Impaired Skin Microvascular Function in Children, Adolescents, and Young Adults With Type 1 Diabetes

FAISEL KHAN, PHD
TARIK A. ELHADD, MRCP
STEPHEN A. GREENE, MD
JILL J.F. BELCH, MD

OBJECTIVE — Vascular disease in type 1 diabetes is a complex and multifactorial process, which probably begins in childhood in association with the onset of diabetes. To determine the possible factors involved, we measured microvascular responses to endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilators in 56 patients with type 1 diabetes (aged 9–22 years) and 22 control subjects.

RESEARCH DESIGN AND METHODS — Skin perfusion was measured at the dorsum of the foot using laser Doppler flowmetry during low-current iontophoresis of acetylcholine and sodium nitroprusside. Maximum vasodilator function was measured during local 44°C skin heating.

RESULTS — Vascular responses were significantly reduced in patients with type 1 diabetes compared with responses in control subjects: acetylcholine ($P < 0.01$, analysis of variance [ANOVA]), sodium nitroprusside ($P < 0.01$, ANOVA), and local heating ($P < 0.02$. Mann-Whitney *U* test). Endothelium-dependent responses were related to duration of diabetes ($r = -0.38$, $P < 0.01$) and to glycemic control ($r = -0.37$, $P < 0.01$). Significant correlations were found in the patient group between responses to acetylcholine and sodium nitroprusside ($r = 0.28$, $P < 0.05$) but not to heating, suggesting that a common factor (e.g., nitric oxide activity) may be responsible for the abnormal vascular responses to these chemicals.

CONCLUSIONS — Early changes in microvascular function are present in young patients with type 1 diabetes, long before the initial clinical presentation. These abnormalities may be related to complex interactions between structural abnormalities and functional changes in the endothelium, smooth muscle, and nitric oxide activity.

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Microvascular disease is a major feature of type 1 diabetes and results from long-standing structural and functional changes. Metabolic control and duration of diabetes seriously influence the onset and progression of microvascular complications (1,2), but the precise onset time is not known. Recently, we (3) and others (4,5) have shown that vasodilator responses are significantly reduced in children with type 1 diabetes who have no clinical evidence of vascular disease.

The development of macrovascular and microvascular disease in type 1 diabetes involves complex and multifactorial processes. The vascular endothelium has a vital and complex role in regulating blood flow by producing important chemicals, such as endothelium-derived relaxing factor/nitric oxide (NO), prostacylin, and endothelin, that regulate both hemostasis and vascular tone (6). Abnormalities in the endothelium/NO pathway have been reported in adults with type 1 diabetes, in both resistance vessels (7–9) and arteries (10,11), although this has not been a consistent finding (12). Assessment of endothelium-dependent vasodilation commonly involves arterial cannulation for infusion of vasoactive chemicals. The invasive nature of this technique, however, makes it undesirable for use in young people. Consequently, there is still little information available regarding the integrity of endothelial cell function/NO activity in young patients with diabetes.

We have previously shown that endothelial and white blood cell function are abnormal in children with type 1 diabetes (13). The aim of the present study was to assess skin microvascular responses to the iontophoresis of acetylcholine and sodium nitroprusside, endothelium-dependent and -independent vasodilators, respectively, in young patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

— We recruited 56 children, adolescents, and young adults with type 1 diabetes from the children's and young adult diabetic clinics at Ninewells Hospital, Dundee, Scotland. We also enrolled 25 healthy normal control subjects. Details are given in Table 1. A total of 49 patients were taking twice-a-day injections of premixture insulin using the pen delivery system, 4 were on a basal bolus regimen, and 3 were using conventional insulin syringes (twice-a-day injections). Ethical approval was obtained from the local medical ethics committee. Patients and control subjects gave written informed consent, and when the participant was <16 years of age, the written consent of a parent or guardian was obtained.

Pubertal status was determined by a consultant pediatric endocrinologist (S.A.G.). The attainment of final adult height was taken as an indication of adulthood. Prepubertal subjects were categorized using the Tanner classification (14). Subjects between the prepubertal and

From the University Department of Medicine, Section of Vascular Medicine and Biology (F.K., T.A.E., J.J.F.B.), and Child Health Ninewells Hospital and Medical School (S.A.G.), Dundee, Scotland, U.K.

