactive substances, this method can be used to assess the vasoreactivity of skin microcirculation. All subjects were evaluated after an acclimatization period of approximately 20 min in a temperature-controlled room. Skin blood flow at the dorsum of the foot was assessed using laser Doppler fluxmetry (PeriFlux System 5000; Perimed AB, Järfälla, Sweden) while the subjects were in the supine position. Microvascular endothelium-(in)dependent vasodilatation was evaluated using iontophoresis (PeriIont 382 Power Supply; Perimed) of 1% ACh and 1% SNP, respectively, according to a previously reported protocol (19). The measurements were always performed in the same order: ACh before SNP. All tests were performed when skin temperature was above 28°C. The foot was heated by means of blankets and warm water if skin temperature did not rise above 28°C on its own; measurements were started when the skin temperature reached 28°C. Blood flow measurements were excluded before analysis if they appeared to be technically incorrect, which was defined as no response to iontophoresis at all and/or a high backscatter value. Technically correct measurements provided data on baseline perfusion, perfusion plateau after iontophoresis, and the relative percentage change from baseline, which was calculated as 100% × ([Perfusion plateau − Baseline flow]/Baseline flow). We previously demonstrated in nine healthy volunteers that the intrasubject coefficients of variation of ACh-dependent and SNP-dependent vasodilation (i.e., relative change from baseline) were 9.8 ± 5.6% and 8.3 ± 5.4%, respectively (20).

Statistical Analysis

SAS 9.4 for Windows was used for statistical analysis. Data are presented as mean ± SD, or median and range when the distribution was not normal, as indicated in the table footnotes and figure legends. In the case of nonnormality (SNAP, vasodilatory responses to ACh and SNP), data were log-transformed before analysis, and the mean difference is expressed as a percentage. All differences among the groups were tested using one-way ANOVA. Statistical analyses were considered significant when $P < 0.05$. In the case of significance, within the ANOVA test, pairwise comparisons were used between all groups to identify which group differences were statistically significant.

RESULTS

Characteristics

The subject characteristics are summarized in Table 1. We did not find any clinically relevant differences in age and blood pressure between the four study groups. The patients with diabetes with and without neuropathy did not show clinically relevant differences in BMI or duration of diabetes. In line with the definition of the groups, HbA_{1c} was higher in both groups with diabetes. In addition, as expected, BMI was higher in both groups with diabetes compared with patients with CAP and HCs. The patients with diabetes without neuropathy had better glycemic control than the patients with diabetes and neuropathy.

Neuropathy Measurements

The results of the skin biopsy and nerve conduction studies are shown in Table 1 and Fig. 1. Compared with the HCs, both the patients with CAP (mean difference 2.4 epidermal nerve fibers/mm; 95% CI 0.9–3.9; $P = 0.003$) and those with diabetic neuropathy (mean difference 1.8 epidermal nerve fibers/mm; 95% CI 0.3–3.3; $P = 0.020$) demonstrated a decreased number of IENFs. Furthermore, compared with HCs, all patient groups showed significant reductions in sural nerve SNAP (DM group: relative mean difference based on log values 146.7% [95% CI 2.1–495.9%], $P = 0.045$; DPN group: relative mean difference based on log values 325.5% [95% CI 89.4–855.7%], $P < 0.001$; CAP group: relative mean difference based on log values 370.6% [95% CI 112.3–943.2%], $P < 0.001$). Compared with the HCs, both the CAP and DPN groups showed an increased NIS-LL (CAP group: mean difference 16.9 [95% CI 20.7–13.1], $P < 0.0001$; DPN group: mean difference 8.8 [95% CI 12.6 – 4.9], $P < 0.0001$), whereas no differences were detected between HCs and the DM group. The increase in NIS-LL was more pronounced in the patients in the CAP group compared with those in the DPN group (mean difference 8.1 [95% CI 4.2–11.9]; $P < 0.001$).

