Impairment of Peripheral Blood Flow Responses in Diabetes Resembles an Enhanced Aging Effect

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OBJECTIVE — To test the hypothesis that skin blood flow responses in the fingertip of diabetic patients are impaired and to examine the role of aging in both healthy control subjects and diabetic patients.

RESEARCH DESIGN AND METHODS — We measured cutaneous blood flow using laser Doppler techniques in 40 people with diabetes and in 20 age- and sex-matched healthy control subjects. To induce vasoconstriction, subjects were asked to perform three 1-min stressor tasks: mental arithmetic, contralateral hand grip, and immersion of the contralateral hand in ice water. To induce vasodilatation, a local heat stimulus of 45°C was applied for 5 min.

RESULTS — Basal blood flow did not differ between groups, but vasoconstrictive responses induced by arithmetic or immersion of the contralateral hand in ice-cold water and vasodilatation induced by local heating were severely impaired in diabetic subjects, compared with healthy control subjects ($P < 0.01$). These responses correlated with autonomic nerve function and deteriorated significantly with advancing age in control subjects, but not in diabetic subjects. Blood flow in younger diabetic subjects resembled that of older control subjects.

CONCLUSIONS — These data demonstrate that diabetes has effects on precapillaries that may be direct or mediated via autonomic nerves, which result in a deficit that resembles premature aging.

Neuropathy remains the most frequent complication of diabetes mellitus and is the major contributor to the increased morbidity associated with this metabolic disorder (1,2). Peripheral symmetric neuropathy, involving large myelinated, small myelinated A-delta, and unmyelinated C fibers, is often accompanied by the development of foot ulcers in the presence of an apparent intact circulation, which can eventually lead to limb loss from gangrene (1–5). Small fiber nerve dysfunction, in particular, is associated with numerous clinical features, including hyperalgesia, diminished pain and warmth perception, and impaired autonomic control of skin temperature regulatory mechanisms (1,6–9).

Sympathetic regulation of blood flow to the skin is complex, involving long descending autonomic fibers that mediate central reflex control of vascular tone, short reflex arcs through the spinal cord, and local reflexes within the skin (10–12). Neural regulation of skin blood flow is further complicated by the presence of arteriovenous anastomoses, which are highly innervated structures involved in thermoregulatory processes. Such shunts provide a low-resistance pathway for blood flow where large volumes of blood can be partitioned to a superficial venous plexus, largely bypassing the nutritive capillaries of the skin (6,11). An attractive hypothesis is that diabetes results in the loss of neural control of these vessels such that there is increased shunt flow creating a deficit in skin blood flow at the nutritive capillary level (6,14,15). Evidence to support this hypothesis includes the finding of increased venous oxygen tension (14), raised pedal skin temperature, and venous distension (16). However, it remains uncertain whether impaired shunt blood flow occurs as a result of a specific nerve fiber–type defect or whether it is a reflection of microvascular damage.

The development of noninvasive methods for assessing skin blood flow (e.g., laser Doppler and thermography techniques) has enabled clinical measurements of the effects of diabetes on microvascular perfusion (15,17–21). To date, measurements of skin blood flow in diabetic subjects have provided conflicting results. Rendell (19) reported that diabetes was associated with decreased blood flow in areas of skin both rich and relatively devoid of arteriovenous shunts, while Hauer et al. (8) found increased palmar blood flow relative to flow in the fingertips. Further, several studies have reported increased skin blood flow in diabetic subjects, compared with that of controls (3,15). Similarly, reports of the effects of diabetes on skin blood flow in situations requiring vasoconstriction or vasodilatation are also conflicting (3,6,13,22). It is likely that the discrepancies indicate that the diabetes-induced changes in skin blood flow result from multiple mechanisms, presumably of both neural and microvascular origin.