Address correspondence and reprint requests to Faisel Khan, PhD, University Department of Medicine, Section of Vascular Medicine and Biology, Ninewells Hospital and Medical School, Dundee, DD1 9SY, Scotland, U.K. E-mail: f.khan@dundee.ac.uk.

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Abbreviations: ANOVA, analysis of variance; mC, millicoulomb; PU, perfusion unit; SkEF, skin erythrocyte flux.

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

Table 1—*Characteristics of the study population*

	Type 1 diabetic patients	Control subjects
n	56	25
Sex (M/F)	23/33	12/13
Age (years)		
Mean ± SEM	14.8 ± 0.5	15.4 ± 0.9
Range	9–22	9–22
Weight (kg)	55.7 ± 1.9	56.5 ± 3.0
Height (cm)	159.7 ± 1.8	163.6 ± 2.7
Blood pressure (mmHg)		
Systolic	119.5 ± 1.6*	107.4 ± 2.8
Diastolic	76.9 ± 1.5*	65.9 ± 3.5
Heart rate (beats/min)	74.7 ± 1.9†	67.5 ± 2.2
Duration of diabetes (years)		
Mean ± SEM	6.6 ± 0.6	—
Range	1–18	—
HbA_{1c} (%)	8.7 ± 0.2	<5.8
Insulin dose (U/kg)	0.91 ± 0.03	—
Total cholesterol (mmol/l)	4.5 ± 0.1	—
Skin temperature (°C)	30.6 ± 0.3	30.5 ± 0.6

Data are means ± SEM unless otherwise indicated. *$P < 0.005$; †$P < 0.05$.

young adult categories were classified as adolescents. Accordingly, in the group with type 1 diabetes, there were 13 prepubertal children, 19 adolescents, and 24 young adults.

Glycemic control was assessed by measuring HbA_{1c} using ion-liquid chromatography. Five of the young adults with diabetes and none of the control group were current smokers. None of the patients had any clinical evidence of diabetic retinopathy, and all but one had urinary albumin excretion values within the normal reference range.

Endothelium-dependent and -independent microvascular responses

Studies were conducted in a temperature-controlled room (25–26°C) in the morning, ~2 h after a light breakfast. Patients took their usual morning insulin. Subjects were lying in the supine position with their feet at heart level. After a 25-min equilibration, skin perfusion (termed skin erythrocyte flux [SkEF]) was measured continuously at the dorsum of the right foot using a single-point laser Doppler flowmeter (MBF3/D; Moor, Axminter, U.K.). Skin temperature was measured using a contact thermistor (YSI model 4098; Yellow Springs Instruments, Yellow Springs, OH).

Endothelium-dependent and -independent vascular responses were measured using low-current iontophoresis of acetylcholine and sodium nitroprusside, respectively, as described previously (15,16). In brief, the dorsum of the right foot was cleaned gently with alcohol and deionized water, and a direct electrode chamber was attached using double-sided adhesive tape. The chamber consisted of a central compartment that held the laser probe in position and contained the solutions (~0.5 ml) for iontophoresing. A control laser probe, without current, was attached 4 cm distal to the iontophoresis chamber. The indifferent electrode was placed around the right ankle to complete the circuit. The leads from the electrodes were connected to a battery-powered iontophoresis controller (MIC 1; Moor).

A stable baseline SkEF was measured for 4 min. Acetylcholine chloride (Sigma, St. Louis, MO) was made up to a 1% solution in deionized, sterile water and iontophoresed using an anodal current of 0.1 mA for 20 s to achieve a dose of 2 millicoulombs (mC) (mC/cm^2). The subsequent vascular response was measured for 4 min, which was sufficient time for SkEF to plateau. SkEF did not return to baseline, and two additional doses were iontophoresed (40 and 80 s = 4 and 8 mC/cm^2) to produce a cumulative dose-response curve. At a different site, on the same foot and not simultaneously, 1% sodium nitroprusside (David Bull, Warwick, U.K.) was iontophoresed to achieve doses of 2, 4, and 8 mC/cm^2. The order of acetylcholine and sodium nitroprusside delivery was randomized. Acetylcholine and sodium nitroprusside vascular response were averaged over each 4-min measuring period, and a ratio of this average over the prestimulus baseline SkEF was calculated for each dose.

Maximal skin microvascular vasodilation

A standard heater (Perimed, Stockholm, Sweden) was attached to an unperturbed area on the dorsum of the right foot (3). Baseline SkEF was measured for 4 min, after which the heater was set to 44°C. The maximal hyperemic response, typically achieved in 15–20 min, was measured over 60 s and expressed as a ratio of the maximal SkEF over baseline.