Microvascular Function Tests

The results of microvascular function tests are shown in Table 2 and Fig. 2. Compared with HCs, the blood flow response to ACh (i.e., the percentage change from baseline) in patients with CAP was not significantly different (relative mean difference based on log values 13.3% [95% CI −35.0–97.7%]; $P = 0.652$), whereas the vasodilatory response to ACh was significantly reduced in both groups with diabetes compared with HCs (DM group: relative mean difference based on log values 174.2% [95% CI 44.2–421.3%], $P = 0.003$; DPN group: relative mean difference based on log values 157.5% [95% CI 42.0–366.7%], $P = 0.003$). There were no significant differences in the blood flow response to SNP ($P = 0.082$). The reduction in ACh-mediated vasodilatation in patients with type 2 diabetes was accompanied by a significantly higher blood flow at baseline in both patients with diabetes without neuropathy (mean difference 5.1 perfusion units [PU] [95% CI 9.4–0.7]; $P = 0.025$) and those with neuropathy (mean difference 5.4 PU [95% CI 9.4–1.3]; $P = 0.010$) compared with HCs.

CONCLUSIONS

Our findings show that patients with CAP demonstrate normal endothelium-dependent and endothelium-independent skin microvascular reactivity. In addition, irrespective of the presence of polyneuropathy, patients with type 2 diabetes show decreased endothelium-dependent vasodilation of skin microvessels. These data suggest that polyneuropathy does not contribute to impaired microvascular endothelium-dependent skin vasodilation in type 2 diabetes and that microvascular dysfunction does not contribute to neuropathy in CAP.

The finding of reduced endothelium-dependent (ACh-mediated) vasodilation in patients with diabetes is in line with previous observations of stimulated hyperemia in the skin (3,21). Our data also suggest a trend toward reduced endothelium-independent (SNP-mediated) vasodilation in patients with diabetes. Whereas the existence of vascular smooth muscle dysfunction in type 2 diabetes is much more a matter of debate than that of endothelial dysfunction, a recent meta-analysis (22) suggested a moderate to large impairment of vascular smooth muscle function, which is

Table 1—Characteristics of study subjects

	HC (n = 16)	CAP (n = 16)	DM (n = 11)	DPN (n = 15)	P value (ANOVA)
Sex					NA
Male	9	12	9	9	
Female	7	4	2	6	
Age (years)	61.8 ± 12.4	64.5 ± 7.8	64.1 ± 6.6	63.8 ± 12.6	NA
BMI (kg/m^2)	23.9 ± 2.3	26.5 ± 3.2	28.2 ± 2.2	28.2 ± 3.5	NA
HbA_{1c}, % (mmol/mol)	5.4 ± 0.2 (36.0 ± 2.0)	5.6 ± 0.3 (37.1 ± 3.5)	7.1 ± 1.1 (54.1 ± 11.8)	7.9 ± 1.7 (62.5 ± 18.2)	NA
Smoker (n)	4	3	3	3	NA
Total cholesterol (mmol/L)	5.7 ± 1.2	5.5 ± 1.2	4.3 ± 1.3	4.8 ± 0.9	NA
Triglycerides (mmol/L)	1.2 ± 0.3	1.7 ± 1.1	1.7 ± 1.0	1.9 ± 1.0	NA
Blood pressure (mmHg)					NA
Systolic	140 ± 20	143 ± 17	140 ± 20	149 ± 14	
Diastolic	82 ± 11	83 ± 11	76 ± 11	78 ± 8	
Duration of diabetes (years)	—	—	12.5 ± 9.8	12.1 ± 8.2	NA
Insulin therapy (n)	0	0	4	6	NA
Metformin (n)	0	0	4	6	NA
SU-derivatives (n)	0	0	9	12	NA
DPP-4 inhibitors (n)	0	0	0	2	NA
Antihypertensive therapy (n)	1	6	5	13	NA
Calcium channel blockers	0	0	1	7	NA
Diuretics	1	5	2	4	NA
β-Blockers	0	4	0	2	NA
Angiotensin II inhibitors	0	1	2	5	NA
ACE inhibitors	0	1	3	7	NA
Statin therapy (n)	0	2	8	10	NA
Intra-ENF density (IENs/mm^2)	5.7 ± 2.3	3.3 ± 2.3*	4.9 ± 2.2	3.9 ± 1.5*	0.014
Sural nerve amplitude (μV)	8.9 (2.8–30.0)	2.5 (2.5–10.7)*	5.3 (0.5–25.0)*	3.0 (0.5–11.0)*	<0.001
Neuropathy Impairment Score–Lower Limbs	0.7 ± 1.4	17.6 ± 9.0*	0.9 ± 1.4†	9.5 ± 4.4*†‡	<0.0001