One factor that has received relatively little attention and may markedly influence microcirculatory responsiveness in diabetes is the effect of the normal aging process. In particular, while many studies of the effects of diabetes on the cutaneous circulation have included appropriately age-matched control groups (23–25), the longitudinal effects of aging on microvascular responses to neural and local stimuli have not been well documented. This is somewhat surprising, given that diabetes has been likened to a state of accelerated aging, and aging is associated with structural microvascular changes such as decreased arterial distensibility and venous compliance as well as

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Abbreviations: ANOVA, analysis of variance.

Table 1—*Epidemiological data including indexes of sensory and autonomic neuropathy*

	Control subjects	Diabetic patients	*P* value
n	20	40	—
Age (years)	45.8 ± 3.1 (19–72)	46.9 ± 2.1 (18–71)	0.7596
Type 1:type 2	—	15:25	—
HbA_{1c} value (%)	—	8.57 ± 0.38	—
Duration of diabetes (years)	—	15.4 ± 1.6	—
Retinopathy	—	16/40 (40)	—
Nephropathy	—	6/40 (15)	—
Somatic nerve function			
Vibration threshold (log microns)	2.75 ± 0.42 (19)	8.06 ± 1.03(29)	<0.0001*
Warm thermal threshold (°C)	3.20 ± 0.45 (19)	8.06 ± 0.65 (24)	<0.0001
Cold thermal threshold (°C)	0.42 ± 0.05 (19)	3.26 ± 0.78 (27)	0.0012*
Autonomic nerve function			
Expiration-to-inspiration ratio	1.24 ± 0.03 (17)	1.11 ± 0.02 (27)	0.0012
Valsalva R-R index	1.60 ± 0.08 (17)	1.31 ± 0.05 (28)	0.0017
Postural R-R index	1.45 ± 0.06 (16)	1.12 ± 0.04 (27)	<0.0001

Data are means ± SE (*n*) or range, *n* (%), or *P*. Significance was determined by one-way ANOVA. *Significance determined by Welch ANOVA (29) because homogeneity of group variance was not met according to O'Brien's test of variance.

changes in peripheral resistance, which contribute to the progressive development of peripheral artery disease (26,27). Alterations in the character of the extracellular matrix include crosslinking secondary to accumulation of advanced glycosylation endproducts. Changes in the composition of the matrix include changes in collagen-to-elastin ratio. Either may contribute to both diabetes and aging-related changes in vascular function. Therefore, the aims of this study were to determine the effect of both diabetes and aging on cutaneous blood flow responses to stimuli acting both locally and through reflex and centrally mediated mechanisms. To accomplish these aims, laser Doppler blood flow measurement was performed on age-matched diabetic patients and control subjects whose age range spanned ~50 years.

RESEARCH DESIGN AND METHODS

Subjects

The study group consisted of a total of 40 subjects with diabetes (age range, 18–71 years; duration of diabetes, 15.4 ± 1.6 years) and 20 age-matched healthy control subjects (age range, 19–72 years). Demographic data and indexes of autonomic and sensory neuropathic function for the study groups are provided in Table 1. Informed consent was obtained from all individuals participating in the studies, and all procedures were approved by the Institutional Review Board of Eastern Virginia Medical School. Retinopathy was determined by routine fundoscopy and was considered positive if at least background retinopathy was present. Nephropathy was considered present when 24-h urine protein excretion exceeded 500 mg total protein. A subset of 13 of the diabetic patients did not have all of the neuropathy tests. These were initially recruited as a pilot study that did not include those tests. All subjects were recruited in the same fashion by responding to flyers placed in a large endocrinology clinic at the Eastern Virginia Medical School or by referral from clinical endocrinologists. These subjects were similar on clinical characteristics and blood flow parameters to those in the diabetic group who had received all of the tests.

Skin blood flow

Skin blood flow was measured via a noninvasive laser Doppler technique, using the Laserflo Blood Perfusion Monitor (Vasamedics BPM 403A, St. Paul, MN). The application of laser Doppler techniques for assessment of skin blood flow has been previously described in detail (19). Measurements were taken at the pulp of the index finger. Skin blood flow was determined under resting conditions (subject seated; ambient temperature, 20–22°C) and after application of several stressors: mental arithmetic (serial subtraction), hand grip (40% of maximal grip of the contralateral hand for 1 min), cold pressor (immersion of contralateral hand in ice water for 1 min), and warming of the measurement site (local heating to 45°C for 5 min). Stressors were chosen to provide a range of central, spinal cord, and locally mediated responses.

