Primary Nociceptive Afferents Mediate the Blood Flow Dysfunction in Non-Glabrous (Hairy) Skin of Type 2 Diabetes

A new model for the pathogenesis of microvascular dysfunction

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OBJECTIVE — To test the independent contributions of vascular endothelium, sympathetic activation and inhibition, vessel distensibility, and nociceptor-mediated vasodilation in both glabrous and hairy skin circulations.

RESEARCH DESIGN AND METHODS — We measured blood flow using laser Doppler techniques in 10 people with type 2 diabetes and 10 age- and BMI-matched healthy control subjects at the pulp of the index finger (glabrous skin) and the dorsum of the hand (hairy skin). A 5-min ischemic block of the arm was used to test vascular endothelium. Warming of the probe site to 45°C tested neurogenic vasodilation in hairy skin only. Vessel distensibility was tested by gravitational pressure.

RESULTS — Basal blood flow and reactive hyperemia did not differ between groups at either skin site. The vasodilative response to local warming ($P < 0.01$) and limb lowering ($P < 0.05$) were significantly different between groups in hairy skin but not in glabrous skin in the absence of objective measured neuropathy. Nociceptor-mediated flow correlated significantly with the warm thermal threshold ($r = -0.50$, $P < 0.05$). Endothelial-mediated blood flow correlated with systolic blood pressure ($r = -0.76$, $P < 0.01$), LDL cholesterol ($r = -0.62$, $P < 0.001$), C-peptide ($r = 0.65$, $P < 0.05$), and triglycerides ($r = 0.47$, $P < 0.05$).

CONCLUSIONS — These data suggest that neurogenic nociceptor-mediated vasodilation is impaired in subjects with type 2 diabetes when endothelial and sympathetic function are relatively intact. Heat-induced vasodilation may be a specific test of small heat-sensitive C-fiber peripheral neurons and may be an integral part of the metabolic syndrome.

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We and others have previously demonstrated impairment of cutaneous blood flow in people with diabetes, measured on the pulpar surface of the fingers (1–9). We have also shown that there is an intrinsic rhythm to the regulation of blood flow (10) and that the reduction in flow resembles a premature aging phenomenon (1). Both defective vasoconstriction (1–4,8) and vasodilation responses (1,5,6,9,11) with different stimuli under various conditions have been observed. It remains uncertain as to what extent this vascular dysfunction is a result of endothelial dysfunction, microangiopathy, or subclinical neuronal defects. Furthermore, the vascular abnormalities could be primary and mediate neuronal damage via oxidative stress or tissue hypoxia.

Structural changes in peripheral vessel beds in diabetes, including hyalinosis (12), thickening of the basal lamina by smooth muscle cell proliferation (13), and impaired myogenic reactivity (14,15), are well known. However, it seems unlikely that structural alterations are compatible with altered rythmicity (10). It is more likely that neurogenic defects underlie the loss of vascular reactivity. Our previous data support the contention that the vascular defects in the upper limb are related to the function of small fiber neurons (1,10). More recently, the role of vascular endothelium in these vessels has been examined using post-ischemic hyperemia (16) and pharmacologic studies (9) using direct (sodium nitroprusside) and endothelium-dependent (acetylcholine) vasodilators in which profound impairments in diabetes have been found. However, the magnitude of this defect and its precise localization (endothelial production of a vasodilator or failure of smooth muscle responsiveness) has remained controversial (17). It would be beneficial to distinguish the extent of each mechanism in these vascular abnormalities.

