Original Articles

Microvascular Blood Flow, Volume, and Velocity Measured by Laser Doppler Techniques in IDDM

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A laser Doppler device with the capability to simultaneously measure skin blood flow, microvascular volume, and erythrocyte velocity was used to assess blood flow changes in 35 insulin-dependent diabetes mellitus (IDDM) subjects, mean age 33 ± 1 yr, with average duration of diabetes 14 ± 1 yr, and in a nondiabetic control group. Blood flow was determined at 35 and 44°C at several sites on the upper and the lower extremities with a temperature-regulated probe. Blood flow was highest at both temperatures on the pulps of the index finger and the first toe, regions of high density of arteriovenous anastomoses. There was significantly greater blood flow at most locations for the nondiabetic than the diabetic group at 35°C, and the differences between the two groups were substantially larger at 44°C. At 44°C, blood flow in the control group was ~40% greater in the upper extremity and 50% greater in the lower extremity than it was in the diabetic subjects. The differences were attributed to decreases of both microvascular volume and velocity in the diabetic group. In the upper extremity, volumes in the diabetic patients were 10–15% lower and velocities 10–40% lower than in the nondiabetic subjects. In the lower extremity, volumes were 20–25% lower and velocities 40–50% lower. We conclude that laser Doppler techniques can be used to assess microvascular changes in the skin of diabetic patients. This approach may be useful to evaluate and model diabetic microangiopathy. *Diabetes* 38:819–24, 1989

Although the most deleterious effects of diabetic microangiopathy are seen in the retina and kidney, there is much evidence that the process is generalized, involving most capillary beds (1–3). The most accessible microvascular bed is that of the skin, and skin blood flow in diabetic individuals has been studied by various techniques including video microscopy (4,5), venous occlusion plethysmography (6–8), and laser Doppler flowmetry (9,10). The laser Doppler technique is the simplest procedure to perform technically and may be applied almost anywhere on the skin. Measurements of skin blood flow by laser Doppler correlate well with those obtained with plethysmography, heat thermal clearance, and Xe-133 washout (11–14). However, laser Doppler measurements reflect information at a more superficial skin depth than plethysmography, xenon clearance, and heat thermal clearance because of limited penetration (~1 mm) of the laser beam in the dermis of the skin (11,12,15,16). Conversely, the laser Doppler beam penetrates to deeper levels than the superficial capillary layer accessible with video microscopy (13,17). Although each technique provides slightly different information about skin blood flow, the various studies with different techniques all suggest abnormalities in capillary blood flow and its regulation in the skin of diabetic patients. These abnormalities increase with duration of diabetes and are more pronounced in patients with poor diabetic control (7,8). Therefore, it appears that skin blood flow is a useful correlate of systemic diabetic microangiopathic disease.

In this article, we describe an extension of laser Doppler analysis of skin blood flow in diabetic subjects. For the first time, the two independent components of blood flow, microvascular volume and erythrocyte velocity, have been separately quantitated by a practical application of laser Doppler theory (18,19). In addition, measurements were performed at a much broader range of anatomic sites than in previous studies. Skin microvasculature varies significantly depending on location. The capillary loops subserving skin nutrition carry much less blood than either the deeper subpapillary plexi or arteriovenous (AV) anastomoses, which play a major role in thermoregulation (20,21). Areas like the pulp of the fingertip and toe have a much larger density of AV anastomoses than other locations, such as the knee or elbow

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(21,22). Most studies have concentrated on comparisons of diabetic and nondiabetic subjects by testing only one location, usually the fingertip or toe, where the density of AV anastomoses is extremely high, and microcirculatory blood flow is dominated by this nonnutritive flow under sympathetic control. In this study, several different sites on both upper and lower extremities were tested for each diabetic and nondiabetic subject, comparing areas with dominant AV flow to those with mainly nutritive capillary flow.

To more fully characterize potential differences in blood flow between diabetic and nondiabetic subjects, both basal and maximal blood flow conditions were measured. Basal skin blood flow is only a tiny fraction of the maximal possible flow. When stimulated, skin blood flow increases massively. Therefore, comparing diabetic and nondiabetic individuals while they are in the basal state may reflect differences only in basal control of the skin microvasculature. Capillary impairment in the basal state could be compensated by control regulation via the large reserve capacity of the skin. Some studies have used postocclusion reactive hyperemia to attempt to generate maximal blood flow. However, Johnson et al. (23) have demonstrated that local heating of the skin to 42–44°C generates a peak response that surpasses that of reactive hyperemia by ~40%, and reactive hyperemia does not further increase flow in skin heated to this temperature. In our study, local heating of the skin to 44°C was used to induce maximal flow.