Subjects were seated in a standard chair with left forearm and hand positioned on an armrest. The right arm was positioned for easy access to the hand-grip dynamometer (Jamar 5030 J1, Jackson, MI) and a container of ice water. After an equilibration period, baseline blood-flow measurements were taken for a 5-min period after which measurements were continued during the 1-min application of a particular stressor. A recovery period of 5 min was interposed between application of each stressor.

Assessment of cardiac autonomic function

Cardiac reflex responses were characterized as previously described (28) by changes in heart rate (determined from R-R intervals) on *1)* deep breathing (expiration-inspiration index) while in the supine position, 2) Valsalva maneuver (forced expiration against a pressure of 40 mmHg) in the supine position, and 3) transition from a supine to a standing position.

Assessment of sensory perception

Previously described psychophysical methods (28) were used to measure sensation at the great toe of all subjects. Thermal sensitivity was assessed by a subject's ability to distinguish temperature differences between two thermal pads (Physitemp Thermal Sensitivity Tester NTE-2, Physitemp, Clifton, NJ). Warm threshold was determined with the reference temperature at 35°C, and the temperature of the test pad raised according to the psychophysical algorithm to a maximum of 45°C. For cold threshold, the reference temperature was 25°C, and the temperature of the test pad was lowered accordingly. Vibration threshold was measured using a Physitemp Vibratron II vibrometer (Physitemp, Clifton, NJ) and the previously described psychophysical procedure (28).

Statistical methods

Values for blood flow and deltas are expressed in laser-Doppler perfusion units, and for each group, the means ± SE are shown in tables and text. Analysis of variance (ANOVA) was used to test mean differences between groups for each variable

Table 2—*Blood flow responses in absolute change (Δ) and percentage change (%) to vasoconstrictor and vasodilator stimuli*

	Control subjects	Diabetic patients	*P* value
n	20	40	—
Mean baseline flow†	20.9 ± 3.4	12.4 ± 1.7	0.0801*
Mental arithmetic (Δ)	−9 7 ± 3.0	−2.8 ± 0.7	0.0040
Hand grip (Δ)	−10.4 ± 3.7	−2.4 ± 0.7	0.0060
Cold pressor (Δ)	−13.5 ± 4.1	−6.0 ± 1.3	0.0333
Warming (Δ)	22.8 ± 5.2	11.3 ± 2.4	0.0224*
Mental arithmetic (%)	−28.6 ± 7.9	−18.6 ± 4.3	0.2313
Hand grip (%)	−31.0 ± 6.6	−18.9 ± 4.7	0.1381
Cold pressor (%)	−58.5 ± 6.0	−37.9 ± 4.2	0.0067
Warming (%)	189.4 ± 62.4	65.7 ± 8.7	0.0101

Data are *n* or means ± SE. Significance was determined by one-way ANOVA. *Significance determined by Welch ANOVA (29) because homogeneity of group variance was not met according to O'Brien's test of variance; †flow units are laser Doppler perfusion units.

where variance was homogeneous, and Welch ANOVA (29) was used for those variables that were heterogeneous according to O'Brien's test of variances.

