Impaired Pressure-Induced Vasodilation at the Foot in Young Adults With Type 1 Diabetes

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Vascular and neurological mechanisms are both likely to be involved in foot ulcer. We recently reported a pressure-induced vasodilation (PIV), relying on unmyelinated afferent excitation. We previously found that cutaneous blood flow in response to locally applied pressure might be impaired in diabetic patients because of the combined effects of low cutaneous temperature and alterations in microcirculatory function. Therefore, we aimed to analyze whether, at a relatively high cutaneous temperature, PIV is present in type 1 diabetes and to assess endothelial-dependent vasodilation and endothelium-independent vasodilation. We measured cutaneous blood flow using laser Doppler flowmetry on the head of the first metatarsus in response to applied pressure at 5.0 mmHg/min in warm conditions (29.5 ± 0.2°C). Responses to iontophoresis of acetylcholine (endothelium dependent) and sodium nitroprusside (endothelium independent) were measured using laser Doppler flowmetry in the forearm. The data indicate that PIV exists at the foot level in normal subjects, whereas it was not found in diabetic patients. In diabetic patients, the nonendothelial-mediated response to sodium nitroprusside was preserved, whereas the endothelial-mediated response to acetylcholine was impaired. These findings might be relevant to the high prevalence of foot ulcer that occurs in diabetic patients. *Diabetes* **53:721–725, 2004**

The physiopathological factors involved in the development of foot ulcerations in diabetes include neuropathy (1), microangiopathy (2), and elevated static and dynamic pressure areas under the feet (3). Specifically, a direct relationship exists between neuropathy in primary afferents and the prevalence of foot ulcers (4), although the underlying mechanisms are unclear. Recently, we showed that cutaneous blood flow initially increased before it decreased in response to a progressive locally applied pressure strain. This transient pressure-induced vasodilation (PIV) appears to be a protective cutaneous response. This response was observed both on the hand in humans (5,6) and on the skin of the head in rats (7). This mechanism disappears after desensitization of primary afferents by capsaicin in animals and humans (6,7). Therefore, we speculated that the PIV, relying on unmyelinated afferent excitation, could be a missing link between neuropathy and foot ulcer in diabetes. We previously showed that skin blood flow in response to locally applied pressure is impeded in diabetic patients compared with control subjects at comparable, relatively low skin temperature (8). However, in this study, we did not observe PIV at the foot level in healthy subjects. Therefore, in the present study, we aimed to analyze whether, in warm conditions, PIV is present in both type 1 diabetic patients and age-matched nondiabetic control subjects. Because endothelial dysfunction is fundamental to the development of any vascular process and occurs early in the development of vascular disease in diabetes (9), we investigated endothelium-dependent vasodilation and endothelium-independent vasodilation and their association with PIV.

RESEARCH DESIGN AND METHODS

Twelve patients with type 1 diabetes and no respiratory or cardiac failure, neuropathy of nondiabetic origin, peripheral vascular disease, psychological disorder, or tremor and 12 age-matched nondiabetic control subjects were studied. Diabetic patients were recruited from the Department of Medicine and Diabetology of the University Hospital of Angers. All measurements were performed while patients were hospitalized for a 1-week educational program. Patients were hospitalized for at least 4 days before the study to attain an optimal daily stabilization of glycemic control. Each measurement was started 1.5–2 h after a standard hospital meal in the last 3 days of the 1-week hospitalization. All patients received conventional treatment, which included three to four subcutaneous injections of insulin per day. Before their participation, they were thoroughly informed of the methods and procedures of the study. All subjects gave their informed consent to participate in this institutionally approved study. Details of the clinical characteristics of subjects are reported in Table 1.