RESEARCH DESIGN AND METHODS

There were 35 diabetic subjects (18 men, 17 women), mean age 33 ± 1 yr, and 30 nondiabetic control subjects (14 men, 16 women), mean age 31 ± 1 yr (NS). The average duration of diabetes was 14 ± 1 yr. The average glycosylated hemoglobin in the diabetic group was 11.3 ± 0.6%.

Patient selection. Insulin-dependent diabetes mellitus patients <45 yr of age were chosen. To clearly delineate a diabetic group at risk for microangiopathy, a minimum duration of diabetes of 5 yr was required. An age- and sex-matched group of nondiabetic individuals served as a control population. Informed consent was secured from all participants. Smokers, patients with anemia, and individuals on drug regimens known to affect blood flow (e.g., anticoagulants, nitrates, Ca^{2+}-channel blockers, vasodilators, adrenergic agents, and pentoxifylline) were excluded from the study. Patients taking antiplatelet agents were not excluded.

Both diabetic and nondiabetic subjects underwent a careful clinical examination, including detailed examination for neuropathy and peripheral vascular disease. Patients with clinical evidence of peripheral vascular disease, either arterial or venous, were excluded. Diabetic patients with neuropathy were included, but nondiabetic volunteers with evidence of neurologic abnormality were excluded.

Laser Doppler flowmetry. The TSI instrument (model BPM403, St. Paul, MN) used in this study has a low-power laser diode with which coherent light (2 mW at 780 nm) is delivered to the tissue by a flexible fiber-optic probe. At 780 nm, the laser light is only minimally absorbed by melanin, so there is no potential problem with testing dark-skinned subjects (24). This low-power light has no measurable effect on the tissue. As light enters the tissue, it is diffusely scattered in a random fashion principally by stationary tissue cells, which because of their lack of motion do not impart Doppler shifts. A fraction of the light is also scattered by moving erythrocytes within the microcirculation, leading to detectable Doppler shifts of 100–1000 Hz. The diffusely scattered light is sampled on the skin surface by a pair of optical fibers located 0.5 mm from the laser delivery fiber. The sampling fibers transmit the light back to the signal processor, which consists of a photodetector and microcomputer. The microcomputer stores the fluctuating light signal in memory and at every 100 ms computes the fraction of the light signal that is Doppler shifted and the mean of the absolute value of the Doppler frequency shift. The product of these two variables is the optimal laser Doppler flow parameter as determined theoretically (18). As long as sufficient light is returned by the sampling fibers, the calculation proceeds to output a result. Because of the wavelength of transmission at 780 nm, deeply pigmented or thick skin does not constitute an obstacle to measurement. If for any reason the fraction of sampled light is insufficient to determine the parameters, the instrument flashes an error message.

The blood volume parameter is derived from the fraction of the light that is Doppler shifted. The detected light has typically had a path of 2–3 mm within the tissue during which its likelihood of being Doppler shifted is proportional to the concentration (number density) of moving erythrocytes within the tissue. Theoretically, m, the mean number of Doppler-scattering events per photon, is given by the product of the concentration of erythrocytes ([erythrocyte]), the scattering cross section of the erythrocyte (S_{mc}), and the photon path length (L_{ph}): $m = [\text{erythrocyte}] \cdot S_{mc} \cdot L_{ph}$ (18). For human skin, both the mean scattering cross section of the erythrocyte (35 μm^2) and the mean photon path length (2.5 mm) are essentially invariant when averaged over $>10^4$ erythrocytes and $>10^8$ photons for each measurement. Therefore, the TSI instrument outputs a blood volume parameter that is actually m, which when multiplied by 1.2×10^4 gives the number of moving erythrocytes per cubic millimeter of tissue.