RESULTS

Effect of diabetes on basal blood flow

Baseline blood flow levels, measured at the pulpar surface of the index finger with the hand at heart level and under ambient temperature conditions (20–22°C), were not significantly different between the study groups (20.9 ± 3.4 in the control subjects vs. 12.4 ± 1.7 in the entire group of people with diabetes, $P = 0.0801$; see Table 2). When type 1 and type 2 diabetic groups were considered separately (see Table 3), several of the multiple group comparisons of blood flow parameters showed differences with control subjects in only the type 2 diabetic group, with the exception of mean (baseline) flow, which differed from control subjects only in the type 1 diabetic group. However, there were no significant differences between type 1 and type 2 diabetic groups on any of the blood flow parameters. The mean age of the type 1 diabetic group (34.9 ± 3.2 years) was significantly less than that of the type 2 diabetic group (54.1 ± 1.5 years), but the duration of diabetes was greater in the type 1 diabetic group (22.3 ± 2.9 vs. 11.8 ± 1.5) than in the type 2 diabetic group. There was, however, no difference in degree of diabetes control between the different groups of diabetic subjects (HbA_{1c} values, 8.7 ± 0.5 vs. 8.5 ± 0.5%, respectively).

Diabetes and vascular responses

Group data illustrating the blood flow responses to the vasoconstrictor and vasodilator stimuli are illustrated in Fig. 1 and Table 2. In general, blood flow responses to the various stressors were found to be blunted in the diabetic subjects. For example, immersion of the contralateral hand in ice water decreased blood flow by 58.5 ± 6.0% in the control subjects, compared with 37.9 ± 4.2% in the diabetic subjects ($P = 0.0067$). Similarly, in response to local warming of the skin to 45°C, blood flow increased by 189.4 ± 62.4% in the control subjects vs. 65.7 ± 8.7% in the diabetic subjects ($P = 0.0101$). The absolute changes, as opposed to the percentage of basal, induced by the stressors were uniformly impaired in the people with diabetes. Significance was lost for handgrip and mental arithmetic when taken as a percentage of basal because of the lower basal flow in diabetic subjects. There was no relation between diabetes control, duration of diabetes, and indexes of blood flow.

Effect of age on basal blood flow

Correlation matrices were generated by groups (control and diabetic) to observe any relationships present in each group. In the control subjects, the basal level of microvascular perfusion, as measured by laser Doppler blood flow, showed a highly significant inverse relationship with age ($r = -0.79$, $P < 0.0001$; see Fig. 2). In contrast, in the diabetic subjects, the relationship between baseline blood flow and age was lost ($r = 0.06$, NS; see Fig. 2). Further analyses performed separately in type 1 and type 2 diabetic subjects showed no significant relationship between baseline blood flow and age in either group (type 1 or type 2 diabetes).

Age and responses to stressors

In the control group, the blood flow response to the vasoconstrictor stimuli, immersion of contralateral hand in ice water, and mental arithmetic showed significant negative correlations ($r = -0.76$, $P < 0.0001$, and $r = -0.78$, $P < 0.0001$, respectively) with age (Fig. 2). As in the case of basal blood flow, age-dependent relationships were not apparent within the diabetic subject group for either constrictor stimuli ($r = 0.03$, NS, and $r = -0.03$, NS, respectively; see Fig. 2). No relationship between age and the ability to increase blood flow in response

Table 3—*Blood flow in type 1 versus type 2 diabetic patients*

	Control subjects	Type 1 diabetes	Type 2 diabetes
Age (years)	45.8 ± 3.1 (20)	34.9 ± 3.2 (15)*	54.1 ± 1.53 (25)*
Duration of diabetes (years)	—	22.3 ± 2.9 (13)	11.8 ± 1.5 (25)†
HbA_{1c} (%)	—	8.7 ± 0.5 (9)	8.5±0.5 (21)
Mean flow§	20.9 ± 4.3 (20)	8.1 ± 2.0 (15)*‡	14.9 ± 2.4 (25)
Mental arithmetic (Δ)	−9.7 ± 3.0	−1.6 ± 0.7 (15)*‡	3.4 ± 1.1 (25)*‡
Hand grip (Δ)	−10.4 ± 3.7	−2.3 ± 1.0 (15)	−2.4 ± 1.0 (25)‡
Cold pressor (Δ)	−13.5 ± 4.1	−5.1 ± 2.0 (15)	−6.5 ± 1.8 (25)
Warming (Δ)	22.8 ± 5.2	13.0 ± 5.2 (14)	10.4 ± 2.2 (24)
Mental arithmetic (%)	−28.6 ± 7.9	−14.9 ± 7.0 (15)	−20.8 ± 5.5 (25)
Hand grip (%)	−31.0 ± 6.6	−26.2 ± 7.5 (15)	−1 4.5 ± 5.9 (25)
Cold pressor (%)	−58.5 ± 6.0	−37.6 ± 6.1 (15)	−38.0 ± 5.8 (25)*
Warming (%)	189.4 ± 62.4	80.2 ± 17.6 (14)	57.2 ± 9.1 (24)*

