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Peripheral Vasoconstrictor Responses to Sympathetic Activation in Diabetic Patients: Relationship With Rheological Disorders

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The sympathetic nervous system regulates peripheral blood flow. This study investigated sympathetic vascular control in diabetic patients by measuring cutaneous blood flow (CBF) with a laser Doppler device at rest and during three sympathetic activation tests: deep-breathing, changing positions from sitting to standing, and using the Valsalva maneuver. The influence of various factors, particularly rheological changes, was also assessed. Forty-two type II diabetes mellitus (non–insulin-dependent [NIDDM]) patients and 14 control subjects were studied. The mean value and standard deviation (SD) of basal CBF at rest were not significantly different in the two groups. In 12 NIDDM patients, the SD was less than the lowest limit found in the controls. During the three tests, the reduction in CBF and its downward slope were lower in NIDDM patients than in controls, with the greatest difference occurring during the deep-breathing test. During this test, the downward slope of CBF was less than the lowest control level in 14 NIDDM patients. The log SD of basal CBF correlated with the decrease in CBF during the sitting-to-standing and Valsalva tests in control subjects and during all three tests in NIDDM patients. In NIDDM patients, log mean basal CBF correlated negatively with the log erythrocyte filtration index ([FI] an index of rigidity) and positively with hemoglobin A_{1c} (HbA_{1c}). The log downward slope of CBF during the deep-breathing test correlated negatively with log erythrocyte FI. The log downward slope of CBF during the sitting-to-standing and Valsalva tests correlated positively with total cholesterol and triglycerides, respectively. Basal CBF and the CBF response to these tests did not correlate with retinopathy, nephropathy, peripheral neuropathy, or heart rate variations during these tests. This study suggests that CBF assessment by laser Doppler flowmetry affords an attractive noninvasive way to investigate sympathetic nervous function in diabetic patients. The amplitude of changes in basal CBF and the decrease in CBF during the deep-breathing test show that this is a simple and sensitive procedure for detecting sympathetic nerve dysfunction. Moreover, rheological blood properties and metabolic factors seem to strongly influence resting CBF and vasomotor reflexes.

CARDIAC AUTONOMIC NEUROPATHY is a frequent complication of diabetes mellitus. Subclinical cardiac autonomic neuropathy may be assessed by standardized tests.[1] These tests are mainly based on measurement of changes in heart rate during different stimuli that involve vagal activation.[2,3] Their good reproducibility has been demonstrated.[4] However, these tests do not constitute a tool sensitive enough to detect sympathetic dysfunction. In particular, postural hypotension is a late complication of diabetic neuropathy.[5] Other methods have been proposed to investigate sympathetic nervous system function. Direct measurement of autonomic nerve fiber conduction in peripheral tissues is an invasive and difficult procedure unsuitable for clinical use.[6] Indirect methods have been used, including measurement of sweat responses[7] and pupillary abnormalities.[8,9] Spectral analysis also seems appropriate when evaluating sympathetic control of heart rate and blood pressure.[10,11]

The sympathetic nervous system regulates peripheral vasculature in the hands and feet, and therefore, sympathetic function can be studied by measuring vasoconstrictor responses in the extremities. Skin blood flow at rest has been found to be slightly reduced in type I (insulin-dependent) diabetic patients,[12] and this abnormality seems to occur early in streptozotocin-induced diabetes mellitus in the rat.[13] The spontaneous fluctuations in hand and foot blood flow are probably mediated by changes in peripheral sympathetic tone, and a reduction in these fluctuations has been found to be related to diabetic autonomic neuropathy.[14] However, blood flow at rest is dependent on several factors such as room temperature, emotional state, ambient noise, and patient cooperation. These factors probably account for a large part of the high intersubject variability in cutaneous blood flow (CBF).[15]

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It has long been recognized that deep-breathing induces a vasoconstriction in the extremities[16] that is mediated by the sympathetic nervous system[17] and disappears in denervated limbs.[16] The cold pressor test,[18-21] the Valsalva maneuver,[19,22,23] and the standing test[19,22] also induce a peripheral skin vasoconstriction mediated by the sympathetic nervous system. Methods used to measure vasoconstrictive responses include plethysmography,[14,21,24] Doppler ultrasound,[25] and thermography.[26] In several reports, laser Doppler flowmetry has been used.[19,20,22,23]

