Neurovascular Response to Pressure in Patients With Diabetic Foot Ulcer

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Diabetic foot ulcer (DFU) is a problem worldwide, and prevention is crucial. We hypothesized that the inability of the skin to respond to pressure is involved in DFU pathogenesis and could be an important predictive factor to take into account. We included 29 patients with DFU and 30 patients with type 2 diabetes without DFU. Neuropathy and skin blood flow at rest were assessed in response to acetylcholine, sodium nitroprusside, local heating (42°C), and to nonnoxious locally applied pressure. Results were compared with those obtained from 10 healthy age-matched control subjects. Vasodilatation in response to pressure was significantly impaired in both groups with diabetes compared with healthy subjects. The vasodilator capacity to pressure was significantly lower in patients with DFU compared with those without DFU, despite the absence of significant difference in cutaneous pressure perception threshold and vascular reactivity to acetylcholine, sodium nitroprusside, and heat. This pronounced alteration of neurovascular response to pressure in patients with DFU is a good marker of skin vulnerability and could be used to better predict individuals at risk.

Diabetic foot ulcer (DFU) is a burden in the management of patients with diabetes worldwide; for instance, it is still the main cause of nontraumatic lower limb amputation (1). This is likely to be an increasing concern owing to the rising prevalence of diabetes (1), and better knowledge about the pathogenesis of DFU is required to improve prevention.

Peripheral neuropathy has a key role in the physiopathology of DFU (2). Neuronal alteration leads to hypoesthesia, dry skin, and foot deformities with abnormal plantar pressure. However, a wide disparity exists in the risk that patients with diabetic neuropathy will develop DFU because other factors are often involved, such as lower limb arteriopathy, articular rigidity, and visual impairment, and also certain social and educational issues (2).

Other than these well-known factors, the ability of the skin to protect against pressure seems determinant to avoid pressure ulcers. We previously described a specific increased skin blood flow (SKBF) in response to local pressure in healthy subjects that contributes to the limitation of skin ischemia and foot ulceration (3). This neurovascular response is impaired in patients with type 1 and type 2 diabetes (4,5), as well as in older adults without diabetes (6), and may be directly or indirectly associated with a higher risk of pressure ulcer. The aim of the current study was to assess the neurovascular response in patients with DFU. We hypothesized that among patients with diabetes, those with DFU have a more severe alteration of the neurovascular response than those without.

RESEARCH DESIGN AND METHODS

The study included 59 patients with type 2 diabetes: 29 without a DFU and 30 with DFU. Patients were recruited prospectively from a single diabetic foot center. We excluded patients with respiratory or cardiac failure, congenital methemoglobinemia, porphyria, cutaneous lesions above the ankle, and those with severe peripheral arterial disease (defined by an ankle-brachial pressure index (ABPI) <0.5) and/or necrosis. The study was approved by the local ethics committee (CPP Sud Est III, Lyon, France) and registered on ClinicalTrials.gov

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(identifier NTC01963559). All participants provided written informed consent before participation. In addition, results were compared with those obtained from 10 healthy age-matched control subjects extracted from a previously studied population (identifier NCT00160927).

The main demographic characteristics of the patients, history of diabetes, and foot ulcer characteristics were collected. Peripheral neuropathy was assessed using the Neuropathy Symptoms Score (NSS), the Neuropathy Disability Score (NDS), the Douleur Neuropathique 4 score, the warm perception threshold, and the cutaneous pressure perception threshold (CPPT) before and after local application of lidocaine.

