Early Decrease of Skin Blood Flow in Response to Locally Applied Pressure in Diabetic Subjects

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Pressure ulcers are common debilitating complications of diabetes that are caused by tissue ischemia. Skin blood flow in response to locally applied pressure might be impaired in diabetic patients because of the combined effects of a typically low skin temperature and alterations in microcirculatory function, and could be worsened by neuropathy. We measured skin blood flow by laser Doppler flowmetry over the internal anklebone in response to local pressure applied at 5.0 mmHg/min in three groups of diabetic patients (with clinical and subclinical neuropathy and without neuropathy) and in healthy matched control subjects at usual room temperature. Compared with in matched control subjects with comparable skin temperatures (29.3 ± 0.4 vs. 28.7 ± 0.4°C), in diabetic patients the skin blood flow response to locally applied pressure was further impeded, even in those without neuropathy. Indeed, skin blood flow decreased significantly from baseline at much lower applied pressure (7.5 mmHg) in diabetic subjects, again even in those without neuropathy, than in control subjects (48.8 mmHg). The large difference between these pressures could partially explain diabetic patients' high risk of developing decubitus and plantar ulcers. ***Diabetes*** **51:1214–1217, 2002**

Pressure ulcers are common debilitating complications of diabetes (1). Diabetes disturbs the autonomic regulation of skin microcirculation, even in the absence of neuropathy. Moreover, sensory diabetic neuropathy mainly affects unmyelinated primary afferent fibers (2) and impairs the vasodilation related to normally functioning unmyelinated C fibers (3,4). We recently reported on cutaneous pressure-induced vasodilation (PIV) in humans (5,6) and rats (7) as a physiological response to an increase in nonnoxious progressive local pressure. This mechanism allows skin blood flow to increase in response to locally applied pressure, whereas in its absence, skin blood flow is progressively decreased with application of increased local pressure. Although it results from nonpainful stimulation, this mechanism has been shown to disappear after chronic treatment with capsaicin in animals and humans (5,7), suggesting that it is dependent on the normal function of capsaicin-sensitive primary nervous afferents. Because temperature and blood flow in cutaneous microcirculation are highly correlated (8), skin temperature may have a major effect on skin blood flow response to applied pressure. Therefore, we hypothesized that the skin blood flow response to locally applied pressure would be impaired in diabetic patients because of the combined effects of their typically low skin temperature and diabetes-induced alterations of the vascular and nervous systems. To verify this hypothesis, we measured the effects of locally applied pressure on skin blood flow over the internal anklebone in diabetic patients with clinically apparent or subclinical neuropathy and without neuropathy and in healthy matched control subjects at usual ambient temperature.

RESEARCH DESIGN AND METHODS

Subjects. Subjects were included in the present study if they were >18 years of age; had no respiratory or cardiac failure, neuropathy of nondiabetic origin, or peripheral vascular disease; took no vasodilator drugs; and were able to remain still for 30 min (e.g., subjects with a psychological disorder or tremor were excluded). After receiving an extensive explanation of the protocol, all participants gave informed consent to the study, which had been approved by our local ethical committee. Diabetic patients were recruited from the Department of Medicine and Diabetology of the University Hospital of Angers, France and studied at a usual ambient temperature (25.6 ± 0.1°C). A control group of healthy volunteers of comparable age and sex was studied under a thermal environment comparable with that of the diabetic patients. The characteristics of the diabetic patients and control subjects are reported in Table 1.

Assessment of neuropathy. Diabetic neuropathy was diagnosed according to the San Antonio Consensus Statement criteria (9). The symptoms were evaluated with a neuropathy symptom score, and the clinical signs were evaluated based on the neuropathy disability score in diabetic patients and control subjects. This latter score included tactile and pain sensitivity (Semmes-Weinstein monofilaments; Stoelting,Wood Dale, IL), vibration sensitivity (neurothesiometer; Horwell, London, U.K.), thermal sensitivity (Peritemp 4,005 heater; Perimed, Järfälla, Sweden), and clinical reflex detection. Diabetic patients and control subjects were considered free from neuropathy if both their neuropathy symptom score and neuropathy disability score were equal to zero. Inclusion in the subclinical neuropathy group was based on a neuropathy symptom score and/or neuropathy disability score of >0 but <5. Subjects were assigned to the diabetic neuropathy group if the neuropathy symptom score and/or the neuropathy disability score was >5. The measurement of peroneal sensory nerve conduction velocity was investigated by way of surface electrodes in diabetic patients only.