Although bedside tests of cardiac autonomic function such as the Valsalva ratio, variations of the RR interval during deep-breathing, heart rate response to standing, and sustained hand-grip give important information, they do not measure skin blood flow, and they detect widespread autonomic failure but not focal abnormality. It seemed appropriate to measure vasoconstrictor responses to different stimuli when studying peripheral sympathetic tone in patients with dysautonomia.[19] One study has also suggested that evaluating skin vasomotor reflexes of diabetic patients by laser Doppler flowmetry is useful.[23] With this method, Tooke et al[27] have shown that insulin exerts effects on skin microvascular hemodynamics independently of hypoglycemic action. Insulin also has an inhibitory effect on norepinephrine- and angiotensin II–induced contraction in both the artery and the vein.[28]

Several studies have found hemorrheological alterations in diabetic patients, consisting of an increase in blood and plasma viscosity, erythrocyte hyperaggregation, and a decrease in erythrocyte filterability.[29-31] The latter two disorders contribute to hyperviscosity and thus to slowing of blood flow. Hemorrheological disorders tend to modify skin blood flow and its changes during sympathetic stimulation.

The aim of the present study was to assess peripheral sympathetic function in diabetic patients using three different stimuli, the deep-breathing test, the Valsalva maneuver, and the standing test, to measure vascular responses by laser Doppler flowmetry, and to evaluate factors that modify the responses, particularly the rheological parameters. Only non–insulin-dependent diabetes mellitus (NIDDM) patients without insulin treatment were investigated, to rule out an exogenous insulin effect.

SUBJECTS AND METHODS

Patients

Skin vasomotor reflexes were studied in 42 NIDDM patients, 15 men and 27 women, without clinical signs of dysautonomia and without postural hypotension. The mean age was 51.2 years (range, 31 to 67). None of them had hypertension, cardiac or pulmonary disease, or anemia, and patients on drugs known to affect blood flow (eg, vasodilators, calcium-channel blockers, angiotensin-converting enzyme inhibitors, adrenergic agents, nitrates, or β-blockers) were excluded from the study. Patients taking pentoxifylline or anti–platelet-aggregation drugs were also excluded.

All patients underwent a careful clinical examination including a detailed examination for neuropathy and peripheral vascular disease. Patients with clinical evidence of arterial or venous disease were excluded. The diagnosis of peripheral neuropathy was made when two or more of the following four evaluations were abnormal[32]: symptoms, nerve conduction studies showing abnormalities in at least two different limb nerves, ankle reflex, and vibration perception threshold. Clinical and biological characteristics of the patients are shown in Table 1.

Table 1. Clinical and Biological Characteristics of the NIDDM Patients

Characteristic	Mean ± SEM
Age (yr)	51.2 ± 1.7
BMI (kg/m^2)	29.0 ± 0.7
Waist to hip ratio	0.96 ± 0.02
Peripheral neuropathy (n)	22
Retinopathy (n)	5
Nephropathy (n)	11
Blood pressure (mm Hg)	
Systolic	131 ± 2.1
Diastolic	80 ± 1.2
Creatininemia (μmol/L)	81.9 ± 1.9
Urinary albumin excretion (mg/24 h)	28.3 ± 10.9
Fasting glycemia (mmol/L)	9.16 ± 0.46
Postprandial glycemia (mmol/L)	10.53 ± 0.75
Fructosamine (nmol/L)	321 ± 12
HbA_{1c} (%)	8.03 ± 0.36
Total cholesterol (mmol/L)	5.72 ± 0.33
HDL cholesterol (mmol/L)	1.27 ± 0.06
Triglycerides (mmol/L)	1.85 ± 0.18
Uricemia (μmol/L)	253 ± 10

The data were compared with data from a healthy control group of 14 subjects aged 20 to 71 years with normal body weight (body mass index [BMI], 23.2 ± 0.8 kg/m^2; range, 18.9 to 28.0).

Laser Doppler Flowmetry

The PF2 Periflux device (Perimed, Stockholm, Sweden) was used to measure CBF. It consisted of a low-power (5 mW) helium-neon laser source. The laser light was delivered to the skin via flexible graded-index fiberoptic light guides. The wavelength of transmission was 632 nm.