For all groups, experiments (microvascular and nerve sensitivities) were conducted at rest in a temperature-controlled room on the lower portion of the medial surface of the tibia to avoid any confounding effects from ointment and bandages used in DFU treatment. Endothelium-dependent vasodilatation was measured in response to acetylcholine (ACh, 2%) (Sigma-Aldrich, Saint Quentin Fallavier, France), endothelium-independent vasodilation in response to sodium nitroprusside (SNP; 1%, nitrate) (SERB, Paris, France), and vasodilation in response to local heating to 42°C (Peritemp PF4005; Perimed, Craponne, France). SKBF in response to a nonnoxious progressive locally applied pressure (11.1 Pa s^{-1}) was continuously recorded, before and after local application of lidocaine as described in detail previously (2,5). Vasodilation capacities in response to pressure in the groups with diabetes were calculated at the time maximal SKBF occurred in the healthy group. Maximal vasodilation was analyzed in response to SNP. The maximal vasodilation occurring at the initial peak and at the late plateau phase was analyzed for ACh and heat stimulations.

Results are presented as mean ± SEM. One-way ANOVA was used to investigate differences among groups.

SKBF response to pressure was also analyzed using a mixed-model approach with repeated-measures ANCOVA fitting main effects of lidocaine (yes/no) and DFU (yes/no), and their interaction. Significant interaction effects were explored using the Tukey method, with and without adjustment for the potentially confounding effect of age and coexisting medical conditions (HbA_{1c}, nephropathy, retinopathy, or cardiovascular disease).

Analyses were performed using the mixed procedure of SAS 9.4 software (SAS Institute, Cary, NC). Differences were considered significant when P was <0.05.

RESULTS

The general characteristics of the population are described in Table 1. Patients with and without DFU were comparable in age, sex, glycemic control, and systolic blood pressure. Patients with DFU had a significantly longer history of diabetes and a lower BMI than patients without DFU. Healthy subjects were age-matched with both groups with diabetes but had a significantly lower systolic blood pressure and BMI. In patients with DFU, the mean ± SEM duration of DFU was 20 ± 28 weeks (Table 1). Peripheral arterial disease, defined by absence of palpable pedal pulse and/or ABPI <0.9, was present in one patient with DFU (ABPI: 0.67). Mean ABPI ± SEM was 1.04 ± 0.2 for patients with DFU and 1.06 ± 0.15 for those without DFU.

Table 1—Population characteristics

	Healthy subjects (n = 10)	Patients without DFU (n = 29)	Patients with DFU (n = 30)
Age (years)	60 ± 2	63 ± 9	68 ± 1
Male (%)	60	83	73
History of diabetes (years)	NA	12 ± 8	18 ± 11
BMI (kg/m^2)	23 ± 1	32 ± 6‖	29 ± 4‖¶
HbA_{1c} (%)	NE	9.1 ± 1.7	8.6 ± 2.1
HbA_{1c} (mmol/mol)	NE	76 ± 16	70 ± 20
Insulin (%)	NA	76	50
Systolic blood pressure (mmHg)	117 ± 5	147 ± 3‖	144 ± 3‖
High blood pressure (%)	NA	76	57
Dyslipidemia (%)	NA	86	80
Retinopathy (%)	NA	45	53
Nephropathy (%)	NA	21	47
Cardiovascular disease (%)	NA	27	40
History of DFU (%)	NA	0	43
Location of DFU	NA	NA	
Toe			37
Metatarsal bone			53
Rear foot			10
DFU surface area (mm^2)	NA	NA	184 ± 224
Duration of DFU (weeks)	NA	NA	20 ± 28

Data are mean ± SEM or %. NA, not applicable; NE, not estimated. ‖Significant difference ($P < 0.05$) between healthy subjects and patients with diabetes. ¶Significant difference ($P < 0.05$) between patients without DFU and patients with DFU.

Patients with DFU had a significantly higher NDS score than those without DFU, but there was no significant difference between the two groups for the NSS score or for CPPT, both before and after local application of lidocaine. CPPT was significantly higher in both groups after lidocaine application than before, indicating the blockade of the small peripheral nerve response. NSS and NDS were significantly higher in both groups with diabetes compared with healthy subjects; there was no significant difference in CPPT (Table 2).