Data are mean ± SD or median (range), unless otherwise indicated. DPP-4, dipeptidyl peptidase 4; IEN, intraepithelial nerve; NA, not applicable; SU, sulphonylurea. *Significant difference compared with HCs. †Significant difference compared with subjects with CAP. ‡Significant difference compared with patients with diabetes without neuropathy.

most prominent in the microcirculation. The absence of a statistically significant difference among the groups in our data set may well be the result of a type 2 error caused by the larger variation in SNP-mediated skin vasodilation, as postulated by others (5). Hence, we suggest that impairments in smooth muscle responsiveness to nitric oxide are most likely present in our patients with type 2 diabetes. Since endothelium-dependent vasodilation depends, at least in part, on vascular smooth muscle function, our data therefore do not differentiate between impaired endothelial or impaired smooth muscle function.

Interestingly, we were unable to detect any difference between the neuropathic and nonneuropathic patients with type 2 diabetes in both ACh- and SNP-mediated vasodilation. When interpreting these data, we should realize that, although our patients in the DM group do not show clinical evidence of neuropathy, they do demonstrate a significant reduction in sural nerve amplitude compared with the HCs. This indicates the presence of some axonal damage in the nonneuropathic patients with diabetes as well. This is not unexpected; neuropathy in patients with diabetes is more a disease continuum rather than a discrete entity. In spite of these findings in the nonneuropathic patients with diabetes, it is clear that our neuropathic patients with diabetes have more axonal damage. They demonstrate a reduction in nerve fiber density and a more pronounced decrease in sural nerve amplitude. Furthermore, a distinct clinical contrast between the neuropathic and nonneuropathic patients is indicated by the NIS-LL. Despite these differences in markers of axonal damage and clinical symptoms, we did not find any differences in microvascular function between the two groups with diabetes, an indication that the perceived relationship between neuropathy and endothelium-dependent and endothelium-independent microvascular dysfunction is most likely the result of parallel damage to nerves and microvessels by diabetes-related factors. As mentioned at the beginning of this article, our results contrast with some other studies (4–6), but not all (21). We suggest that differences between neuropathic and nonneuropathic patients with diabetes previously demonstrated in other studies are most likely a result of patient selection: patients with diabetes with neuropathy are often older, more obese, and exposed to diabetes for a longer period than patients with diabetes without neuropathy. Most studies do not correct for those baseline differences. Although our small sample size did not allow for corrections for