While the subject was seated for at least 10 minutes in a quiet room with the temperature set at 22° to 24°C, the laser probe was attached to the right index finger with the beam aimed at the palmar side. Velocimeter flow was monitored. Basal CBF was recorded for 5 minutes. The mean and standard deviation (SD) for basal CBF were obtained. Three vasomotor tests were then performed; a deep-breathing test consisting of six deep respirations in 1 minute, a sitting-to-standing test with the subject remaining in the standing position for 1 minute, and the Valsalva maneuver with a forced exhalation of 40 mm Hg for 10 seconds in the sitting position. Before the recording, the patient was asked to practice these tests. CBF was expressed in arbitrary perfusion units (PUs), and all data were recorded by a computerized system.

During the deep-breathing test, CBF decreased during each inspiration and then increased during exhalation (Fig 1). The mean value for the three cycles that showed the greatest decrease was calculated. During the Valsalva maneuver and after a few seconds in the standing position, a decrease in CBF occurred (Fig 1). During each test, the maximum decrease in CBF was expressed as a percentage of the previous basal value and as the downward slope of CBF between the basal level and the minimum value of CBF. The reproducibility of these tests was studied in 10 controls or in patients by evaluating the three vasomotor reflexes twice in the same morning 1 half-hour apart. Coefficients of variation for the percentage of CBF decrease did not exceed 25.9% (Table 2).

Assessment of Heart Rate Variability

In another investigation, three standardized tests, deep-breathing, lying-to-standing, and the Valsalva maneuver, were performed as previously described by means of a computerized system (Autocaft;