Basal SKBF was significantly lower in both groups with diabetes compared with healthy subjects ($P < 0.05$) (Fig. 1*A*), and there was no significant difference in vasodilation capacities after SNP stimulation (data not shown). After ACh stimulation, the initial peak of vasodilation in both groups with diabetes was not significantly different from that found in healthy subjects, but the plateau response

Table 2—Results of the assessment of neuropathy for patients with type 2 diabetes and healthy control subjects

	Healthy subjects (n = 10)	Patients without DFU (n = 29)	Patients with DFU (n = 30)
NSS	0.2 ± 0.1	3.4 ± 0.5‡	4.2 ± 0.5‡
NDS	0.2 ± 0.2	2.8 ± 0.6‡	6.0 ± 0.5‡§
Douleur Neuropathique 4 score	NE	1.6 ± 0.4	2.4 ± 0.3
Tactile perception threshold			
Before lidocaine (g/mm^2)	3.6 ± 0.2	2.94 ± 0.12	3.32 ± 0.18
After lidocaine (g/mm^2)	NE	4.02 ± 0.11	4.20 ± 0.21
Foot skin temperature (°C)	NE	31.5 ± 0.2	31.8 ± 0.3
Warm perception threshold (°C)	NE	41.0 ± 0.6	41.3 ± 0.6

Data are mean ± SEM. NE, not estimated. ‡Significant difference ($P < 0.05$) between healthy subjects and patients with diabetes. §Significant difference ($P < 0.05$) between patients without DFU and patients with DFU.

was significantly lower ($P < 0.01$) (Fig. 1*B* and *C*). After heat stimulation, the initial peak of vasodilation was significantly lower in patients with DFU compared with healthy subjects ($P < 0.05$), but the plateau response was not significantly different (Fig. 1*D* and *E*). Differences between the groups with diabetes in response to ACh, SNP, and heat stimulations were not significant.

In addition, the vasodilatation in response to pressure was significantly lower in both groups with diabetes compared with healthy subjects ($P < 0.05$). The vasodilator capacity to pressure was significantly lower in patients with DFU compared with those without DFU, and lidocaine did not further decrease the vasodilation capacity to pressure in the DFU group whereas it did so significantly in patients without DFU (Fig. 2). These differences remained significant after adjustment for age and coexisting medical conditions ($P < 0.05$).

DISCUSSION

The presence and the severity of diabetic neuropathy has been well established as a cornerstone for the development of DFU (2). However, the current risk stratification system for DFU is mainly based on 10 g monofilament perception and/or vibration perception, and the quality of evidence supporting the predictive value of these parameters is low (7,8). Neuropathy only explains part of the risk of DFU; the frequency of new DFU at 3 years ranges from 14 to 19% in those with neuropathy alone and from 50 to 64% in those with a previous history of DFU (9). This suggests that other factors, such as foot plantar pressure (10), are involved in the pathogenesis of DFU, but which by themselves are reported to be a poor tool to predict foot ulcers. There is therefore clearly a need to find other parameters to better identify people with the higher risk of DFU.