The situation is further complicated by differences in neural regulation of vascular reactivity on different beds. An active neurogenic vasodilative mechanism is present in hairy skin (18–21), as outlined in Fig. 1. The activation of small-fiber polymodal nociceptors by heat or some noxious stimulus is the necessary event. The initial vasodilation is due to relaxation of sympathetic tone. The primary nociceptive affer-

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Abbreviations: ANOVA, analysis of variance; VIP, vasoactive intestinal polypeptide.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

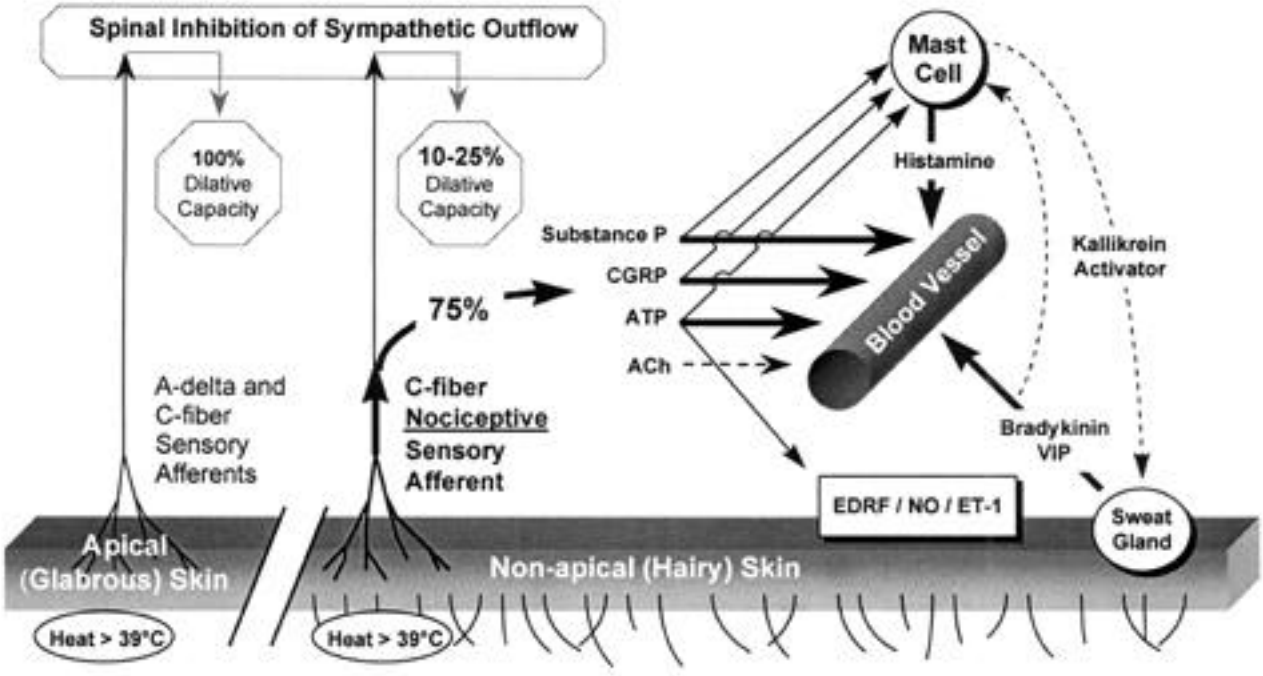

Figure 1—*Diagram of the "axon reflex" in non-glabrous (hairy) skin showing the contribution of local nociceptors to vasodilation and inflammation derived from models using antidromic activation of sensory collaterals.*

ents then release several peptides, including substance P, calcitonin gene–related peptide, and the adenosine analog ATP, which cause a more intense vasodilation (22). When peripheral processes are injured, these neurons switch to synthesize other peptides such as vasoactive intestinal polypeptide (VIP), galanin, and cholecystokinin (23). Some of these compounds may act directly on vascular smooth muscle or through secondary pathways that include mast cell release of histamine and sweat gland secretion of bradykinin and further release of VIP, although their specific roles have yet to be described (18). While this mechanism accounts for 75–90% of the dilatory capacity in human hairy skin (24), the effects of heat in glabrous skin reflect release of sympathetic tone and a passive distension of those skin vessels (25).