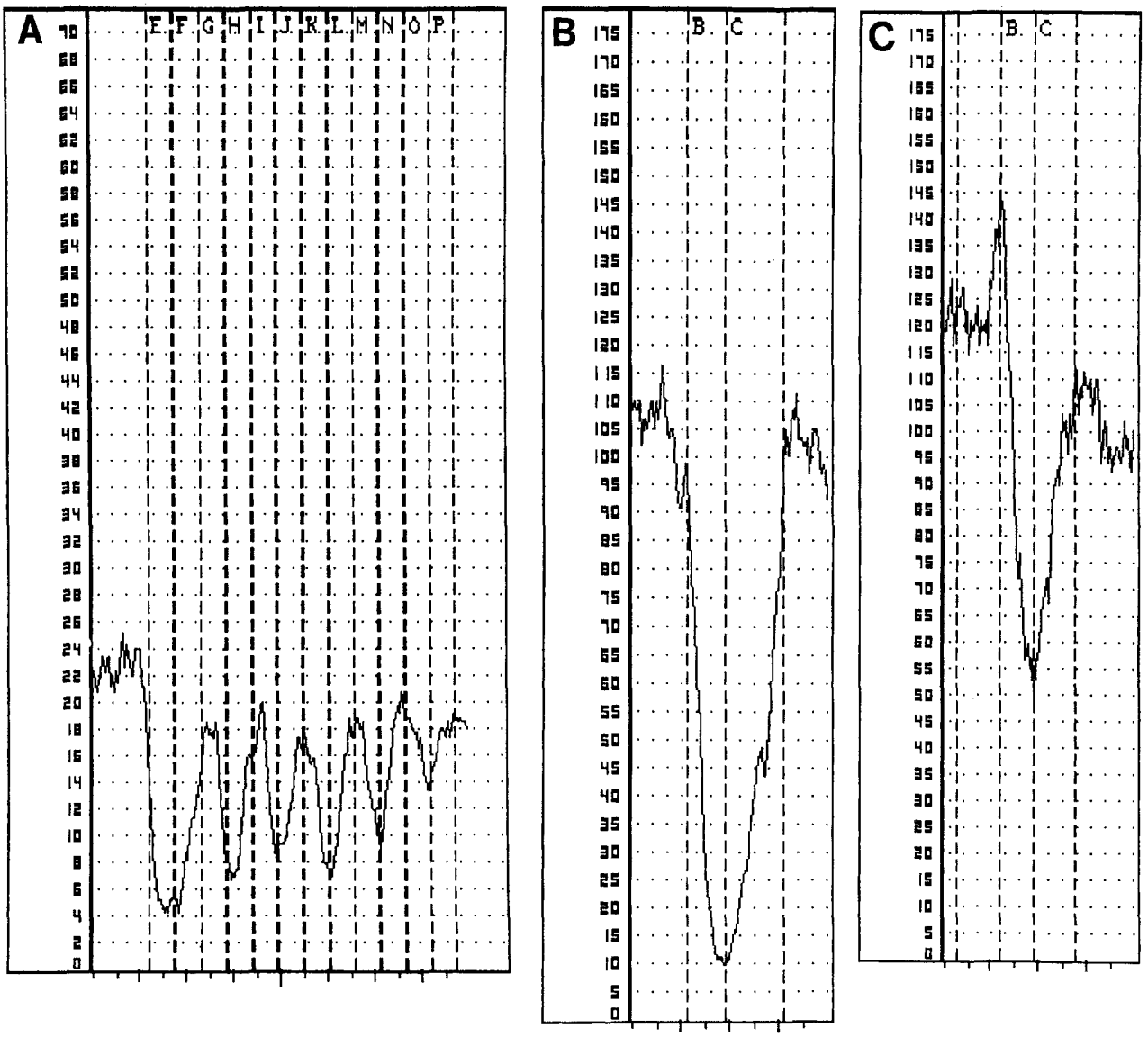

Fig 1. Changes in CBF during (A) the deep-breathing test, where CBF decreases at each inspiration and increases during exhalation; (B) the Valsalva maneuver, where CBF decreases during forced exhalation and increases at the release; and (C) the sitting-to-standing test, where CBF decreases within a few seconds in the standing position.

BBC Master, Edinburgh, UK)[4,33] in 16 NIDDM patients. The data were compared with an age-matched control series.[34]

Rheological Investigations

Measurements were made within 1 hour following venous blood sampling. Erythrocyte filterability was measured using the Hanss hemorrheometer. The initial flow rate of erythrocyte filtration was thus determined as previously described.[29,30] Briefly, the device consisted of an upper plastic block with a central capillary. The end of the capillary was cone-shaped and attached to the Nuclepore membrane (diameter, 13 mm; pore diameter, 5 μm; Pleasanton, CA). The central capillary was filled with a red blood cell (RBC) suspension or the buffer alone (Hanks, pH 7.40). Two level detectors, 9 mm apart, at the top of the capillary activated and then stopped an electronic chronometer when the meniscus of the liquid moved in front of them during the filtration procedure. The time lapse first measured was proportional to the flow rate and therefore to the overall fluidity of the liquid filtered. For the liquid as a suspension, the time lapse was called ts, and for the buffer alone it was called tb. The time lapse was proportional to the initial flow rate. The result was expressed in terms of a filtration index (FI), defined as FI = ([ts − tb]/tb) × (100/H), where H was the hematocrit. High FI values indicated low RBC deformability. The FI was determined in 31 NIDDM patients of the present series.

Erythrocyte aggregation was evaluated by means of an erythrocyte aggregometer (MA2; Myrenne, Roetgen, Germany), the principle of

Table 2. Coefficients of Variation (%) for the Percent Decrease of CBF During Three Tests of Sympathetic Activation in 10 Subjects Tested Thrice in the Same Morning at Half-Hour Intervals

Test	Mean ± SEM
Deep-breathing	24.9 ± 3.6
Standing	25.9 ± 7.0
Valsalva	25.4 ± 5.7

which is based on analysis of the incident infrared light transmitted through the blood sample.[31] The device consisted of a transparent cone-plate chamber in which blood cells were first sheared by the rotation of the cone (shear rate, 600 s^{-1}) for 10 seconds to dissociate RBC aggregates. The rotation of the cone was then either abruptly stopped to obtain the formation of rolls in stasis (shear rate, 0) or greatly slowed (shear rate, 3 s^{-1}) to obtain the formation of rolls at a very low shear rate. A photoelectrical device analyzed the variation of light transmitted through the blood sample. The mean erythrocyte aggregation indexes (MEA1 and MEA2) were obtained, representing aggregation at shear rate 0 or 3 s^{-1}. These indexes were obtained in 24 NIDDM patients in the present study.

Other Investigations

Fasting and postprandial blood glucose, fructosamine (Roche Diagnostic System, Neuilly-sur-Seine, France; normal < 285 nmol/L), HbA_{1c} (microcolumn chromatography, normal < 6.25%), serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, creatininemia, and fasting and postprandial insulinemia were assayed. Diabetic retinopathy was evaluated by means of fundus ophtalmoscopy and fluorescein angiography. Urinary albumin excretion rate was measured by immunonephelometry (BNA; Behring Institute, Mahrburg, Germany) as previously described.[35] Clinical and biological characteristics of the NIDDM patients are shown in Table 1.