The instrument also computes the mean absolute value of the Doppler frequency shift. Because both the microcirculation and the incident light is random, Doppler shifts that increase the frequency are equally likely to occur as those that decrease the frequency (unlike Doppler ultrasound of large-vessel flow). It has been shown theoretically that the mean absolute value of the Doppler shift is linearly proportional to the root-mean-squared speed of all the moving erythrocytes within the sampled volume (18). The constant of proportionality or conversion factor is dependent on invariant optical constants of the instrument and the mean angle at which the $>10^8$ photons are scattered by the $>10^4$ erythrocytes. This factor may be computed theoretically to be ~100 $Hz \cdot mm^{-1} \cdot s^{-1}$. However, alternative techniques to measure erythrocyte speed in the same tissue volumes do not exist. Therefore, the calibration factor has not been experimentally verified by other techniques, and we report velocity in the original frequency units (Hz).

The TSI flow parameter is the product of the mean number of Doppler events per photon and the mean absolute magnitude of the Doppler shift in hertz. This flow parameter, in units of hertz, has been correlated with values obtained

via Xe and H_2 clearance and microsphere deposition in various tissues. The calibration factor of 6 ml · 100 g^{-1} · min^{-1} · 100 Hz^{-1} has been well verified against these alternative techniques.

A temperature-control module, TSI model TCM 420, was used for local skin temperature control. The ends of the laser Doppler fiber-optic probes are inserted into a 19-mm-diameter thermal head attached to a separate solid-state controller. The controller accurately maintains the temperature within ±0.5°C of set point. This module allows for temperature control within a range of 35–45°C.

The probe was placed on the skin, taking care to avoid placing the fiber-optic ends directly over a superficial vein or hair follicle. Blood flow was first obtained at 35°C. Mean flow was measured with a 5-s averaging time to encompass cardiac pulsatile activity. Pulse peak blood flow was measured with a 0.2-s averaging time. With this time constant, cardiac cycles were easily visualized. Simultaneous readout of the volume and velocity was obtained. The temperature was then raised to 44°C. Blood flow increased in response to thermal stimulation, usually in a series of stepwise elevations. When equilibration was obtained at the plateau value, the flow parameters were recorded at both 5.0- and 0.2-s averaging times. In practice, it is sometimes difficult to recognize the maximal plateau value because a series of plateaus may occur over a period of 5–10 min. Furthermore, a fall from the maximal plateau may occur after several minutes. Therefore, the readout is followed continuously by the operator who switches to the 0.2-s averaging time whenever a plateau appears. Volume and velocity were determined at a 5-s averaging time to exclude the effect of cardiac pulsations.

The following locations were tested: the pulp of the tip of the index finger; the dorsal surface of the distal phalanx of the index finger; the ventral surface of the wrist, in the midline; the ventral surface of the elbow; the face, 1 cm in front of the tragus of the ear; the pulp of the tip of the great toe; the extensor surface of the distal phalanx of the great toe; the extensor surface of the ankle, at the midline; and the extensor surface of the leg, 2 cm below the knee, in the midline.

The pulp of the fingertip and toe and the facial region are areas of very high blood flow because of a high density of