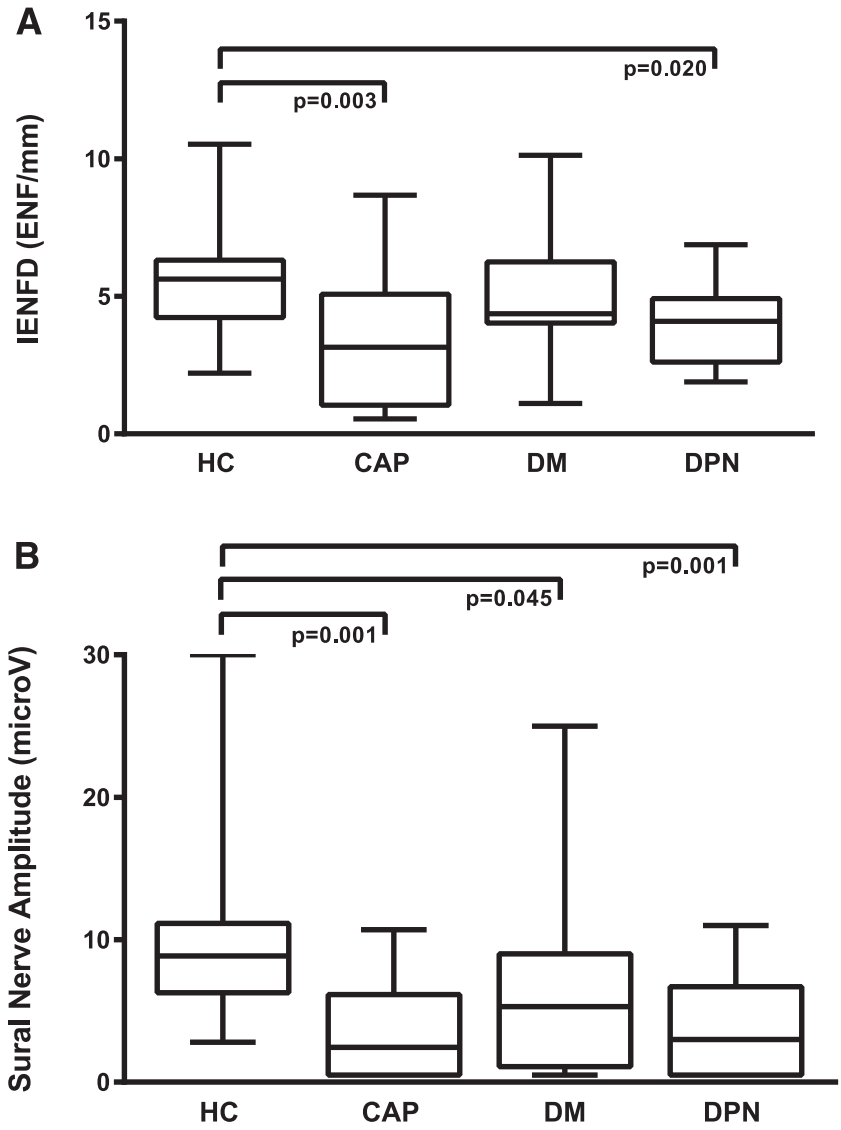

Figure 1—Measurements of axonal damage. *A*: Intra-ENF density (IENFD). Data are mean ± SD (P = 0.014, one-way ANOVA). Brackets indicate significant differences between the groups. *B*: SNAP of the sural nerve. Data are mean ± SD ($P < 0.001$, one-way ANOVA). Brackets indicate significant differences between the groups.

baseline differences, most baseline characteristics, such as duration of diabetes, age, and BMI, were equal among the two groups with diabetes, except for glycemic regulation, which was worse in patients with diabetes and neuropathy.

In our study, baseline blood flow measurements before iontophoresis are higher in subjects with diabetes with and without neuropathy compared with HCs. Data on baseline blood flow in patients with type 2 diabetes are inconsistent: increased (23,24), similar (25,26), and reduced (27,28) blood flow at baseline have all been reported. It is important to realize that baseline skin blood flow measurement using laser Doppler fluxmetry is subject to large spatial and temporal variations (29). This is probably because of large heterogeneity in skin perfusion and the relatively small sample area of the single-fiber laser Doppler probe. Laser Doppler fluxmetry data on baseline blood flow has a large day-to-day coefficient of variation (30), and vasoreactivity can thus be best defined as the relative change from baseline. The increased blood flow at baseline in patients with type 2 diabetes could partly explain the diminished microvascular responsiveness. Higher blood flow at baseline may result in a reduced capacity to respond to vasodilator substances. Both groups with diabetes also demonstrated lower, although nonsignificant, plateau perfusion after ACh iontophoresis, which also contributes to the diminished microvascular responsiveness.

Our findings that patients with CAP do not demonstrate peripheral skin microvascular dysfunction despite a slightly higher BMI, age, and HbA_{1c} are of interest since the etiology of CAP is still under debate. It is currently thought that several mechanisms can lead to a similar neuropathic phenotype; impaired glucose tolerance, dyslipidemia, hypertension, obesity, and increased oxidative stress have all been identified as potential risk factors (31). Our data suggest that generalized microvascular dysfunction is not an additional risk factor or mechanistic explanation that links features of the metabolic syndrome to axonal damage, as was suggested by Visser et al. (11) and Teunissen et al. (32). However, our data do not per se represent local microvascular function of endoneural and epineural vessels, and structural damage to the microcirculation cannot be excluded based on our study.

A few limitations of our study should be considered. The measurements performed in this study were secondary measurements of a larger trial that focused on the development and validation of noninvasive measures of small-fiber density and function in patients with polyneuropathy. The sample size was therefore determined based on a power calculation of this primary research question, not the microvascular data. However, the difference that we found between patients with diabetes versus HCs and patients with CAP is similar to an observation we made in the past. We repeatedly demonstrated a reduction in ACh-mediated vasodilatory response of approximately 300 to 400% points in several disease entities: hypertension (33), chronic kidney disease (34), and systemic sclerosis (35). We defined such a reduction as clinically relevant. A post hoc power analysis based on the difference that was demonstrated in our study between HCs (788 ± 649) and patients with diabetes (221 ± 76), with an α of 0.05, results in a power of 85.1%. We thus conclude that we had enough power to identify a clinically relevant difference.