Data are means ± SE (*n*). *Significant difference from control group ($P < 0.05$ by ANOVA and Tukey-Kramer post hoc); †significant difference from type 1 diabetic group ($P < 0.05$ by ANOVA and Tukey-Kramer post hoc); ‡omnibus significance determined by Welch ANOVA (29) because homogeneity of group variance was not met according to O'Brien's test of variance; §flow units are laser Doppler perfusion units.

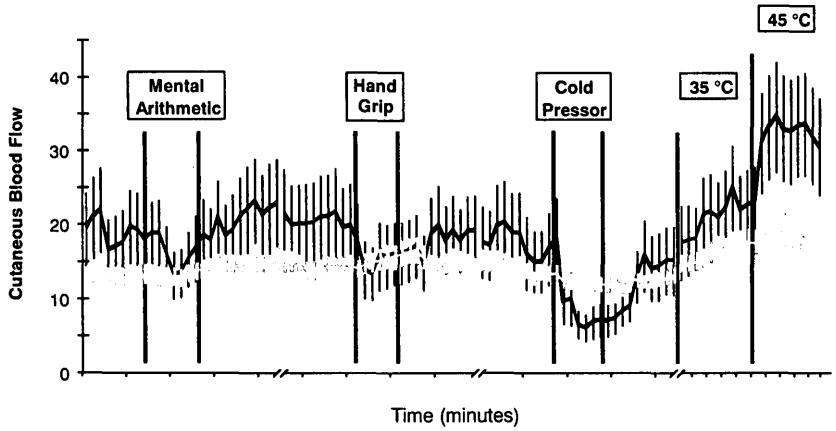

Figure 1—*Skin blood flow measured from the index finger in 40 diabetic subjects (shaded line) and 20 age-matched healthy control subjects (solid line). Data are means ± SE for each time point.*

to local heating was observed for either group. Further analyses performed separately in type 1 and type 2 diabetic subjects yielded no significant relationships between any of these parameters and age in either group (type 1 or type 2 diabetes).

Effects of neuropathy

In the healthy control subjects, the blood flow responses to arithmetic, hand grip, and warming correlated significantly ($r \geq 0.50$, $P < 0.05$) with cold thermal sensation, a measure of somatic neuropathy. However, in the diabetic subjects, the only significant correlation of blood flow with neuropathy measures was between the blood flow response to warming and the warm thermal threshold, another measure of somatic neuropathy.

CONCLUSIONS — The results of the present studies demonstrate that diabetes is associated with impaired cutaneous vasomotor responses to stimuli acting through local, reflex, and centrally mediated mechanisms. Diabetic subjects typically exhibited both a decreased ability to vasoconstrict and to vasodilate, suggesting a generalized alteration in microvascular reactivity. The additional major finding was the marked differences in the effect of aging on vascular responsiveness between the control and diabetic subjects. Nondiabetic control subjects demonstrated significant age effects on both baseline blood flow and vasoconstriction with either contralateral cold or mental arithmetic. These data suggest that under normal conditions, aging per se has a negative effect on microvascular function. In contrast, no similar relationship was apparent in the diabetic subjects. As the slope was lost and the intercept considerably lowered in the diabetic subjects compared with the control subjects, it is conceivable that the impairment in diabetes reflects a state of advanced aging. That the complications of diabetes may, at least in part, represent an advanced aging process is consistent with a previous report (30).