Characterization of the neuropathy. Diabetic neuropathy was diagnosed according to the San Antonio Consensus Statement criteria (10). The symptoms were evaluated by using the neuropathy symptom score (NSS). Specifically, subjects were questioned about the presence or absence and possible nocturnal exacerbation of the following symptoms in the lower legs and feet: muscular cramps, numbness, abnormal hot or cold sensations, tingling sensations, burning pain, aching pain, and irritation by bed clothes. Symptoms were scored as 1 point if present or 2 points if present with nocturnal exacerbation. The clinical signs were evaluated by using the neuropathy disability score (NDS), which was based on the examination of knee and ankle tendon reflexes and sensory modalities. Sensory tests included a pinprick, light touch, and vibration and cold perception. Quantitative sensory testing included the assessment of vibration perception threshold using a neurothesiometer (Horwell, London, U.K.), cutaneous perception threshold using Semmes-Weinstein monofilaments (Stoelting, Wood Dale, IL), thermal sensitivity (temperature-controlled homemade thermode), and clinical reflex detection. An NDS of 0 indicated absence of neuropathy, 1–5 mild neuropathy, 6–16 moderate neuropathy, and 17–28 severe neuropathy (11).

TABLE 1
Characteristics of diabetic patients and control subjects

	Diabetic patients	Control subjects
n	12	12
Age (years)	22 ± 1	23 ± 1
Women/Men	6/6	4/8
Diabetes duration (years)	8.9 ± 1.8	—
Height (cm)	170.9 ± 2.8	177.2 ± 2.9
Weight (kg)	65.3 ± 3.2	67.1 ± 2.2
Fasting glycemia at inclusion (mmol/l)	9.6 ± 1.0*	5.0 ± 0.2
HbA_{1c} (%)	9.2 ± 0.8	—

Data are means ± SE. *$P < 0.01$ vs. control subjects.

Macrovascular investigations. Peripheral vascular disease was ruled out based on the absence of vascular claudication or resting pain and on normal ultrasound Doppler blood flow velocity profiles.

Blood flow response to local pressure application. Diabetic patients and control subjects were tested one time in a quiet temperature-climatized room ($29.5 ± 0.2$°C). Experiments were performed with the subjects resting in the supine position. After at least 45 min of acclimatization, cutaneous blood flow was measured with two laser Doppler probes connected to a two-channel laser Doppler flowmeter (Periflux PF4001; Perimed, Stockholm, Sweden). A reference laser Doppler probe was positioned 5 cm from the laser Doppler probe used for pressure application. This latter probe was positioned on the head of the first metatarsus and attached to an apparatus that has been extensively described elsewhere (12), allowing for a 5.0-mmHg/min rate of pressure increase. Data collection began with a 2-min control period before the onset of pressure increase. Cutaneous blood flow was continuously recorded for 22 min. The laser Doppler flow signal was digitized with a 3-Hz sample frequency using a computerized data acquisition system (Biopac, Santa Barbara, CA). Local cutaneous temperature was monitored simultaneously close to the stimulation site and measured by a surface thermocouple probe positioned 5 cm from each of the two laser Doppler probes. The thermocouple was connected to an electronic thermometer (BAT-12; Physitemp Instruments, Clifton, NJ). Systemic blood pressure was monitored noninvasively using a Dynamap (Ohmeda, Englewood, NJ) positioned on the arm contralateral to the sites of laser Doppler flow measurements.

Laser Doppler iontophoresis. The cutaneous blood flow response to iontophoresis was assessed at the volar aspect of the forearm using three laser Doppler probes positioned 5 cm apart to form an equilateral triangle. We used two specially designed "active" probes (PF 481-1; Perimed) to allow for current application, local heating, and simultaneous cutaneous blood flow recording. The thermostatic holder has a circular chamber of 1 cm², allowing for the positioning of the specially designed disposable sponge of the iontophoretic electrode. Cutaneous laser Doppler flowmetry was measured through a multifibre laser probe (780 nm, 1 mW maximal emission, bandwidth for Doppler shift 20-20000 Hz) at the center of the sponge. Probes were connected to laser Doppler flowmeters (Periflux PF4001; Perimed). The two "active" probes were also connected to temperature-regulated heating systems (Peritemp PF4005; Perimed) and to regulated current suppliers (Periiont, Micropharmacology System, PF 382; Perimed), allowing for the delivery of regulated-intensity currents for programmable durations. A third probe (PF408; Perimed) was used as a reference to confirm the absence of response to current application at an adjacent unstimulated site. Local cutaneous temperature was measured by a surface thermocouple probe positioned 5 cm from each of the two laser Doppler probes. The thermocouple was connected to an electronic thermometer (BAT-12; Physitemp Instruments, Clifton, NJ). Systemic blood pressure was monitored noninvasively using a Finapres 2350 (Ohmeda) positioned on the second or third finger of the hand contralateral to the sites of laser Doppler flow measurements.