The small sample size was also a limiting factor in correcting for confounding factors such as sex, age, blood pressure,

Table 2—Results of iontophoresis

	HC	CAP	DM	DPN	*P* value (ANOVA)
ACh					
Patients (*n*)	12	16	9	12	
Skin temperature (°C)	29.9 ± 1.8	30.7 ± 1.5	31.2 ± 1.4	30.4 ± 1.6	0.228
Baseline blood flow (PU)	5.1 ± 2.1	8.2 ± 4.3	10.2 ± 4.5*	10.5 ± 7.4*	0.044
Perfusion plateau (PU)	40.3 ± 23.9	47.6 ± 19.1	34.1 ± 16.0	33.0 ± 17.0	0.202
Relative change in PU after ACh (%)	687.8 (110.5–2155.1)	517.6 (172.1–1438.8)	233.6 (53.0–305.2)*†	209.7 (64.9–589.8)*†	0.001
SNP					
Patients (*n*)	10	15	8	(a) 7	
Skin temperature (°C)	29.4 ± 1.8	30.9 ± 1.7	30.8 ± 1.5	30.6 ± 1.9	0.063
Baseline blood flow (PU)	5.5 ± 2.4	10.1 ± 3.9	15.2 ± 12.5*	7.5 ± 2.3‡	0.016
Perfusion plateau (PU)	32.2 ± 14.5	68.6 ± 43.2*	32.6 ± 12.2†	36.1 ± 23.1†	0.010
Relative change in PU after SNP (%)	648.5 (126.9–1097.0)	665.3 (134.7–1090.3)	201.3 (110.4–665.2)	406.1 (66.03–871.4)	0.082

Data are mean ± SD or median (range), unless otherwise indicated. *Significant difference compared with HCs. †Significant difference compared with subjects with CAP. ‡Significant difference compared with patients with diabetes without neuropathy.

cholesterol profile, smoking, and medication use. Importantly, most of these factors were comparable among the four groups. However, the four groups are unbalanced when it comes to sex distribution and medication use. To our knowledge, only a few studies have investigated the effect of sex on ACh- and SNP-mediated vasodilation, and these studies demonstrated no significant differences between male and female participants (36,37). We therefore have no indication that sex is an effect modulator. Furthermore, it was previously demonstrated that statin therapy does not influence ACh- and SNP-stimulated vasodilation in patients with type 2 diabetes (38,39). Moreover, insulin, although vasoactive in lean, insulin-sensitive individuals, does not increase skin microvascular blood flow in obese, insulin-resistant individuals when measured by laser Doppler fluxmetry (40). However, antihypertensive medication, mainly ACE inhibitors and angiotensin receptor blockers, may positively influence microvascular endothelial function (41). It was demonstrated that the use of antihypertensive medication restores nitric oxide–mediated vasodilation toward normal in subjects with hypertension (42). However, it should be remembered that we were a priori not interested in microvascular dysfunction caused by (untreated) hypertension. On the contrary, we aimed to identify the influence of neuropathy on microvascular function, independent of confounding factors such as hypertension. The use of antihypertensive medication thus reduces the confounding effect of hypertension itself on microvascular function.