Statistical Analyses

Results are expressed as the mean ± SEM. Statistical studies included ANOVAs or Mann-Whitney tests, respectively, for parameters with a gaussian or nongaussian distribution, chi-square test, Pearson linear correlations, and multiple linear regression analyses (with forward-variable selection). For the correlations, values of some parameters were log-transformed to obtain a gaussian distribution. All statistical calculations were made on a Hewlett-Packard (Evry, France) personal computer using the SPSS statistical software (SPSS Inc, Chicago, IL).

RESULTS

CBF at Rest and During Vasomotor Tests in Controls and NIDDM Patients

In the control group, as in the NIDDM patients, there was no influence of sex or age on basal CBF or on CBF changes during the three vasomotor tests. The decrease in CBF and the log downward slope of CBF during these tests correlated significantly with each other. For the sitting-to-standing and Valsalva tests, they also correlated with log mean basal CBF (data not shown). In the control group, log SD of basal CBF also correlated significantly with the absolute decrease in CBF during the standing test ($r = .702$) and log slope of CBF during the Valsalva test ($r = .691$). In NIDDM patients, log SD of basal CBF correlated significantly with the decrease in CBF during the standing, Valsalva, and deep-breathing tests ($r = .442$, $r = .494$, and $r = .531$) and log slope of CBF during these tests ($r = .550$, $r = .449$, and $r = .537$).

Mean basal CBF at rest and the SD were not significantly different in NIDDM patients and the control group (Table 3). In 12 NIDDM patients, the SD was less than the lowest limit (6.00 PU) found in the controls, whereas mean basal CBF was less than the lowest control value (19.00 PU) in only two NIDDM patients.

Table 3. Mean Basal CBF and SD of CBF

Group	Mean CBF	SD
NIDDM patients (n = 42)	100.3 ± 10.6	11.2 ± 1.3
Controls (n = 14)	126.7 ± 23.1	14.3 ± 1.9

During the deep-breathing test, the reduction in CBF and the CBF downward slope were significantly lower in NIDDM patients than in controls (Table 4). Five NIDDM patients had a decrease in CBF and 14 had a CBF downward slope less than the lowest values found in controls (23% and 4.3%, respectively). During the sitting-to-standing test, the decrease in CBF was lower ($P = .09$) in NIDDM patients. During the Valsalva maneuver, there was a trend toward a smaller decrease in CBF and in the CBF downward slope in NIDDM patients (Table 4).

Correlations of CBF at Rest and During Vasomotor Tests in NIDDM Patients With Clinical and Biological Parameters

In NIDDM patients, mean basal CBF, SD, and CBF changes during vasomotor tests were not significantly different according to the presence or absence of retinopathy or nephropathy (defined by urinary albumin excretion rate > 30 mg/24 h). Seven of 15 patients with a smaller decrease in CBF downward slope during the deep-breathing test had peripheral neuropathy, and of 22 patients with peripheral neuropathy, seven had a smaller decrease in the CBF slope.

Parasympathetic cardiac control studied by analyzing heart rate variability during the three standardized tests appeared to be abnormal in seven of 16 patients who were tested, with an abnormality in at least one of these tests compared with values in the age-matched controls. There was no apparent link between the presence of an abnormal cardiac autonomic function test and a reduced decrease in the percentage of CBF during the deep-breathing test. Nor was there any significant correlation between heart rate variations and changes in CBF during the three tests.

Log mean basal CBF correlated significantly with BMI ($r = .356$, $P = .02$), creatininemia ($r = -.367$, $P = .02$), log FI ($r = -.500$, $P = .004$; Fig 2), MEA2 ($r = .482$, $P = .02$), and HDL cholesterol (n = 27, $r = .382$, $P = .05$), and the correlation with log HbA_{1c} ($r = .289$, $P = .07$) was close to statistical significance. We have taken into account colinearities and have therefore built a model of multiple linear regression analysis entering log mean basal CBF as the dependent variable and log FI and log HbA_{1c} as independent variables. Both parameters were significantly associated with log mean basal CBF (adjusted $R^2 = .488$).

Log SD of CBF correlated significantly with log HbA_{1c} ($r = .330$, $P = .040$; Fig 3) and creatininemia ($r = 0.387$, $P = .010$). Controlling for log HbA_{1c}, the partial correlation coefficient between log SD and creatininemia was $-.287$ ($P = .085$).