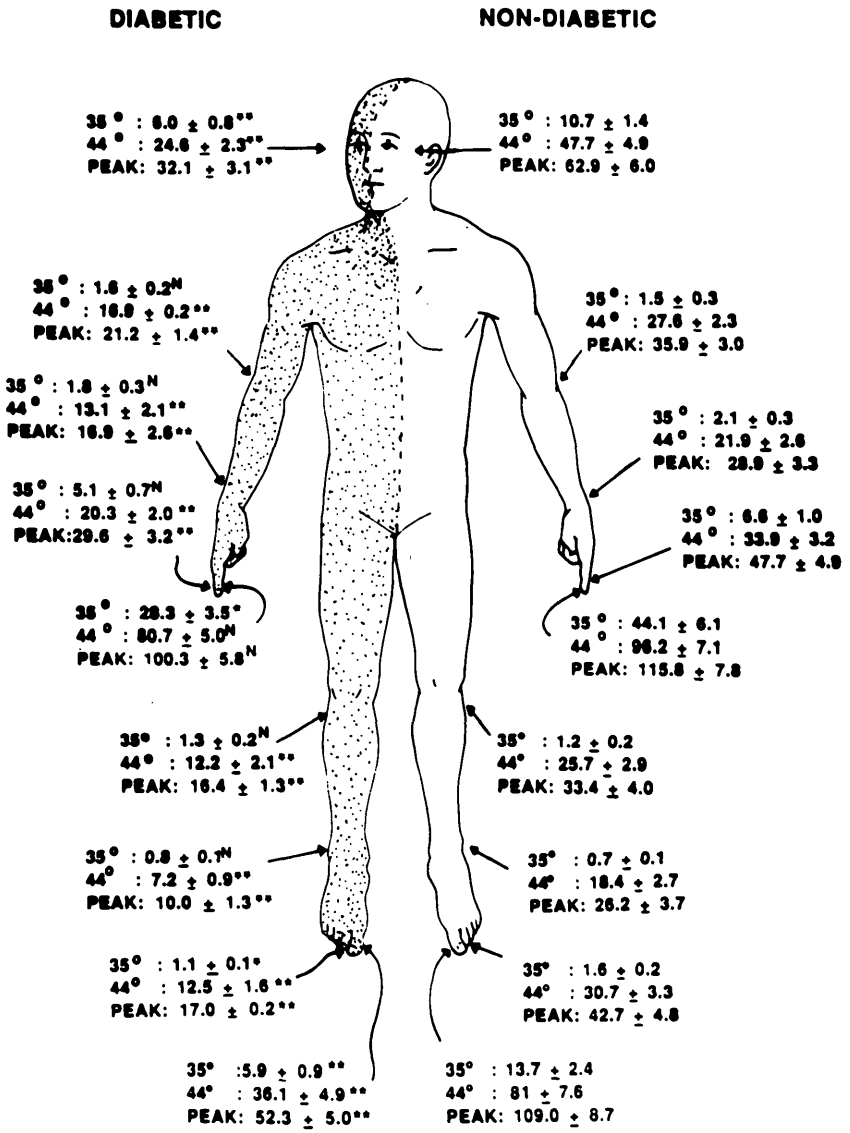

FIG. 1. Comparison of skin blood flow changes in diabetic and nondiabetic subjects. Blood flow (ml · min^{-1} · 100 g^{-1}) was measured at 35 and 44°C with 5-s averaging and at peak blood flow (44°C) with 0.2-s averaging. Results are means ± SE. N, not significant; *$P < .05$; **$P < .01$.

AV anastomoses. The other areas studied have mainly a nutritive capillary supply. At these various sites, the thickness of the nonperfused stratum corneum varies dramatically. For all but the pulps of the fingertip and the toe, the thickness of this highly transparent layer has virtually no effect on laser Doppler measurements. However, for thick layers of callus on the fingertips and toes, there may be sampling of the dead epidermal layers, and flow values are consequently reduced. For this reason, we avoided measuring sites when callus was present. Increased local blood flow around hair follicles might also increase measurement variability. We selected sites where apparent hair follicles were not present.

RESULTS

At 35°C, flow was greatest in areas with high densities of AV anastomoses, i.e., the face and fingertip and toe pulps (Fig. 1). There was significantly greater blood flow at 35°C in the nondiabetic than the diabetic subjects on the face, fingertip pulp, dorsum of the toe, and toe pulp but not at the other locations (Fig. 1). Thermal stimulation to 44°C produced a substantial increase in blood flow at all locations for both contrast groups (Fig. 1). Flow was highest on the fingertip and toe pulps, where values >100 ml · min^{-1} · g^{-1} were recorded in the control group. Although blood flow at the other locations did not attain these high values, the proportional increase was much greater, in many cases 15–20 times the 35°C value. There was no significant difference in blood flow between men and women at either 35 or 44°C.

In the diabetic group, the thermally stimulated response was significantly lower than that of the control group at all sites except the fingertip pulp (Fig. 1). The comparative difference was ~40% on most upper-extremity locations and >50% on the lower extremity, including the toe pulp.

Despite these significant differences, there was also considerable variation in both groups. The variability was greater at 35°C (at which coefficients of variation [C.V.] at most sites ranged from 80 to 100%) than at the maximal flow state of 44°C, at which C.V. still reached 50–60%. The variation in the derived flow parameter is attributed to the combined variability of its two components. Comparison of microvascular blood volume and velocity in the two contrast groups showed that variations in velocities were much greater than in volumes, and variations were greater for both parameters at 44 than at 35°C (Figs. 2 and 3). At 44°C, C.V. of velocities ranged 40–50%, whereas those of volumes were 20–30%.