We measured the blood flow changes in response to iontophoresis of 2% acetylcholine chloride solution (a substance that induces endothelium-dependent vasodilatation) and 1% sodium nitroprusside (a substance that induces endothelium-independent vasodilatation). These measurements were performed in two consecutive experiments separated by at least 1 day. For each patient, iontophoresis of deionized water was performed simultaneously with the drug diffusion using the same current modality on the second "active" probe. In each experiment, the sponges were moistened with 0.2 ml of the solutions. The current application consisted of a 10-s, 100-μA anodal current for the experiment with acetylcholine chloride solution and a 20-s, 100-μA

cathodal current for the experiment with sodium nitroprusside. Two disposable Ag/AgCl electrodes served as the opposite pole in each experiment.

A stable baseline blood flow was measured for 2 min before current application was performed. Twenty min after the end of the current application, the site was locally warmed to 44°C to cause maximal cutaneous vasodilatation (13–16). The data were recorded on a computer via an analog-to-digital converter (Biopac Systems, Santa Barbara, CA), with a sample rate of 3 Hz, on 16 bits. To take into account possible changes in systemic hemodynamic conditions, cutaneous blood flow was indexed as cutaneous vascular conductance (CVC), calculated as the ratio of cutaneous blood flow expressed in arbitrary units to mean arterial blood pressure. Maximal CVC in response to local heating represented the mean cutaneous blood flow values observed over the last minute of the heating period. Then, CVC was normalized for each subject using the maximal CVC as 100% to better reflect changes in cutaneous blood flow (17,18), and results for iontophoresis were expressed in percent of maximal CVC. Vasodilation to iontophoresis of deionized water was subtracted from the effects of the diffused drugs (19,20).

Statistical analyses. The distribution of age, sex, and glycemia was analyzed with unpaired t test. χ^2 test was used to assess the difference in prevalence of nil/not nil NSS and NDS between the diabetic patients and control subjects. Due to instantaneous variability of the laser Doppler flow signal, the results were averaged over 30-s periods for future analysis. All results are expressed as means ± SE.

Values at baseline were calculated as the average over the 2-min control period. A two-way ANOVA was used to identify differences from iontophoretic responses to drug from baseline among the diabetic patients and the control subjects, as well as their interaction. Within each group, a paired t test was performed to determine the level of significance at peak value in response to progressive locally applied pressure and in response to sodium nitroprusside and acetylcholine chloride solution compared with baseline. We evaluated different peak values in response to sodium nitroprusside and acetylcholine chloride solution between the two groups by unpaired t test. For all statistical analyses, a P value <0.05 was regarded statistically significant. Nonsignificant results are reported denoted as NS.

RESULTS

The results from the neuropathy assessment are presented in Table 2. Based on the NDS results, mild neuropathy was present in diabetic patients. Sensitivity to Semmes-Weinstein monofilaments, neurothesiometry, and thermal sensitivity were not different between diabetic patients and control subjects. Cutaneous temperatures did not significantly differ between diabetic patients ($34.5 ± 0.3$°C) and control subjects ($34.5 ± 0.2$°C). In all experiments, mean laser Doppler flow on the reference probe was stable. No hemodynamically significant changes occurred, since systemic arterial blood pressure throughout the different experiments was unchanged compared with starting values.