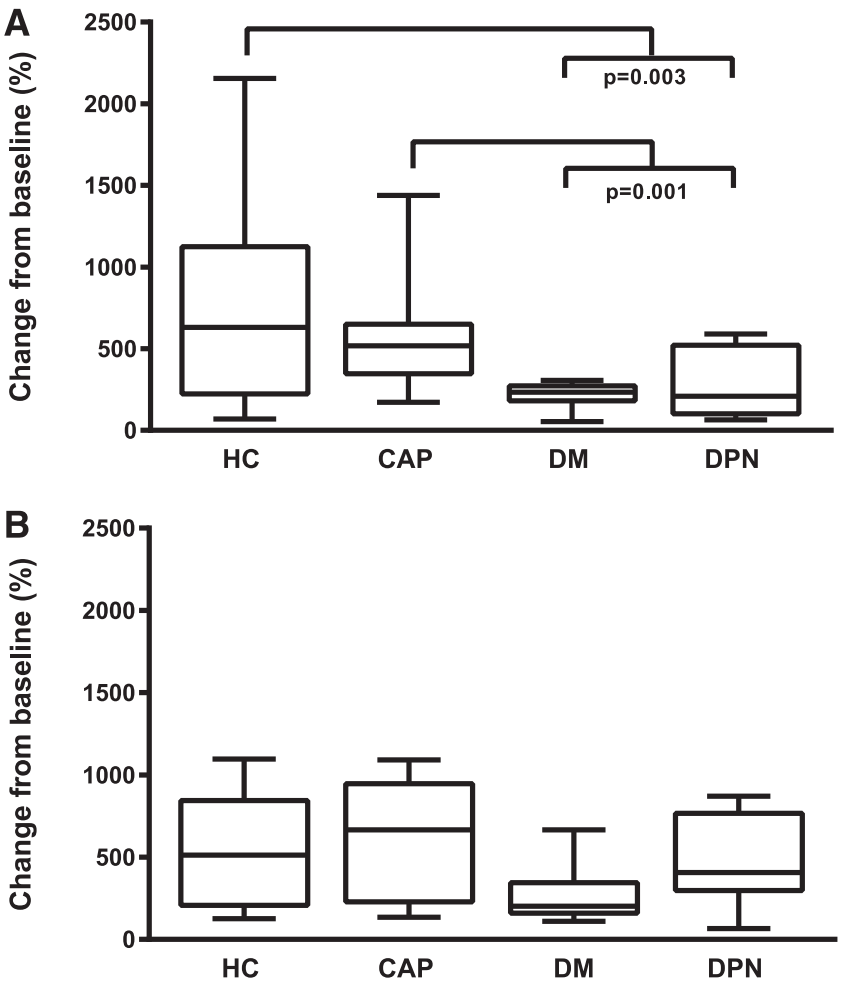

Figure 2—Microvascular function measurements. *A*: Results of ACh iontophoresis as relative change from baseline (P = 0.001, one-way ANOVA). Brackets indicate significant differences between the groups. *B*: Results of SNP iontophoresis as relative change from baseline (P = 0.082, one-way ANOVA).

In addition, this study has a cross-sectional design, which in general makes it impossible to infer causality. However, given our main findings, patients with CAP show no reduction in skin microvascular reactivity compared with HCs, and

neuropathic patients with diabetes show no reduction in microvascular skin reactivity compared with nonneuropathic patients with diabetes; we find it most likely that the association often found between microvascular dysfunction and diabetic neuropathy is caused by a confounding factor. Hyperglycemia seems the most obvious candidate, although other diabetes-related factors such as dyslipidemia and low-grade inflammation may also play a role (43).

Of note, we did not measure the C-fiber-mediated neurovascular response, which is responsible for vasodilation of the skin in the case of injury or inflammation. This flair response—also called the Lewis triple response—is important in wound healing and, when impaired, is a major risk factor of diabetes-related foot problems (4,44). This response has been extensively investigated in previous studies and was shown to be reduced in several neuropathies, including a large population of axonal neuropathies and diabetic neuropathy (44–47). We did, however, examine endothelial and smooth muscle microvascular function of the skin independent of C-fibers, which seems to be unimpaired in patients with CAP and decreased in patients with diabetes with and without polyneuropathy.

Finally, microcirculatory beds of different organs are not equal. Therefore, we should be cautious when extrapolating conclusions from observations of the skin microcirculation to the (dys)function of other microvascular beds. For instance, we did not measure the function of epineural or endoneural vessels, and local impairments of those vessels could have contributed to peripheral nerve damage. Nevertheless, the skin microcirculation is easily accessible and often a good representation of generalized vascular dysfunction (48).

In summary, we did not find differences in either ACh- or SNP-mediated skin vasodilation between patients with CAP and HCs. We confirmed the presence of impaired endothelium-dependent vasodilation in patients with type 2 diabetes but did not find differences between patients with diabetes with and without polyneuropathy. These results suggest that polyneuropathy in patients with CAP or type 2 diabetes does not contribute to the development of skin microvascular dysfunction and, vice versa, that impaired skin microvascular vasodilation does not contribute to polyneuropathy in CAP. Furthermore, the data also suggest that it is unlikely that impaired microvascular endothelium-dependent and -independent vasodilation in the skin in type 2 diabetes plays a major role in the development of polyneuropathy. Therefore, we suggest that the perceived relationship between impaired vasodilation and polyneuropathy often found in patients with type 2 diabetes is probably not causal, but may be the result of diabetes-related factors such as hyperglycemia, dyslipidemia, or inflammation. However, given the statistical limitations resulting from the small sample size in this study, we feel that future trials with larger sample sizes should confirm our results.

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