We sought to test the independent contributions of vascular endothelium, sympathetic activation/deactivation, vessel distensibility, and nociceptor-mediated dilation in the skin circulation of patients with type 2 diabetes and designed a sequence of provocative tests to evaluate the function of each component. The hyperemic response to an ischemic block is thought to primarily reflect endothelial function mediated by nitric oxide (26), prostacyclin (27), and possibly ATP-sensitive potassium channels (28). Peripheral sympathetic activity is dramatically increased by exposure to cold and decreased with either local or whole-body warming. To test the distensibility of peripheral vessels, we exposed the warmed vascular bed to hydrostatic pressure by lowering the arm. We chose to study the upper extremity to observe activation and release of sympathetic tone that is not confounded by the postural autoregulatory activity of lower limbs. In glabrous skin of the upper limb, the release of sympathetic tone by warming should allow for maximal vessel distension in the presence of hydrostatic pressure. However, in hairy skin, maximal dilation will occur only if the nociceptor-mediated mechanism is intact. Thus, we measured the skin blood flow response to each of these stimuli in the fingertip pulp and dorsum of the hand in type 2 diabetic subjects and matched healthy control subjects.

RESEARCH DESIGN AND METHODS

Subjects

We studied 10 type 2 diabetic subjects and 10 normal healthy control subjects that were matched for age (±2 years) and BMI (±10%). Clinical details are outlined in Table 1. These patients were recruited by responding to community advertising for diabetes research studies, and they were selected for this study based on their lack of neuropathic symptoms. We excluded any patients with clinically significant retinopathy (greater than background level on routine fundoscopy), nephropathy (presence of >20 µg/ml protein in a random urine sample), history of major macrovascular events, clinical signs of peripheral vascular disease, and patients who were taking any calcium channel–blocking agents known to affect vascular responsiveness (29). None of the subjects were smokers at the time of testing, and nine of them had smoked at some time in their lives. None of the subjects drank more than four alcoholic beverages per week, and none had drunk for 48 h before testing.

Procedures

Blood flow testing. Blood flow was measured noninvasively at the pulp of the index finger (glabrous) and the dorsal aspect of the left hand (hairy skin) using a Laserflo Blood Perfusion Monitor (BPM 403A; Vasamedics, St. Paul, MN). Flow values recorded by the blood flow monitor were temporally averaged during data collection every second to act as a low-pass filter. The output voltage data from the blood flow monitor was sampled every second by custom-written data acquisition software that interfaces with an analog to digital data acquisition board (DAS-8 board; Keithley Metrabyte, Taunton, MA). Voltage data were converted mathe-

matically back to flow values. This experiment requires repeated measurements from the two different sites. Therefore, the order in which the two sites were tested was randomly determined. The patients were allowed 30 min to acclimate to room temperature and sat quietly in a closed room at 20–22°C with their arm at heart level and listened to taped instructions. Their hand temperature was measured before beginning testing at the dorsum of the hand, and all were >28°C. The following stimuli were presented sequentially:

1. Ischemic block: after a 2-min baseline period, a standard sphygmomanometer positioned over the brachial artery was inflated to 40 mmHg above the subject's systolic blood pressure and maintained for 5 min, then released.
2. Cold pressor: 10 min after the release of the ischemic block, the subject immersed the contralateral (right) hand in ice water for 30 s.
3. Local warming: 2.5 min after the cold pressor, a small area of skin around the probe (2 cm diameter) was warmed and maintained at 35°C for 5 min, then raised to 45°C and maintained there for 5 min.
4. Limb elevation and lowering: the subject raised their forearm (from the elbow distally) for 2 min, then lowered the entire arm for an additional 10 min while maintaining the local skin temperature at 45°C throughout.

Sensory tests. We measured sensory thresholds at the pulpar aspect of the dominant great toe including vibration, cold thermal, and warm thermal thresholds using the VSA 3000 and TSA 3000 (Medoc, Minneapolis, MN) device. This device consists of a computer-driven set of thermal and vibratory probes and patient response unit. Each modality was tested using the method of limits with six trials separated by a random interval between 4 and 20 s long. This method involves a ramped stimulus and requires the subject to respond by pushing a handheld button when the stimulus is recognized. For vibration, the stimulus began at zero vibration units (log base 10 of peak-to-peak amplitude) and increased at the rate of 0.5 vibration units per second. There was a maximum level of 25 vibration units for each trial. For cold thermal sensation, the initial temperature was 25°C, and during each stimulus, the temperature decreased at the rate of 0.5°C per second to a minimum of 0°C. For warm thermal sensation, the initial temperature was 32°C, and during each stimulus, the temperature increased at the rate of 0.5°C per second to a maximum of 50°C. Each threshold was calculated as the mean stimulus level of the six trials.