Microvascular response to local pressure application. Mean resting laser Doppler flow in diabetic patients

TABLE 2
Results of the assessment of neuropathy

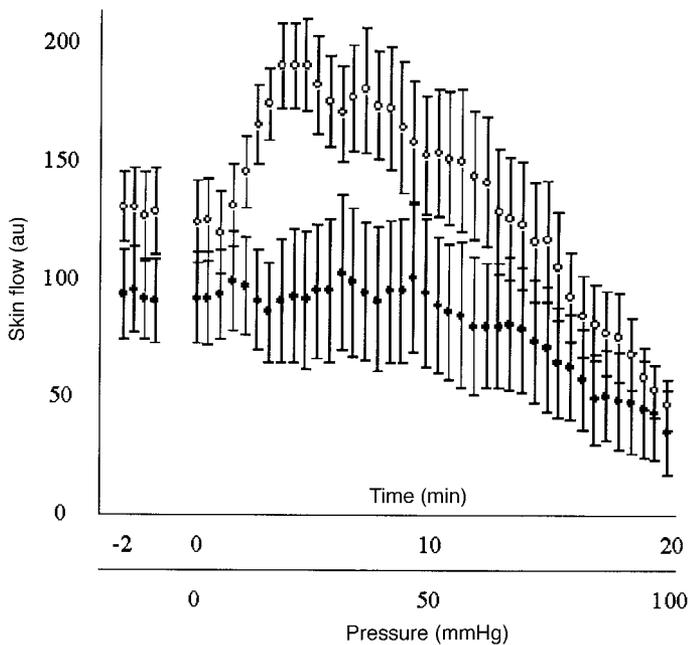|	Diabetic patients	Control subjects
n	12	12
NSS	0.5 ± 0.2	0.2 ± 0.2
NDS	1.6 ± 0.8*	0 ± 0
Semmes-Weinstein monofilaments (g)	2.4 ± 0	2.4 ± 0
Ankle vibration threshold (V)	7.1 ± 1.0	6.9 ± 0.6
Forefoot vibration threshold (V)	5.2 ± 0.7	4.5 ± 0.5
Hand		
Cold threshold	30.8 ± 0.3	31.0 ± 0.2
Warm threshold	34.1 ± 0.6	34.0 ± 0.2
Foot		
Cold threshold	29.2 ± 0.7	30.5 ± 0.2
Warm threshold	34.3 ± 0.3	34.2 ± 0.5

Data are means ± SE. *$P < 0.05$ vs. control subjects.

FIG. 1. Means ± SE of skin blood flow measured with laser Doppler flowmetry during progressive 5.0-mmHg/min local pressure increase in diabetic patients (●) and control subjects (○).

(87.0 ± 15.2 au) was not significantly different from that in control subjects (117.7 ± 14.4 au, NS). In control subjects, laser Doppler flow values increased in response to progressive locally applied pressure versus baseline and reached a maximal value of 169.3 ± 16.2 au at the 22.5–25-mmHg pressure interval ($P < 0.001$ vs. baseline). Mean laser Doppler flow did not increase in diabetic patients and was 85.9 ± 24.5 at the 22.5- to 25-mmHg pressure interval (NS vs. baseline) (Figs. 1 and 2).
Laser Doppler iontophoresis. Two-way ANOVA analysis showed that a significant vasodilation occurred under both acetylcholine and sodium nitroprusside. However, the vasodilator response was significantly attenuated in diabetic patients compared with control subjects for acetylcholine ($P < 0.001$) but not for sodium nitroprusside.