At 35°C, significant differences between diabetic and nondiabetic subjects existed only on the fingertip pulp, face, and toe pulp (Fig. 2). Thermal stimulation to 44°C produced

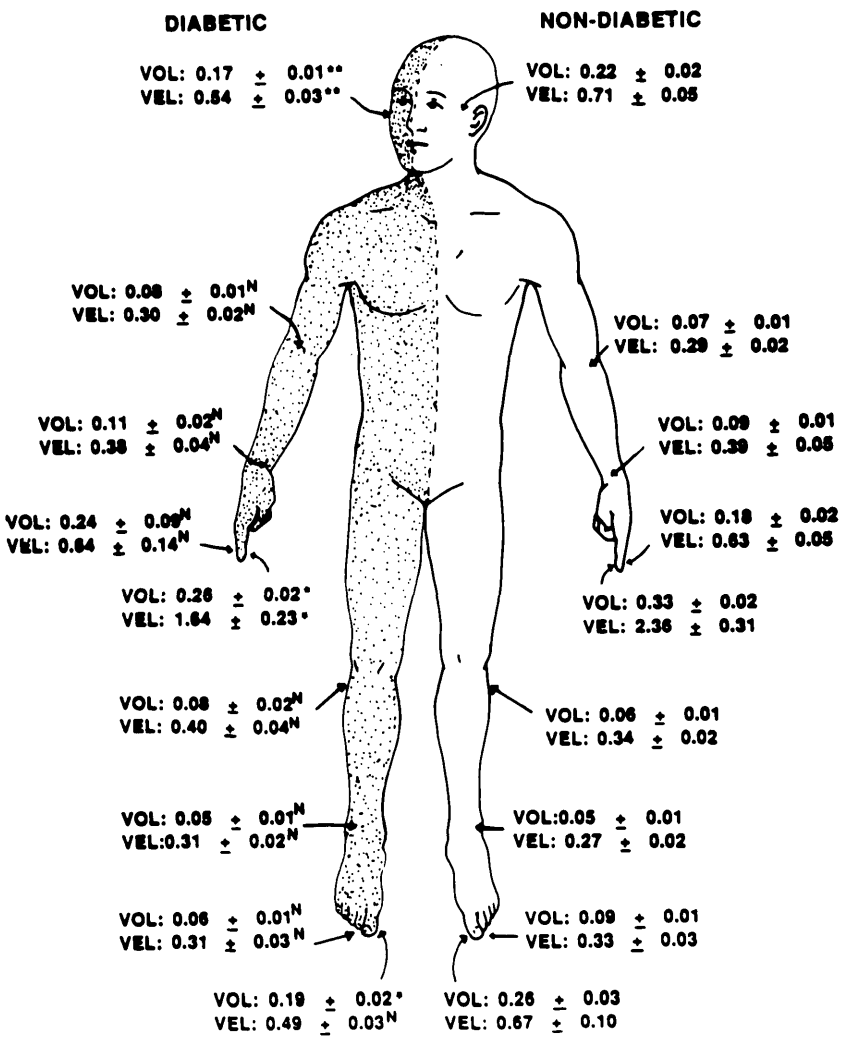

FIG. 2. Comparison of volume and velocity changes in diabetic and nondiabetic subjects at 35°C. Microvascular volume (erythrocytes/mm^3 × 1.2 × 10^4) and velocity (Hz × 10^3) were measured at 35°C with 5-s averaging. Results are means ± SE. N, not significant; *$P < .05$; **$P < .01$.