Autonomic testing. The heart rate (measured as R-R interval) response to deep breathing was measured using the QMED NDx device. This response requires the supine subject to perform paced breathing while an electrocardiogram recording is made. This test reflects cardiac autonomic neuropathy and has previously been described in detail (30).

Data analysis

Data are expressed as means ± SEM. Paired *t* tests were used to compare the epidemiologic variables of the matched groups. Likelihood ratio χ^2 was used to analyze categorical variables. The data for blood flow was found to be parametric and approximated a random normal distribution for both study groups using the Shapiro-Wilk *W* test ($P = 0.1919$), so parametric analyses were performed on those variables. One-way analysis of variance (ANOVA) was used to determine significance of blood flow data, and a mixed (within × between) ANOVA with contrast testing was performed on the blood flow data to confirm the univariate tests.

Table 1—*Summary data for 20 age-matched subjects*

	Control subjects	Diabetic subjects
n	10	10
Age (years)	59.3 ± 2.2	59.7 ± 2.2
BMI (kg/m²)	30.0 ± 1.3	30.5 ± 1.2
Sex (M/F)	4/6	6/4
Duration of diabetes	N/A	10.1 ± 2.0
Systolic blood pressure	130.6 ± 3.54	136.6 ± 13.9
Diastolic blood pressure	83.3 ± 3.12	83.4 ± 2.49
HbA_{1c}	5.42 ± 0.12	8.09 ± 0.69†
C-peptide	2.93 ± 0.28	3.40 ± 0.22
Lipid profile		
Total cholesterol	216.9 ± 5.10	185.5 ± 13.1*
LDL	132.1 ± 5.31	92.0 ± 13.4*
HDL	48.5 ± 3.77	38.1 ± 2.93
Triglycerides	181.6 ± 28.3	299.7 ± 57.2*
Sensory variables		
Vibration threshold	4.9 ± 1.6	12.1 ± 2.8
Cold thermal threshold (°C)	2.7 ± 0.5	11.9 ± 2.3†
Warm thermal threshold (°C)	6.8 ± 1.3	16.5 ± 0.8†
E:I ratio (autonomic variable)	1.14 ± 0.02	1.09 ± 0.03

Data are means ± SEM. *$P < 0.05$, †$P < 0.01$, each derived from paired *t* tests (two-tailed), except for sex ratios, which were tested using the likelihood ratio χ^2 test. No adjustments were made here for multiplicity of planned comparisons.

RESULTS

Blood flow in dorsum of the hand

Blood flow results from the dorsum of the hand are illustrated in Fig. 2 and outlined in Table 2. Baseline blood flow was not significantly different between groups. The reduction in blood flow during ischemia is expected because blood velocity is expected to approach zero; therefore, this segment was not tested statistically for any group differences. The hyperemic response to the ischemic block lasted ~3 min in most subjects. The peak hyperemic response was slightly diminished in the type 2 diabetic subjects. However, this difference did not reach significance in the maximum hyperemic flow value, time to the maximum value, or area under the curve (Table 2). There was very little response to the cold pressor stimulus in either group. In contrast, warming produced a 10-fold rise in blood flow in control subjects, while type 2 diabetic subjects had a significantly diminished ($P < 0.01$) response of only about a threefold increase. Upon lowering of the limb, there was a 20-fold increase in flow in control subjects but an eightfold increase in diabetic subjects. This difference was significant ($P < 0.05$) between the type 2