In spite of technical consensus (18,20), caution is still warranted when using laser Doppler techniques to provide estimates of absolute blood flow because of uncertainty in the exact population of vessels being studied in a particular subject (21). To control for this, we utilized data from a reasonable number of heterogeneous controls (n = 20) and doubled the number of age-matched diabetic subjects, thereby allowing the full range of variation to be uniformly distributed within groups. In light of this, these effects of diabetes and age deserve attention.

While the techniques used in the present study do not provide definitive evidence as to the site of impairment (i.e., neural vs. microvascular), it is tempting to speculate the involvement of a microvascular abnormality. This is based on the fact that responses were impaired to stimuli that act through a combination of local, reflex, and centrally mediated pathways with the microvasculature representing a common endpoint. Further, in a recent study, we reported that diabetic subjects also show a diminished amplitude and frequency of spontaneous vasomotion, the periodic variation in blood flow resulting from rhythmic vasoconstriction of arterioles (31). Collectively, these data suggest an alteration at the level of the arteriolar wall. There are numerous biochemical mechanisms that could contribute to altered vascular reactivity. This may be an endothelium-dependent functional abnormality in people with diabetes due to the overproduction of protein kinase C (γ) (32) or due to impaired nitric oxide–induced vasodilatation (33,34). To date, studies into these mechanisms are ongoing but have yet to be thoroughly described in diabetes.

Another possible mechanism involves processes of advanced nonenzymatic glycosylation with resultant changes in mechanical properties of the vessel wall (35). This is supported by studies demonstrating that advanced glycosylation reactions can result in matrix protein cross-linking and may contribute to the excessive accumulation of matrix proteins within the vessel wall (30). Consistent with this suggestion, we have shown that induction of diabetes in the rat, with streptozotocin, leads to a decrease in arteriolar distensibility and impaired vascular smooth muscle mechanotransduction (26,27). Further, these abnormalities were found to be prevented by treatment with aminoguanidine, an inhibitor of advanced nonenzymatic glycosylation reactions (36). The possible involvement of advanced glycosylation reactions in the impaired microvascular responsiveness described in the present studies is also consistent with an accelerated aging process. Thus, in vitro data indicates that advanced glycosylation reactions proceed at a rate dependent on both the time of exposure and the type of glycating agent (30), while in vivo data has shown that in normal subjects advanced glycation endproducts accumulate on lens crystallin protein as a function of aging (37) and may be independent of the concentration of the glycating agent (38). It seems therefore feasible that aging within the diabetic population may contribute independently to the microvascular dysfunction.

Previous studies have suggested that aging is associated with loss of superficial nutritional dermal capillaries, while blood flow through the deeper subpapillary plexi containing arteriovenous anastomoses is relatively unaffected (24,39). In contrast, the literature contains conflicting data as to the effect of aging on cutaneous microvascular reactivity. For example, there are reports both of advancing age leading to unchanged or impaired vasodilator responses to local heating (23,25,39). In the present study, while not finding an age-dependent reduction in the response to local heating in either study group, there was a marked reduction in the diabetic