During the deep-breathing test, the log CBF slope decrease correlated significantly with log FI (n = 31, $r = -.407$, $P = .023$) and the percent decrease of CBF correlated negatively with MEA2 (n = 24, $r = -.533$, $P = .007$).

During the standing test, the log CBF slope decrease correlated with total cholesterol ($r = .301$, $P = .050$).

During the Valsalva test, the percent decrease of CBF correlated negatively with systolic blood pressure ($r = -.311$, $P = .048$). The log CBF slope decrease correlated with BMI

Table 4. Decrease in CBF During Deep-Breathing, Standing, and Valsalva Tests Expressed as Percent of the Previous Basal Level and Downward Slope Between the Basal Level and the Minimum Value of CBF

	Deep-Breathing		Standing		Valsalva	
Group/Parameter	%	Slope	%	Slope	%	Slope
NIDDM patients (n = 42)	43.4 ± 6.0	7.4 ± 0.8	75.5 ± 6.6	10.8 ± 1.5	67.4 ± 7.4	9.6 ± 1.3
Controls (n = 14)	79.1 ± 23.0	11.9 ± 1.3	99.9 ± 14.2	16.9 ± 3.4	83.3 ± 21.0	15.6 ± 3.8
P	.04	.003	.09	NS	NS	NS
Lowest level in controls	23	4.3	35	1.7	30	2.0
NIDDM patients with levels < lowest control level (n)	5	15	7	2	5	7

(r = .322, P = .038) and log triglycerides (r = .369, P = .015) and negatively with creatininemia (r = −.420, P = .005). We have taken colinearities into account and have therefore built a model of multiple linear regression analysis entering the log CBF slope as a dependent variable and log FI and log triglycerides as independent variables, and found a significant correlation with log triglycerides (adjusted R^2 = .223).

DISCUSSION

The present study was performed in NIDDM patients without clinical signs of autonomic neuropathy and without postural hypotension, to detect early signs of cardiovascular sympathetic dysfunction and the factors that can modify the peripheral vascular response to sympathetic activation.

Basal CBF was not significantly different in NIDDM patients and control subjects. This result is in agreement with previous results reported in type I diabetic patients.[12] However, the wide intersubject variability of resting blood flow[15] limits the significance of these results.

The standardized tests involving cardiovascular autonomic reflexes proposed by Ewing and Clarke[1] have helped in better understanding autonomic dysfunction in diabetes. But they are not sensitive enough for detection of sympathetic dysfunction. Skin vasomotor reflexes have been studied with plethysmography, and more rarely with laser Doppler flowmetry.[23] They involve complex mechanisms. The response to standing is mediated by a local venoarteriolar reflex,[36] low-pressure baroreceptors,[37,38] arterial baroreceptors,[38] and increases in epinephrine, norepinephrine,[39] and renin[40] levels. By recording blood flow at multiple sites and using several tests involving different afferent pathways (baroreceptors for the Valsalva maneuver,[41] chest-wall and lung-inflation receptors for the inspiratory gasp,[16] and somatic afferents for the cold pressor test), all of which share the same sympathetic efferent system as the final common pathway, we obtained a sensitive procedure capable of evaluating the sympathetic deficit in peripheral nerves. For instance, the study of changes in skin blood flow during the standing test provides a sympathetic index, since it has been shown that the time of CBF recovery after the decrease induced by standing correlates with the change in blood pressure and heart rate immediately after standing and that this time is prolonged by phentolamine, a sympatholytic agent.[23]

The study of peripheral vasoconstrictor responses by venous-occlusion plethysmography has shown that in more than half of the diabetic patients with peripheral neuropathy, both cardiovascular and sympathetic vasoconstrictor functions are impaired.[21]

In the present study, the reproducibility of the skin vasomotor reflexes studied by laser Doppler flowmetry seemed acceptable. The most striking result found in NIDDM patients consisted of a smaller decrease in CBF and in the CBF downward slope during the deep-breathing test. The most frequent (33.3%) abnormal result consisted of a reduced CBF downward slope during the deep-breathing test. There was also a smaller CBF decrease during the sitting-to-standing test and a trend toward a reduction in the CBF downward slope during the Valsalva maneuver.