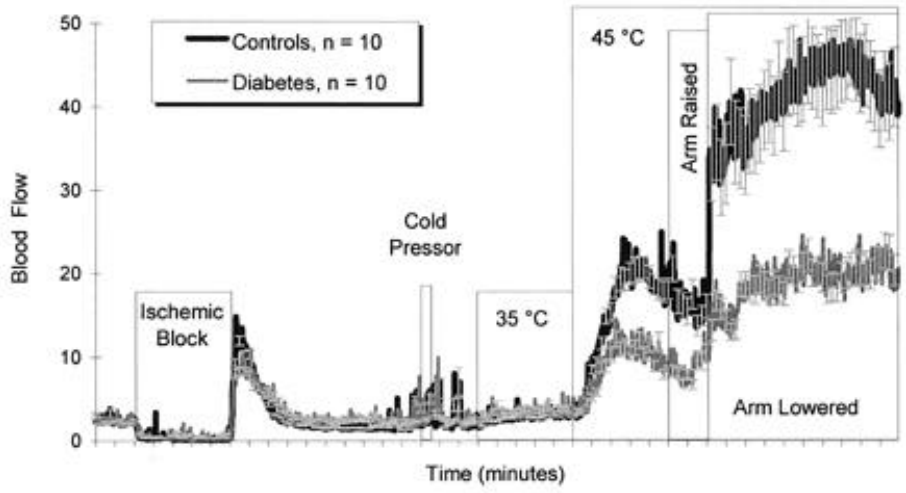

Figure 2—*Skin blood flow measured from the dorsum of the hand in both groups of subjects. Data are displayed as group means ± SEM* (n = *10).*

diabetic and control groups. Repeated-measures ANOVA with contrast testing for baseline versus heated and versus lowered limb conditions was performed to confirm these findings while allowing for multiplicity of planned comparisons. This analysis also showed significant differences between control and type 2 diabetic subjects during both warming and lowering of the limb ($P < 0.05$ each).

There were significant inverse correlations between systolic blood pressure and the hyperemic response to ischemia ($r = -0.76$, $P < 0.01$) and the heated arm lowering ($r = -0.52$, $P < 0.05$) and between warm thermal threshold and the response to warming ($r = -0.51$, $P < 0.05$). There were also significant correlations between flow at 35°C and the LDL cholesterol ($r = -0.62$, $P < 0.001$), C-peptide ($r = 0.65$, $P < 0.05$), and triglycerides ($r = 0.47$, $P < 0.05$).

Table 2—*Skin blood flow measurements in glabrous and non-glabrous skin of the upper limb in control and diabetic subjects*

	Control subjects	Diabetic subjects
Dorsum of hand (hairy skin)		
Baseline blood flow	2.4 ± 0.7	2.3 ± 0.6
Peak post-ischemic hyperemia	23.9 ± 4.9	21.1 ± 3.5
Area under curve for hyperemia	854 ± 118	840 ± 220
Time to peak hyperemia (s)	35.5 ± 2.3	44.7 ± 7.7
Cold-induced vasoconstriction (change)	−0.59 ± 0.5	−0.39 ± 0.3
Baseline thermal stimulus (35°C)	2.8 ± 0.4	3.4 ± 0.8
During heat stimulus (45°C)	16.1 ± 2.6	6.7 ± 1.6†
Arm raised (2 min at 45°C)	16.4 ± 4.6	8.6 ± 2.0
Arm lowered (10 min at 45°C)	57.8 ± 15.3	18.9 ± 4.8*
Finger pulp (glabrous skin)		
Baseline blood flow	20.8 ± 7.7	14.6 ± 3.8
Peak post-ischemic hyperemia	41.4 ± 8.3	52.6 ± 17.3
Area under curve for hyperemia	2,165 ± 559	2,135 ± 621
Time to peak hyperemia (s)	133.4 ± 47.7	186.7 ± 36.4
Cold-induced vasoconstriction (change)	3.8 ± 2.0	13.8 ± 10.2
Baseline thermal stimulus (35°C)	22.3 ± 4.4	30.7 ± 7.9
During heat stimulus (45°C)	49.8 ± 12.3	42.5 ± 8.0
Arm raised (2 min at 45°C)	39.1 ± 10.0	22.4 ± 4.8
Arm lowered (10 min at 45°C)	86.5 ± 21.0	81.1 ± 18.8

Data are means ± SEM. *$P < 0.05$; †$P < 0.01$, derived from one-way ANOVA. No adjustments were made here for multiplicity of planned comparisons.