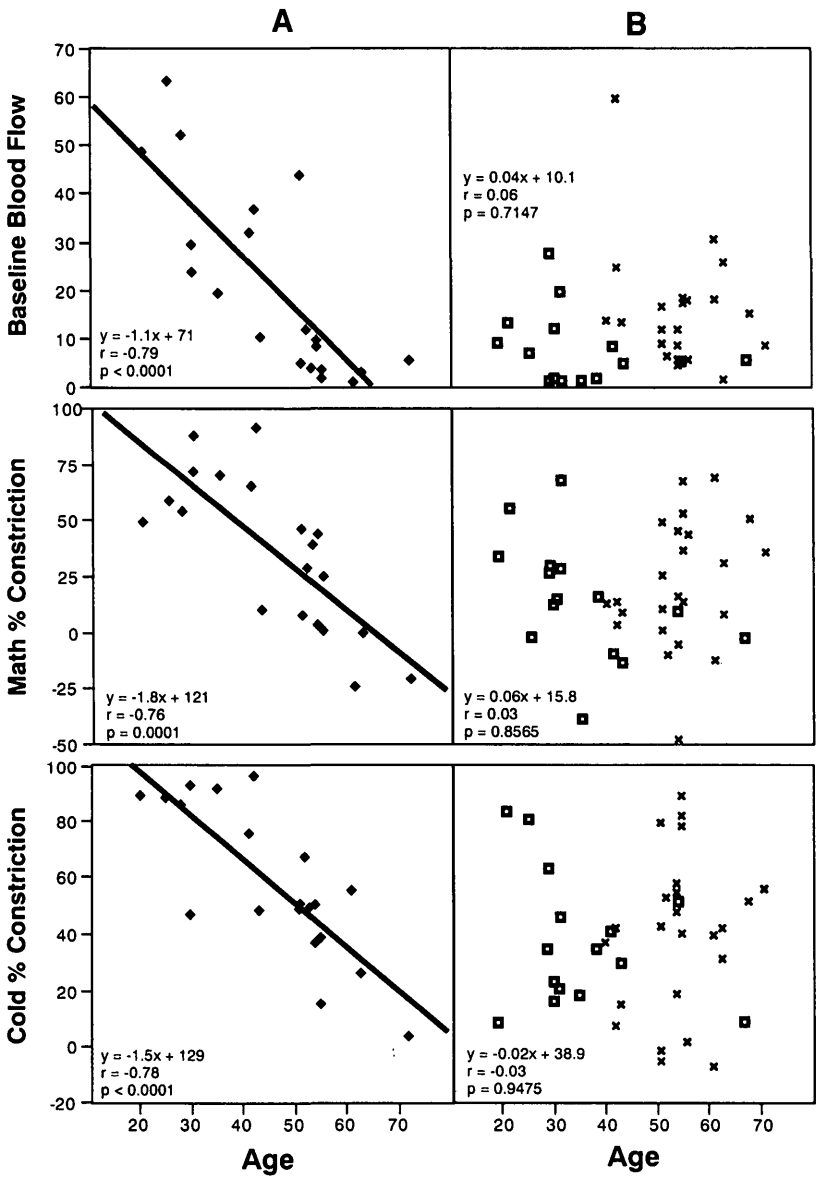

Figure 2—*Correlation of blood flow measures with age in control subjects (A) and diabetic patients (B). In the diabetic group, open squares represent subjects with type 1 diabetes and the x symbols represent subjects with type 2 diabetes.*

subjects, relative to that of the control subjects. The vasodilator response to the heating stimulus would be expected to involve both relaxation of precapillary resistance vessels and/or capillary recruitment. The velocity component of blood flow is indicative of upstream vascular relaxation, and the volume component in part reflects capillary recruitment. Since the major change measured in laser Doppler blood flow studies is velocity, with virtually unchanged effects of provocative stimuli on volume (41), it seems reasonable to infer that the effects of aging on altered responsiveness are due to impaired modulation of precapillary vessels. This could be at the level of the vessel wall per se or altered sympathetic regulation of precapillary sphincter tone.

With respect to a relationship between aging and sympathetic activity, several studies have demonstrated an increase in catecholamine levels with age (40,41). However, in older subjects (without coexisting vascular disease), any increase in norepinephrine availability may be compensated for by changes at the level of adrenergic receptors or at a postreceptor level (42). The correlation between vasoconstriction and age in normal subjects suggests that the precapillary elements or arteriovenous anastomoses are indeed under sympathetic control and that this deteriorates with age. In contrast, the impairment in people with diabetes suggests either that the vessels are unable to respond or that the loss of autonomic nerve supply obscures the aging effect.

Whatever the mechanism, be it loss of vascular reactivity or impaired autonomic function, it is clear that diabetes is an advanced form of aging with respect to vascular reactivity. It would be of interest to determine whether the reversal of the glycation of proteins that affect vascular status (37) or the reversal of autonomic dysfunction would restore vascular reactivity in people with diabetes.

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