The SD of basal CBF, ie, the amplitude of changes in basal CBF, was also reduced in many NIDDM patients (28.6%), which again suggests a change in peripheral sympathetic tone, since it has been shown that spontaneous fluctuations in blood flow of the extremities are under sympathetic control,[14] and we found a significant correlation between the SD and the decrease

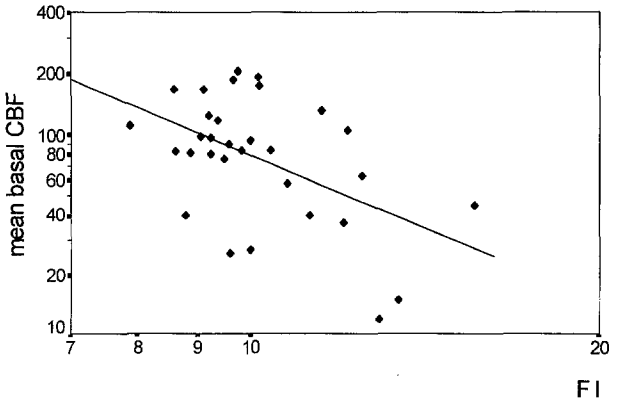

Fig 2. Linear regression between log mean basal CBF and log FI of RBCs in NIDDM patients.

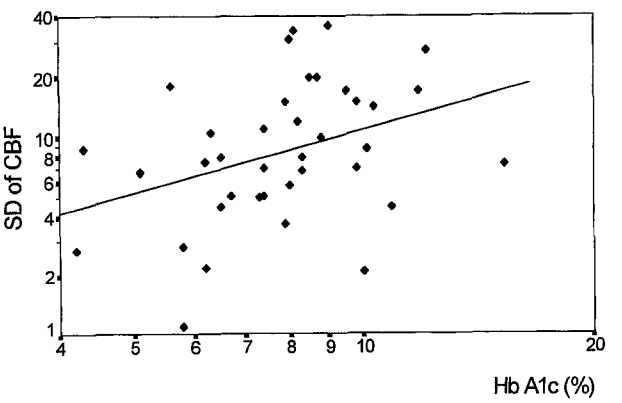

Fig 3. Linear regression between log SD of CBF and log HbA_{1c} in NIDDM patients.

in CBF during tests of sympathetic activation both in controls and in NIDDM patients.

In NIDDM patients, mean basal CBF correlated negatively with the RBC FI, which means that the lower the RBC deformability, the lower the mean basal CBF. This suggests that either both abnormalities coexist or that rheological changes have a slowing effect on CBF. To our knowledge, this result was suspected but never clearly established in diabetes. The decrease in CBF during the deep-breathing test also correlated negatively with the FI and the aggregation index, which suggests either that both abnormalities coexist or that alterations in RBC rheological properties are responsible for a reduction in the CBF changes induced by sympathetic activation. This may result from the slowing effect of rheological alterations on resting CBF and may therefore limit a further CBF decrease after sympathetic activation.

The mean basal value of CBF correlated positively with HbA_{1c}. This suggests that prolonged poor glycemic control might increase CBF at rest, since an increase in muscle and skin blood flow has been reported in short-term type I diabetes.[42,43] Changes in CBF during the postural and Valsalva tests correlated positively with total cholesterol and triglycerides, respectively, which suggests that increases in these plasma lipid parameters help to amplify changes in CBF during sympathetic activation. It can be hypothesized that the increase in cholesterol and triglycerides increases plasma viscosity.[44]

The SD of CBF may simply reflect abnormal peripheral sympathetic function, but it also seems to depend on glycemic control. The vascular responses to the sympathetic activation tests, particularly during the deep-breathing test, show that these tests are simple, sensitive, and reproducible. However, the reduction in the mean resting CBF, spontaneous changes in CBF at rest, and changes in CBF during the deep-breathing test are also associated with RBC rheological alterations, whereas changes in plasma viscosity secondary to hyperglycemia and high levels of cholesterol and triglycerides may increase the mean resting CBF, spontaneous changes in CBF at rest, and changes in CBF during the postural and Valsalva tests. Moreover, rheological blood properties seem to strongly influence resting CBF and vasomotor reflexes. There was no apparent link between peripheral neuropathy or the results of cardiac autonomic function tests and the results of sympathetic vasoconstrictor responses. These data suggest that when autonomic function is being assessed in diabetic patients, both cardiac autonomic responses and sympathetic vasoconstrictor function should be tested, since alterations in sympathetic tone likely play an important role in the development of severe diabetic complications such as foot ulceration.[45]

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