Blood flow in finger pulp

Blood flow results from the finger pulp are illustrated in Fig. 3 and outlined in Table 2. Mean basal blood flow in control subjects was 20.8 ± 7.7. This was slightly reduced in type 2 diabetes (14.6 ± 3.8), but was not significantly different from control subjects. After the experimental ischemia, there was a twofold increase in blood flow in both control and diabetic subjects. Flow returned toward basal within ~4 min in control subjects, but in three of the diabetic subjects, it remained elevated for at least 10 min. Nonetheless, there were no significant differences in the maximum hyperemic flow value, time to the maximum value, or area under the curve (Table 2). Cold pressor stimulation elicited a similar degree of reduction in both groups, and warming to 45°C produced equivalent vasodilation in each group to almost three times basal flow, which peaked after ~3 min. Raising the arm above heart level reduced flow equivalently, and lowering it caused a fivefold rise that was not dissimilar in diabetic and control subjects. A repeated-measures ANOVA with contrast testing as described above also showed no significant differences between control and type 2 diabetic subjects during either warming or lowering of the limb. There were significant correlations of vibration perception with the basal blood flow response ($r = 0.45$, $P < 0.05$) and cold-induced vasoconstriction ($r = 0.48$, $P < 0.05$) at this measurement site.

CONCLUSIONS — In type 2 diabetes, we found the predominant abnormality in skin blood flow was the loss of the active neurogenic vasodilative mechanism in non-glabrous (hairy) skin (20,25). In the glabrous skin of the finger pulp, the absence of any clear abnormality indicates that these distal vessels retained their reactivity and distensibility in the presence of diabetes. In stark contrast, the heat-induced dilative response in hairy skin of the hand was impaired in the type 2 diabetic group to one-half that of normal healthy control subjects, similar to that reported by others (3,24). In hairy skin, this mechanism is mediated through the action of small fiber nociceptors (Fig. 1). The preservation in glabrous skin of normal heat-induced vasodilation and distension with hydrostatic pressure suggests that the distal vessels are not yet angiopathic,

while the profound abnormality in adjacent non-glabrous skin directly implicates a subclinical neuronal defect simply because the major component of heat-induced vasodilation in this type of skin is neurogenic and there were no clinical findings of neuropathy in the upper limbs.

More recently, the role of endothelium and nitric oxide has been implicated in the impaired vasodilation in diabetes (31). There are conflicting reports in the literature. Tur et al. (16) showed a 23% reduction in peak post-ischemic flow in type 2 diabetes, whereas another study (2) in type 1 diabetes found no significant difference. In the present study, there is little evidence for a defect in this mechanism in either glabrous or non-glabrous skin of the upper extremity. Tur et al. used higher brachial artery pressure (300 mmHg) but slightly shorter duration (4 min). It is also possible that our laser Doppler technique fails to detect large changes in healthy subjects, obscuring differences between them and diabetic subjects. Of great interest, we find correlations between blood flow and systolic blood pressure and LDL cholesterol. How these affect the regulation of blood flow is obscure and may further complicate the factors important for regulation of blood flow. Because hypertension and abnormalities in lipid metabolism are precursors for the development of type 2 diabetes (32), intriguing possibilities arise. Our patients had low systolic blood pressure, LDL, and total cholesterol and moderately raised triglycerides. It is beneficial to determine the utility of this test to show endothelial pathology that may be present in humans with diabetes or elements of the metabolic syndrome that could be indirectly perturbed, e.g., hypertension and hypertriglyceridemia (33).