CVC, expressed in percent of maximal CVC at baseline, was 5.2 ± 2.9 vs. 2.6 ± 2.4 (NS) before iontophoresis of
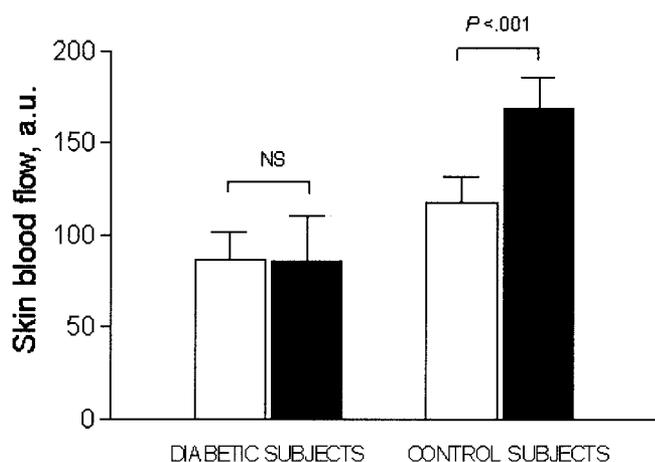

FIG. 3. Laser Doppler flow before (□) and after (■) iontophoresis of sodium nitroprusside. PMVC, percent of maximal vascular conductance.

sodium nitroprusside and 1.8 ± 2.8 vs. 5.7 ± 2.8 (NS) before iontophoresis of acetylcholine, in diabetic patients versus control subjects, respectively. There was no significant difference at peak CVC response after iontophoresis of sodium nitroprusside between diabetic patients and control subjects (17.2 ± 5.7 vs. 21.2 ± 7.1, NS) (Fig. 3). However, peak CVC response after iontophoresis of acetylcholine chloride solution was impaired significantly in diabetic patients (9.8 ± 3.5) compared with control subjects (33.7 ± 5.8, $P < 0.01$) (Fig. 4).

DISCUSSION

In the present study, we have shown that skin vessels vasodilate in response to moderate progressive externally applied pressure strain in control subjects but not in diabetic patients. Since cutaneous blood flow is regulated by humoral, endothelial, and neural factors (21), the absence of PIV, a vascular and neuronal mechanism (6,7), could be related in diabetic patients to both impaired primary afferent neural function and endothelial dysfunction.

The vascular endothelium is a major actor in the control

FIG. 2. Means ± SE of skin blood flow measured with laser Doppler flowmetry during rest (□) and 22.5- to 25-mmHg pressure interval (■) in diabetic patients and control subjects.
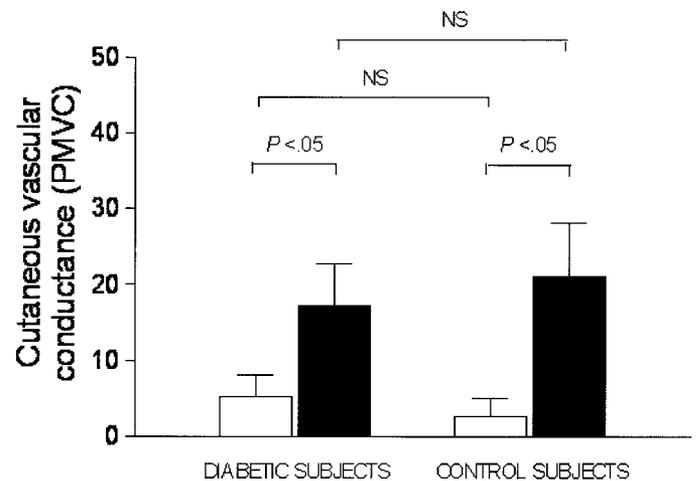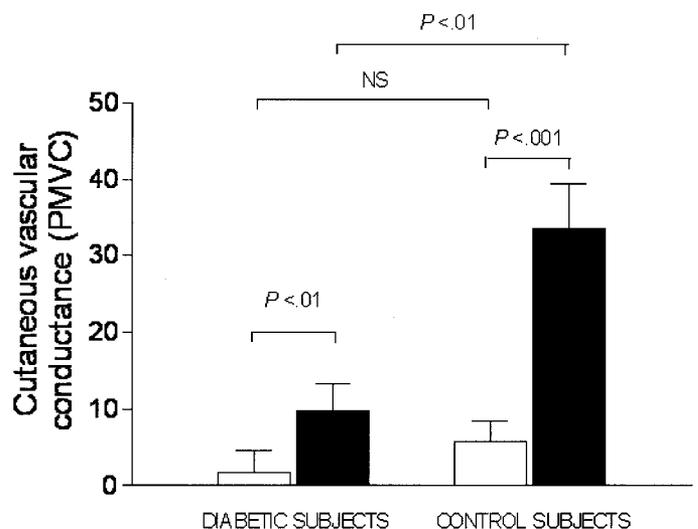