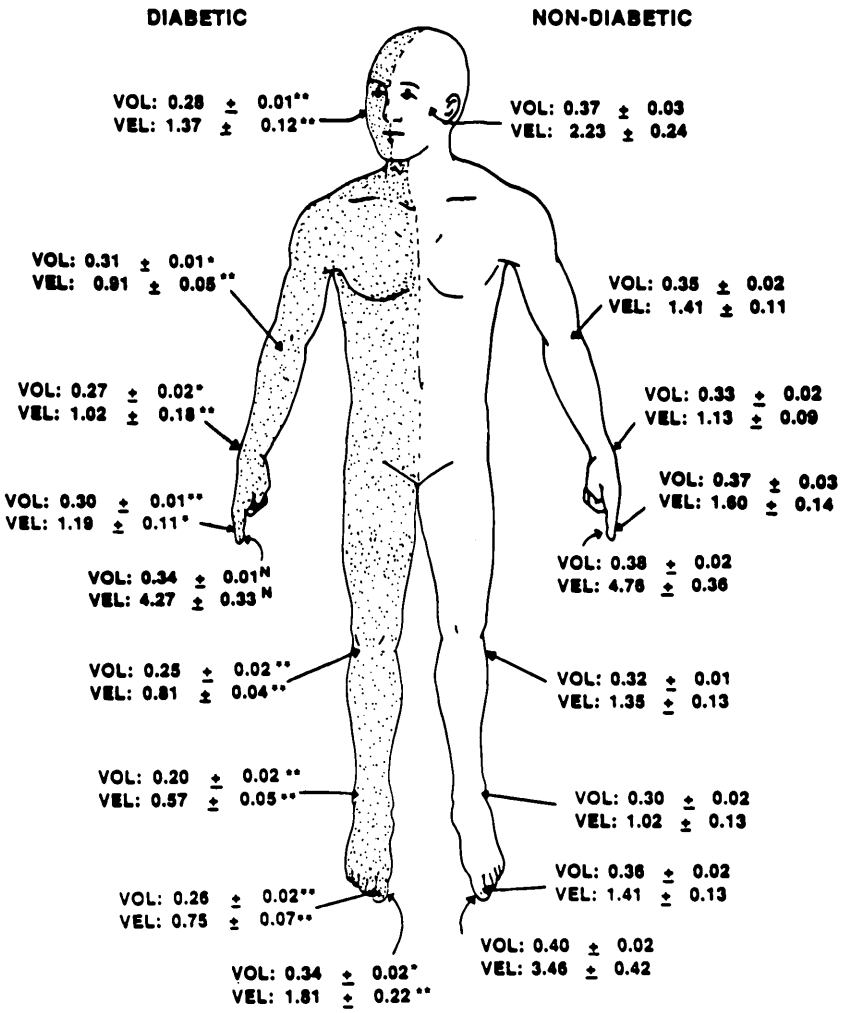

FIG. 3. Comparison of volume and velocity changes in diabetic and nondiabetic subjects at 44°C. Microvascular volume (erythrocytes/mm^3 × 1.2 × 10^4) and velocity (Hz × 10^3) were measured at 44°C with 5-s averaging. Results are means ± SE. N, not significant; *P < .05; **P < .01.

increases in both volume and velocity in both groups (Fig. 3). In the nondiabetic group, the increase was greater in velocity than volume on the face, fingertip pulp, and toe pulp. At the other sites, volume and velocity increased in tandem, usually three- to fourfold. The diabetic subjects showed significantly smaller thermally induced increases than the control subjects in both volume and velocity at all sites but the fingertip pulp. The relative decreases in the upper extremity in the diabetic group with respect to the control group were 10–15% in volume and 10–40% in velocity. In the lower extremity, the diabetic subjects showed volumes that were 20–25% lower, with velocities 40–50% lower. There were 7 patients in the diabetic group with neither symptoms nor physical findings of diabetic neuropathy. There were 14 diabetic subjects who had both symptoms and physical findings indicating diabetic neuropathy. There were no significant differences in blood flow between those with and without diabetic neuropathy.

DISCUSSION

Findings from our study confirm those from earlier investigations that involved various techniques to demonstrate impairment in skin blood flow in diabetic patients. We have gone further by showing that this abnormality extends to most sites on the extremities and face. The deficit in skin blood flow appears to be greatest in the lower extremities, perhaps reflecting a contribution of occult large-vessel disease. However, that deficits appear on both upper and lower extremities with both a proximal and distal distribution argues against accelerated large-vessel disease as the principal cause of the demonstrated abnormality.

That no distal predominance exists also argues against diabetic neuropathy as a major cause of the blood flow abnormalities. A further argument is furnished by the demonstration of an identical degree of blood flow abnormality in a diabetic subgroup without neuropathy. Although peripheral neuropathy does not appear to explain the blood flow abnormalities observed, it could still be argued that a local neural response may be deficient. Both local and reflex influences mediate skin blood flow (25,26), and thermally induced vasodilation occurs despite denervation (27). These local mechanisms could conceivably be disrupted in diabetes despite no evidence of neuropathy.