Most other studies of glabrous skin in diabetes have concentrated on the lower limb because of the likely relationship of skin blood flow with neuropathy (34) and the development of lower limb ulcers and amputations (35). The glabrous skin of the foot (toe pulp) was affected by diabetes much more than the glabrous skin of the hand (finger pulp) (3,5). Rendell and Bamisedun (3) have argued that the higher systemic pressure in the lower limb might accelerate microangiopathic processes. Arora et al. (36) have recently reported that the abnormalities of blood flow in hairy skin of the upper and lower limb in diabetes are very similar. Because we show a loss of neurogenic vasodilation in the upper limb, which was spared of severe neuropathy and ulceration, this process may precede other microangiopathic processes that are accelerated in the lower limb because of increased systemic pressure. Our findings of no defect in sympathetic responsiveness to cold pressor in glabrous skin also support this notion. Capillary density has been shown to be unrelated to these types of functional defects (37). Therefore, it is likely that neurogenic vasodilation is decreased in most of the body, and a functional pressure-related angiopathy may be superimposed on this defect in the glabrous skin of the foot, making it particularly susceptible to the types of damage encountered in diabetic patients with repeated minor trauma in insensate feet.

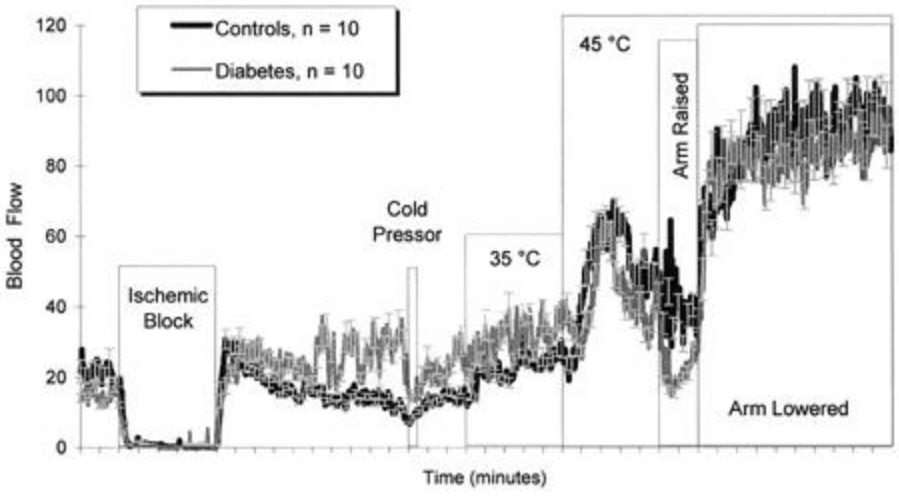

Figure 3—*Skin blood flow measured from the pulpar surface of the index finger in both groups of subjects. Data are displayed as group means ± SEM* (n = *10).*

In 1994, Jaap et al. (31) suggested that failure of skin vasodilation occurs before the onset of type 2 diabetes and that this failure is related to insulin resistance (38). Only very recently, the same group has suggested that sensory neurons play a role in the dilation that follows iontophoresis of acetylcholine or sodium nitroprusside (39). Our findings are compatible with this view, but based on the differences we show between glabrous and hairy skin, we suggest that the sensory neurons in this model are more important than previously thought.

Very recent evidence in animals suggests that capsaicin-sensitive sensory neurons play a role in regulation of glucose tolerance and insulin sensitivity as well (40,41). Thus, defective regulation of the neurovascular unit may be a further component of the metabolic syndrome (33). If the same proves true in humans, then this may represent a primary defect in type 2 diabetes, compounding or even causing insulin resistance, hypertension, or dyslipidemia. In light of this possibility, however remote, it becomes extremely attractive to fully investigate the role of these neurons and the capsaicin receptor specifically in diabetes.

In summary, we have described defective vasodilation in hairy skin of diabetic humans, which strongly suggests a small fiber neurogenic defect in the absence of a microangiopathic defect. We suggest that heat-induced vasodilation should be regarded as a very specific test of small heat-sensitive peripheral sensory nerve fibers regulating the neurovascular unit. The role of microvascular regulation in the complications of the diabetic foot remains crucial, and this theoretical model of neurogenic vasodilation provides a testable, falsifiable hypothesis that is of considerable value in understanding the complications of diabetes.

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