FIG. 4. Laser Doppler flow before (□) and after (■) iontophoresis of acetylcholine. PMVC, percent of maximal vascular conductance.

of the microvascular tone, acting through the release of several vasodilator substances such as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor. NO is the most important vasodilator substance responsible for endothelium-dependent vasodilation (22). There is now substantial evidence that endothelium-dependent vasodilation is impaired in patients with type 1 diabetes as a result of the decreased availability of endothelial NO (23,24). As our previous studies demonstrated involvement of endothelial NO in PIV development (7), the decreased production of these vasoactive mediators might explain the absence of PIV in young diabetic patients.

In the present study, we confirmed an impaired vasodilation to acetylcholine (endothelium dependent) but not sodium nitroprusside (endothelium independent) in our group of diabetic patients at the forearm level. We assume that the impairment found at the arm level reflects a general dysfunction, since previous studies have shown that if a difference may be found between arm and foot, the impairment found at the foot level in diabetic patients is comparable (25) or more severe (26,27) than the impairment found at the arm level. Although controversial results can be found in the literature for endothelium-independent vasodilation (21,27–30), an impairment of endothelium-dependent vasodilation is consistently observed in diabetic patients compared with control subjects. Indeed, these findings suggest that there is normal vascular smooth muscle function in diabetic subjects and that defective endothelial cell acetylcholine receptor excitation (31) and a reduction in NO production (32) are seen relatively early in type 1 diabetes. Abnormalities in the endothelium/NO pathway have been reported in young adults with type 1 diabetes who have no clinical evidence of vascular disease, and these complications develop early (9,30,33). In diabetes, prolonged hyperglycemia is a primary cause of most long-term complications. There is substantial evidence to indicate that hyperglycemia-induced endothelial dysfunction is mediated by free radicals through glucose auto-oxidation and nonenzymatic glycation (34,35). Hyperglycemia could induce increased NO production and reduced NO availability due to inactivation, mediated by free radicals. Recent work suggests that in type 1 diabetes, there could be specific defects in signal transduction mechanisms linked to NO synthase (receptors, ion channels), NO synthase expression, or destruction of NO after it has been produced (36). Skin biopsies from the dorsum of the foot of patients with diabetes have also shown a significant decrease in endothelial NO synthase expression, and this may prove to be the potential cause of reduced endothelial function (29,37).

Neural factors are other mechanisms for the regulation of the microcirculation (27). Several recent studies have demonstrated that damage to unmyelinated fibers has a great impact on skin, with disordered blood flow predisposing to foot ulcers (4,38). Some authors have considered small nerve fibers to be the first fibers to be affected in diabetes (39–41). Their function is not detectable using standard electrophysiology and requires measurement of sensory, neurovascular, and autonomic thresholds and cutaneous nerve fiber density (4). In our study, NDS could suggest the presence of a mild neuropathy in some diabetic patients. Thus, in these patients, an impaired neural response may have further impaired the response to pressure.

In conclusion, the present study confirms the absence of a cutaneous vasodilator response to a progressive pressure strain at the foot level in young diabetic patients, as compared with control subjects, in warm conditions. This abnormality may be related to an interaction between functional changes in unmyelinated C fibers and the endothelium, since a dysfunction of endothelium would be responsible for the absence of PIV. This finding is also relevant to the high prevalence of foot ulcer that occurs in diabetic patients.

ACKNOWLEDGMENTS

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