Macrovascular investigations. Peripheral vascular disease was ruled out based on the absence of vascular claudication or resting pain, with the ankle brachial index >0.95 on both sides (10), and normal ultrasound Doppler blood flow velocity profiles. Transcutaneous oxygen pressure ($TcPO_2$) (TCM3 Tyna; Radiometer, Copenhagen, Denmark) was performed at the dorsum of the foot and on the forearm.

Microvascular response to local pressure application. Studies were performed with the subject in the supine position, which was assumed 20 min

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CGRP, calcitonin gene-related peptide; PIV, pressure-induced vasodilation; $TcPO_2$, transcutaneous oxygen pressure.

TABLE 1
Characteristics of study subjects

	Neuropathic patients	Subclinical neuropathic patients	Nonneuropathic patients	Control subjects
n	15	15	15	15
Age (years)	50 ± 3	58 ± 4	40 ± 3	47 ± 2
Men/women	8/7	11/4	8/7	7/8
Type of diabetes (1/2)	5/10	8/7	9/6	—
Diabetes duration (years)	13.1 ± 2.6	11.9 ± 2.9	7.2 ± 2.0	—
Height (cm)	167 ± 2	168 ± 2	166 ± 2	172 ± 3
Weight (kg)	77 ± 4	73 ± 2	66 ± 4	67 ± 3
Glycemia (g/l)	1.6 ± 0.2	1.3 ± 0.1	1.6 ± 0.2	—
$TcPO_2$				
Forearm (mmHg)	69 ± 3	64 ± 3	68 ± 3	69 ± 2
Foot (mmHg)	64 ± 4	62 ± 4	64 ± 3	65 ± 3

Data are means ± SE.

before the experiments were started. Skin blood flow was assessed with a laser Doppler probe (PF801 Periflux; Perimed) connected to a laser Doppler flowmeter (PF4000; Perimed). A reference probe was positioned 5 cm from the probe used for pressure application. This latter probe was positioned over the middle of the internal anklebone and attached to an apparatus extensively described elsewhere (11), allowing for a 5.0 mmHg/min rate of pressure increase. Data collection began with a 2-min control period before the onset of increasing pressure. Skin blood flow was continuously recorded for 32 min. The laser Doppler flux signals were digitized with a 12-Hz sample frequency using a computerized data acquisition system (Biopac, Santa Barbara, CA). The blood flow signals were averaged every 15 s to reduce the instantaneous variability of the signals attributable to vasomotion. The mean value calculated over the last minute of the experiment, corresponding to application of 150.0 mmHg pressure, was assumed to represent zero blood flow and was subtracted from all skin blood flows recorded from the area of progressive pressure application.

Statistical analyses. All results are expressed as means ± SE. Distribution of age, sex, and type of diabetes was analyzed with unpaired *t* test and χ^2 test. Values at baseline were calculated as the average over the 2-min control period before local pressure was applied. To test for significant differences among groups, we performed one-way ANOVA with Newman-Keuls multiple comparison test over the baseline period. Within each group, a paired *t* test was performed to determine the level of significance at a level of applied pressure compared with baseline. A two-tailed *P* value <0.05 was regarded as statistically significant.

RESULTS

The results from the neuropathy assessment in all groups are presented in Table 2. In brief, sensitivity to Semmes-Weinstein monofilaments and neurothesiometry were not different between diabetic patients without neuropathy and control subjects, whereas sensitivity and neurothesiometry in diabetic patients with subclinical or clinical neuropathy were significantly different from those of the control subjects. No significant change in skin blood flow occurred during the application of local pressure at the unperturbed control site in any of the studied groups, indicating that no systemic hemodynamic changes occurred during the experiments.