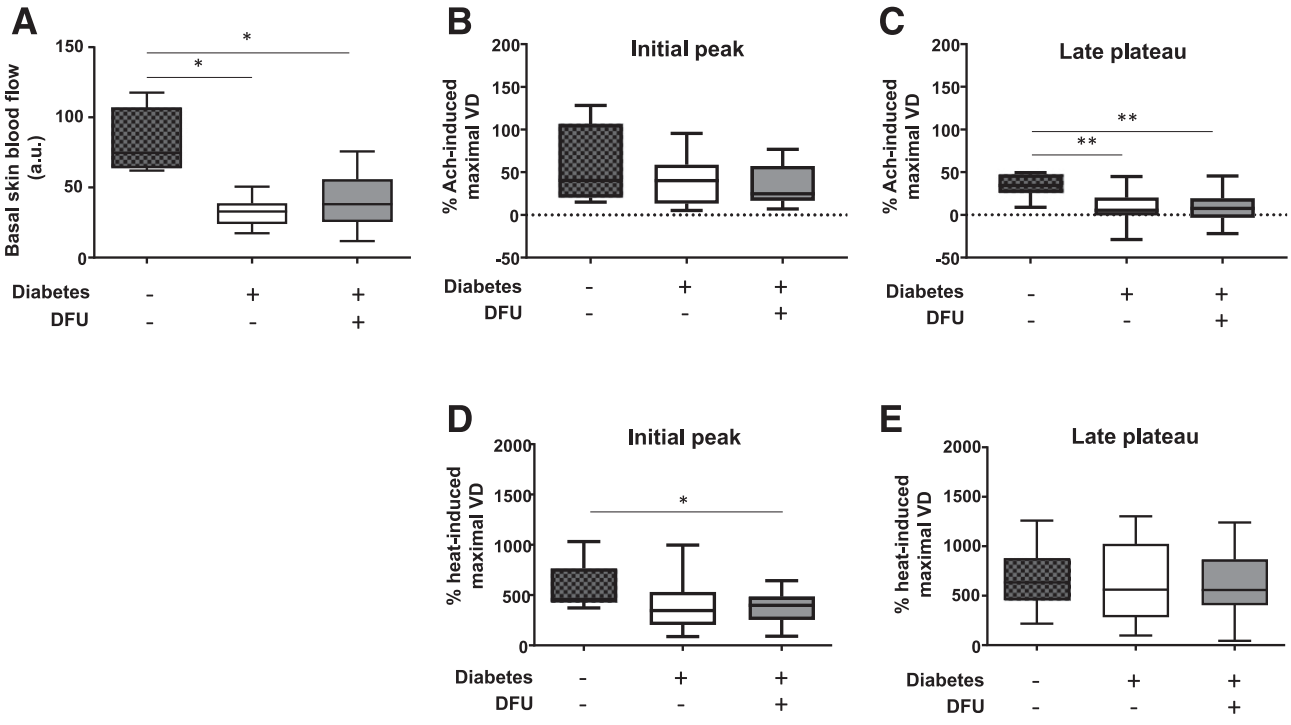

Figure 1—Basal SKBF and response to thermal and pharmacological stimulation. Basal SKBF (*A*), maximal initial peak response to heat (*B*), and maximal late plateau response to heat (*C*). Maximal initial peak response to acetylcholine (ACh) (*D*) and maximal late plateau response to ACh (*E*). Data are median ± interquartile range and minimum-maximum. a.u., arbitrary unit; VD, percentage of vasodilation compared with baseline (%). *$P < 0.05$, **$P < 0.01$.

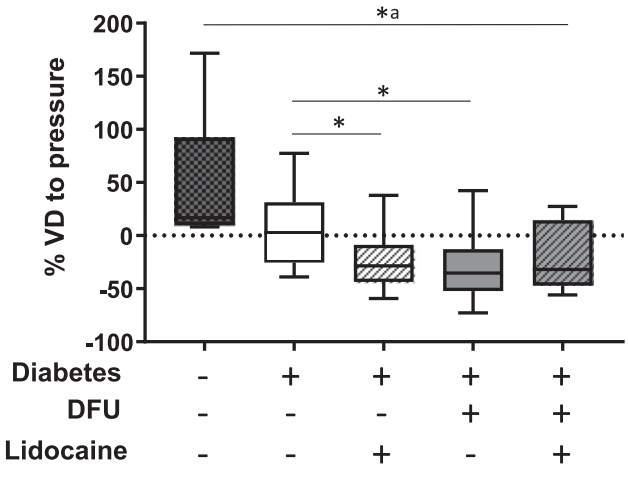

Figure 2—SKBF response to locally applied pressure. Data are median ± interquartile range, minimum-maximum ($P < 0.05$, one-way ANOVA; posttest $P < 0.01$ all diabetic groups vs. healthy control group). VD, percentage of vasodilation compared with baseline (%). *$P < 0.05$; *a: a significant difference ($P < 0.05$) was found between healthy subjects and each of the other groups.