The most important finding in our study is that microvascular volume is decreased in the skin of diabetic patients. There are two possible explanations for this apparent loss of volume. One is capillary dropout, as occurs in diabetic retinopathy or diabetic glomerulopathy. Loss of cutaneous capillaries has not been demonstrated by video microscopy (7). However, the penetration of laser light is greater than

that of video microscopy, which only visualizes superficial capillaries. Perhaps capillary loss is at a deeper level than can be visualized by video microscopy. Another problem with the results of video microscopy is that this technique is performed on unheated skin, where the number of perfused capillaries might be expected to be substantially lower than after thermal stimulation. At 35°C, the laser Doppler technique demonstrated minimal volume differences between diabetic and nondiabetic subjects. It was only at 44°C that volume differences were manifest at most sites.

The alternative explanation for volume loss in the diabetic group is failure to thermally autoregulate the opening of channels to part of the viable capillary bed. Our data cannot distinguish between these two possibilities. Thermal stimulation produces maximal blood flow in normal skin. Reactive hyperemia causes no added effect. We verified that this was also true for the diabetic patients we studied. Therefore, we have no alternative to heat to stimulate blood flow beyond the values obtained at 44°C to test our hypotheses.

The decreased velocity in the diabetic group could be explained by multiple pathogenic mechanisms. Large-vessel disease upstream from the capillary bed may certainly contribute, particularly to the greater decreases on the lower extremity. However, the impairment in velocity showed no distal predominance. On the contrary, the greatest decrease on the upper extremity was at the elbow. Furthermore, we chose relatively young individuals for our study and specifically excluded anyone with clinical evidence of peripheral vascular disease.

A second possible explanation might be selective loss of high-velocity AV shunts. That no difference was found between diabetic and nondiabetic subjects on the fingertip pulp, which has a high density of shunts, seems to argue against this possibility.

A third possible cause of decreased erythrocyte velocity in diabetic individuals is increased resistance to flow in the microvascular bed. Structural changes in the capillaries or pre- or postcapillary vessels could be responsible. Alternatively, increased viscosity of the erythrocytes themselves, known to occur in diabetes, could cause a decrease in mean erythrocyte speed, particularly for capillary flow (28).

Finally, decreased cardiac output caused by occult diabetic cardiomyopathy could also account for decreased microvascular velocity. Variability in cardiac output might also represent one of the major sources of variation encountered in measurement of velocities.

The results of this study demonstrate that microangiopathy affects the microvascular bed in a widespread distribution in the skin of diabetic patients. The process involves a decrease in both microvascular volume and erythrocyte velocity. It is too early to say that skin microangiopathy mirrors the processes occurring in retinal and renal microvasculature. It will be necessary to study a much larger group of diabetic patients to assess the correlations between known diabetic retinopathy or nephropathy and skin blood flow changes. The possibility of using the skin as a model for diabetic microangiopathy would have great practical importance, both experimentally and in clinical practice. In this regard, it is important that microvascular volume measurements carry less inherent variability than erythrocyte velocity or derived flow determinations. Therefore, it appears that the volume parameter would be ideally suited to serial contrast analyses.