Although mean skin blood flow tended to decrease slowly in the control subjects, a marked decrease appeared in the diabetic patients without neuropathy with initial increasing applied pressure (Fig. 1). At baseline, the mean skin blood flow in diabetic patients without neuropathy (14.1 ± 0.2 arbitrary units [a.u.]) was similar to that in control subjects (14.4 ± 0.3 a.u.; NS) and was lower than that in diabetic patients with subclinical (17.2 ± 0.2 a.u.; $P < 0.001$) and clinical neuropathy (16.7 ± 0.2 a.u.; NS).

The mean skin blood flow decreased slowly and reached significance from baseline at 7.5 mmHg in diabetic patients without neuropathy (12.0 ± 1.3 a.u.; $P < 0.05$), at 6.3 mmHg in diabetic patients with subclinical (13.2 ± 2.6 a.u.; $P < 0.01$) or clinical neuropathy (13.8 ± 2.4 a.u.; $P < 0.01$), and at 48.8 mmHg in the matched control subjects (7.9 ± 3.9 a.u.; $P < 0.05$).

Skin temperatures did not differ significantly between diabetic patients without neuropathy (29.3 ± 0.4°C), diabetic patients with subclinical neuropathy (27.7 ± 0.4°C), diabetic patients with clinical neuropathy (28.4 ± 0.4°C), and control subjects (28.7 ± 0.4°C).

DISCUSSION

The main results of our study indicated that in response to applied local pressure, the skin blood flow over the anklebone decreased significantly from baseline, with much lower applied pressure in diabetic patients without neuropathy (7.5 mmHg) or with subclinical or clinical neuropathy (6.3 mmHg) than in matched control subjects (48.8 mmHg) studied in the same thermal conditions.

TABLE 2
Results of the assessment of neuropathy

	Neuropathic patients	Subclinical neuropathic patients	Nonneuropathic patients	Control subjects
Neuropathy symptom score	2.1 ± 0.7	0.7 ± 0.2	0 ± 0	0 ± 0
Neuropathy disability score	8.7 ± 1.4	2.2 ± 0.5	0 ± 0	0 ± 0
Semmes-Weinstein monofilaments (g)	3.8 ± 0.1†	3.7 ± 0.2*	2.7 ± 0.1	3.1 ± 0.2
Neurothesiometer (a.u.)	20.4 ± 4.7‡	16.8 ± 1.8‡	8.8 ± 1.2	8.7 ± 0.9
Peroneal nerve conduction velocity (m/s)	38.6 ± 1.6	40.4 ± 0.7	44.9 ± 0.9	—
Measurable velocities	8 (53)	8 (53)	13 (87)	—

Data are means ± SE or *n* (%). *$P < 0.05$ vs. control subjects; †$P < 0.01$ vs. control subjects; ‡$P < 0.001$ vs. control subjects.

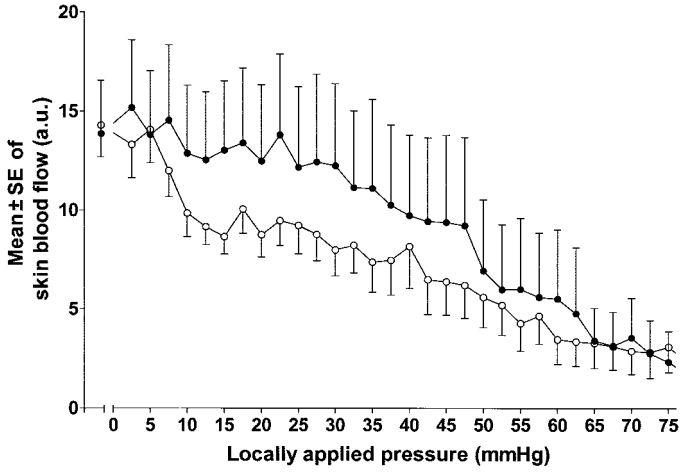

FIG. 1. Means ± SE of skin blood flow measured with laser Doppler flowmetry during progressive 5.0 mmHg/min local pressure increase in diabetic patients without neuropathy (○) and control subjects (●) in usual ambient temperature. The mean skin temperatures were 29.3 ± 0.4 and 28.7 ± 0.4°C, respectively. Data are expressed as arbitrary units.