Microcirculation might be an important factor to take into account because a large body of data describes early functional and anatomical microvascular abnormalities in the feet of patients with diabetes (11,12). A study conducted by Newrick et al. (13) in 1988 found that maximal hyperemic response to 3 min standing measured at a site of high plantar foot pressure and the time to recover baseline SKBF were altered in patients with diabetic neuropathy and could contribute to the vulnerability of skin to pressure. A more recent pilot study found that high-pressure areas in patients with diabetes presented a reduced responsiveness to an endothelium-dependent vasodilator compared with low-pressure areas (14). The SKBF response to ACh application and heating is bimodal, with an initial peak followed by a late plateau phase. Several studies have reported that the initial ACh peak is mediated by endothelial nitric oxide release, whereas the late phase is mediated by small sensory nerves and/or prostaglandin (15,16). Interestingly, regarding the initial peak and late plateau phase induced by heat, the order of the mediators is inverted with respect to the ACh bimodal response (17,18). Therefore, the results presented here regarding the ACh-induced late plateau phase and heat-induced initial peak indicate an alteration in the sensory nerves in patients with diabetes and that this impairment is more severe in the presence of DFU in accordance with NDS results. In the current study, endothelial dysfunction in both groups with diabetes was not significant compared with age-matched healthy subjects, suggesting an age effect (6,19) with no change in smooth muscle capacity to relax, as already reported (6,20,21).

The microvascular results presented here are concordant with previous studies showing that impaired microvascular reactivity in the elderly (6) and in patients with diabetes is independent of neuropathy (20). In contrast, when measuring the indirect nerve-axon–related response that results from stimulation of the C nociceptive nerve fibers after ACh stimulation, Hamdy et al. (21) reported an impaired response dependent of neuropathy. However, although it is reported that microcirculation alteration is more important in patients with a history of DFU (22), microcirculation assessment has not been demonstrated to be useful, beyond neuropathy, to predict the risk of DFU (23). The current study reveals an incremental defect in SKBF response to locally applied pressure in patients with type 2 diabetes and a significantly more severe impairment in those with DFU. This effect occurs despite the absence of a significant difference in the CPPT and vascular reactivity to ACh and SNP between the two populations with diabetes, suggesting a specific pathway. Moreover, basal SKBF was not significantly different between the groups with diabetes, which excludes local vasodilation induced by DFU that could affect the conclusions.

Fromy et al. (3) highlighted an original vasodilatory axon reflex response to nonnoxious pressure strain in the human skin, which is impaired in animal models of induced diabetes as well as in type 1 and type 2 diabetes (4,5,24). Interestingly, pharmacological interventions that restore this neurovascular response in diabetic mice with severe neuropathy limit cutaneous pressure ulcer development (25). Similarly, Fromy et al. (26) also demonstrated that the inactivation in a mouse model of acid-sensing channel 3, an essential neuronal sensor for neurovascular response to pressure, leads to a higher skin vulnerability to ischemia induced by prolonged high-pressure application. These data and the results presented here underline the crucial role of this neurovascular adaptation to pressure to limit skin ischemia and wound genesis.

A potential limitation of the study is the long duration of DFU at baseline in the population. This is consistent with that reported in other specialized centers (27–29), that late referral and poor compliance to offloading explain the major part of the delay to healing (29). However, only one patient with DFU had associated peripheral arterial disease, and none had uncontrolled infection that could contribute to the absence of wound closure and affect microcirculation analysis.

Taken together, the data presented here indicate that the pronounced alteration of neurovascular response to pressure in patients with DFU is a good marker of skin vulnerability and could be used to better predict individuals at risk. With a view to confirm the clinical pertinence of this parameter, a large prospective study including patients with grade 1 or 2 diabetic neuropathy according to the International Working Group on the Diabetic Foot (IWGDF) risk classification for DFU is on-going (ClinicalTrials.gov identifier NTC03213093).

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Data Availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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