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REFERENCES

1. Gundersen HJG: Peripheral blood flow and metabolic control in juvenile diabetes. *Diabetologia* 10:225–231, 1974
2. McMillan DE: Pathophysiology of diabetic micro and macrovascular disease. In *Diabetes Mellitus: Theory and Practice*. Ellenberg M, Rifkin H, Eds. New Hyde Park, NY, Med. Exam., 1983, p. 343–60
3. McMillan DE: The microcirculation: changes in diabetes mellitus. *Mayo Clin Proc* 63:517–20, 1988
4. Fagrell B, Hermansson IL, Korlander SG, Ostergren J: Vital capillary microscopy for assessment of skin viability and microangiopathy in patients with diabetes mellitus. *Acta Med Scand* 687:25–28, 1984
5. Tooke JE, Lins PE, Ostergren J, Adamson U, Fagrell B: The effects of insulin infusion on skin microcirculatory flow in type I diabetes. *Int J Microcirc Clin Exp* 4:69–83, 1985
6. Christensen NJ: Spontaneous variations in resting blood flow, post ischemic peak time and vibratory perception in the feet of diabetics. *Diabetologia* 5:171–78, 1969
7. Tooke JE, Lins PE, Ostergren J, Fagrell B: Skin microvascular autoregulatory responses in type I diabetes: the influence of duration and control. *Int J Microcirc Clin Exp* 4:249–56, 1985
8. Jorgensen RG, Russo L, Mattioli L, Moore WV: Early detection of vascular dysfunction in type I diabetes. *Diabetes* 37:292–96, 1988
9. Rayman G, Hassan A, Tooke JE: Blood flow in the skin of the foot related to posture in diabetes mellitus. *Br Med J* 292:87–91, 1986
10. Rayman G, Williams SA, Spencer PD, Suge LH, Wise PH, Tooke JE: Impaired microvascular hyperaemic response to minor skin trauma in type I diabetes. *Br Med J* 292:1295–98, 1986
11. Saumet JL, Dittmar A, Leftheriotis G: Non-invasive measurement of skin blood flow: comparison between plethysmography, laser Doppler flowmeter and heat thermal clearance method. *Int J Microcirc Clin Exp* 5:73–83, 1986
12. Engelhart M, Nielsen HV, Kristensen JK: The blood supply to fingers during Raynaud's attack: a comparison of laser Doppler flowmetry with other techniques. *Clin Physiol* 5:447–53, 1985
13. Fagrell B: Dynamics of skin microcirculation in humans. *J Cardiovasc Pharmacol* 7 (Suppl. 3):S53–58, 1985
14. Winsor T, Haumschild DJ, Winsor DW, Wang Y, Luong TN: Clinical application of laser Doppler flowmetry for measurement of cutaneous circulation in health and disease. *Angiology* 38:727–36, 1987
15. Nitzan M, Fairs SLE: Simultaneous measurement of skin blood flow by the transient thermal clearance method and laser Doppler flowmetry. *Med Biol Eng Comp*. In press
16. Johnson JM, Taylor WF, Shepherd AP, Park MK: Laser Doppler measurement of skin blood flow: comparison with plethysmography. *J Appl Physiol* 56:798–803, 1984
17. Tyml K, Ellis CG: Evaluation of a laser Doppler flow meter by video microscopy. *IEEE 7th Annu Conf Eng Med Biol Soc, 1985*, p. 528–31
18. Bonner R, Nossal R: Model for laser Doppler measurements of blood flow in tissue. *Appl Optics* 20:2097–107, 1981
19. Bonner RF, Clem TR, Bowen PD, Bowman RL: Laser-Doppler real time monitor of pulsatile and mean blood flow in tissue microcirculation. In *Scattering Techniques Applied to Supramolecular and Nonequilibrium Systems*. Chen SH, Chu B, Nossal R, Eds. New York, Plenum, 1981, p. 685–702 (NATO ASI Ser. B, Vol. 73)
20. Zweifach BW: Structural and functional aspects of the microcirculation in the skin. In *The Microcirculation*. Reynolds SKM, Zweifack BW, Eds. Urbana, Univ. of Illinois Press, 1959, p. 144–50
21. Rowell LB: Reflex control of the cutaneous vasculature. *J Invest Dermatol* 69:154–66, 1977
22. Popoff NW: The digital vascular system. *Arch Pathol* 18:295–330, 1934
23. Johnson JM, O'Leary DS, Taylor WF, Kosiba W: Effect of local heating on forearm reactive hyperemia. *Clin Physiol* 6:337–46, 1986
24. Rodgers GP, Schechter AN, Noguchi CT, Klein HG, Nienhius AW, Bonner RP: Periodic microcirculatory flow in patients with sickle-cell disease. *N Engl J Med* 311:1534–38, 1984
25. Ahmad A: Response of the blood vessels of the upper extremity to prolonged local heat. *Clin Sci* 15:609–16, 1956
26. Barcroft H, Edholm OG: Temperature and blood flow in the human forearm. *J Physiol* (Lond) 104:366–76, 1946
27. Crockford GW, Hellon RF, Parkhouse J: Thermal vasomotor responses in human skin mediated by local mechanisms. *J Physiol* (Lond) 161:10–20, 1962
28. McMillan DI: The effect of diabetes on blood flow properties. *Diabetes* 32 (Suppl. 2):56–63, 1983