Capillary pressure has been shown to be higher in diabetic patients than in control subjects (median values, 20.4 mmHg vs. 16.7 mmHg) (12). Taken together, these results show that the arterial wall and surrounding tissues are very compressible in diabetic patients, which could be an alternative explanation for diabetic patients' high risk for pressure ulcers.

Although much attention has been focused on the role of thermal receptors in the control of skin blood flow, the role of cutaneous mechanical receptors has not been as extensively studied. We recently reported a significant transient increase of skin blood flow during local pressure application in the skin of humans (5,6). Because a skin temperature close to 34°C was considered the optimal temperature for the evaluation of skin vasomotor reflexes in the lower limb (13), the absence of PIV in healthy subjects may be attributable to a low skin temperature. This suggests that the nervous receptors involved in the PIV development are mechanothermal, and not only mechanical.

Vasodilation in diabetic subjects is attenuated in response to occlusive ischemia (14), local heating to 44°C (15), and indirect heating (16). The inability of skin microcirculation in diabetic patients to respond normally to injury (17,18) and even to nonnoxious stimulation, such as the local pressure applied in the present study, may be an important factor in the development of ulceration.

In agreement with results from other studies (19,20), we found no significant differences in basal skin blood flow between diabetic patients without neuropathy and control subjects. Nevertheless, it has been shown that an acute glucose load in healthy subjects (21) and glycemic dysregulation, such as is found diabetes (22–24), may lead to impaired vasodilation in both macro- and microcirculation. The PIV mechanism involves both nervous and microvascular tissues, which could be impaired together or separately in diabetes. Cacciatori et al. (25) observed that peripheral sympathetic adrenergic and cholinergic fibers undergo early alterations in diabetic patients, even when there is no clinical neuropathy. The dysregulation of skin neurovascular function, largely regulated by peripheral C fiber neurons, may be a component of the metabolic syndrome associated with type 2 diabetes. (26). Damage to unmyelinated C fibers in diabetic neuropathy would contribute to abnormalities in cutaneous blood flow (2) and impairment of the vasodilation related to those fibers (3,4).

Capsaicin desensitization in both humans and rats has resulted in the total disappearance of PIV (5,7), which demonstrates that PIV is dependent on capsaicin-sensitive nerve terminals. PIV is totally absent in diabetes. Other mechanisms mediated by these fibers, such as a C fiber–mediated axon reflex, are also altered in diabetic patients with neuropathy (27). The loss of axon-reflex vasodilation and diminution of pain perception contribute to diabetic foot ulceration (19). Although a dysfunction of unmyelinated C fibers occurs in diabetic patients with neuropathy, our results failed to demonstrate that clinical or subclinical neuropathy had an additive aggravating effect on the response of skin blood flow to local application of pressure.

From the foregoing, we could hypothesize that an impairment in the mechanisms of vasodilation underlying the PIV phenomenon, such as a reduction of release of local vasoactive mediators, may have implications for the pathophysiology of pressure ulcers. It has been shown that the release of neuropeptides, such as calcitonin gene-related peptide (CGRP), is diminished (26,28) and that endothelial-derived NO production is altered in diabetes (29–31). Given that our previous studies demonstrated major roles for CGRP and NO (particularly endothelial-derived NO) in PIV development (7), the decreased production of these vasoactive mediators might partially explain the early decrease of skin blood flow at low applied pressures in diabetic patients. Nevertheless, not all published data are consistent with the formulation outlined above. The impaired neurogenic nociceptor-mediated vasodilation found in hairy skin of people with diabetes does not occur as a consequence of a decrease in NO production. Indeed, endothelial and sympathetic function in diabetic subjects have been shown to be relatively equivalent to that in healthy subjects (2). There is evidence that NO production may be normal or actually increased in patients and/or animal models of type 2 diabetes (32). Therefore, endothelial NO production in diabetes is still controversial (29).

In conclusion, skin blood flow in response to locally applied pressure is further impeded in diabetic subjects, even in those subclinical or clinical neuropathy, compared with in control subjects with comparable skin temperature.

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