Statistical analysis

Data are presented as means ± SEM. SkEF is expressed in arbitrary perfusion units (PU). For statistical analysis of vascular responses, we used logarithmic transformations to normalize distributions. Group differences for dose-response curves were compared using two-way analysis of variance (ANOVA) for repeated measures, followed by *t* tests (after Bonferroni corrections for multiple testing) at each dose when a significant difference between groups was found. The significance of difference between groups for other data was tested using nonparametric tests (Mann-Whitney *U* test). For correlations using acetylcholine and sodium nitroprusside responses, a mean ratio over the three doses was calculated. Correlations on logarithmic transformed data were performed using Pearson's correlation. The null hypothesis was rejected at $P < 0.05$. Statistical analyses were performed using SPSS software (SPSS, Chicago).

RESULTS

— Baseline SkEF was similar in control subjects and patients with type 1 diabetes at the iontophoresis site, 15.3 ± 2.8 and 14.0 ± 1.7 PU ($P = 0.51$, Mann-Whitney *U* test), and control site, 17.5 ± 2.2 and 18.7 ± 2.1 PU ($P = 0.59$), respectively. SkEF did not change significantly at the control site throughout the experiment.

Endothelium-dependent and -independent microvascular responses

Figure 1*A* shows that vascular responses to acetylcholine were significantly reduced in patients with type 1 diabetes ($P < 0.01$, ANOVA). Post-hoc testing showed signifi-

cant differences at all three doses: 0.52 ± 0.06, 0.83 ± 0.05, and 1.03 ± 0.05 in control subjects compared with 0.39 ± 0.03 ($P < 0.05$), 0.64 ± 0.04 ($P < 0.01$), and 0.83 ± 0.03 ($P < 0.005$), respectively, in type 1 diabetic patients. There were significant correlations between the mean response to acetylcholine (averaged over three doses) and the duration of diabetes ($r = -0.38$, $P < 0.01$, Fig. 2*A*) and HbA_{1c} levels ($r = -0.37$, $P < 0.01$, Fig. 2*B*) but not with acetylcholine and age, total cholesterol, systolic and diastolic blood pressure, heart rate, insulin dose, or skin temperature.

Figure 1*B* shows significantly reduced vascular responses to sodium nitroprusside in patients with type 1 diabetes compared with responses in control subjects ($P < 0.01$, ANOVA). At the three doses, SkEF ratios were 0.41 ± 0.06, 0.79 ± 0.07, and 0.93 ± 0.07 PU in control subjects compared with 0.30 ± 0.03 ($P = 0.09$), 0.56 ± 0.04 ($P < 0.005$), and 0.73 ± 0.04 ($P < 0.01$), respectively, in type 1 diabetic patients. There were no significant correlations between the mean vascular response to sodium nitroprusside (averaged over three doses) and any of the parameters listed in Table 1.

Acetylcholine and sodium nitroprusside were significantly correlated in the patient group ($r = 0.28$, $P < 0.05$) but not in control subjects ($r = 0.17$, $P = 0.42$).

Maximal skin microvascular vasodilation

The maximal hyperemia to local 44°C heating was significantly reduced in type 1 diabetic patients compared with responses in control subjects (maximal SkEF over baseline, 18.42 ± 1.26 vs. 25.48 ± 2.60; $P < 0.02$, respectively). There were no correlations between maximal hyperemia and duration of diabetes or glycemic control.

In the patient group, there were no significant differences in vascular responses between males and females (acetylcholine, $P = 0.42$, ANOVA; sodium nitroprusside, $P = 0.60$, ANOVA; and heating, $P = 0.37$, Mann-Whitney *U* test) or between the five smokers and the nonsmokers.

Acetylcholine and sodium nitroprusside responses as ratios of the maximal vasodilator capacity

Heating is an indicator of maximal vasodilator capacity. Because maximal vasodilator capacity was reduced in the patient group, responses to acetylcholine and sodium nitroprusside were expressed as ratios. Ratios for acetylcholine were significantly lower for patients with type 1 diabetes than for control subjects (0.17 ± 0.01, 0.29 ± 0.02, 0.37 ± 0.02 vs. 0.22 ± 0.03, 0.35 ± 0.02, 0.43 ± 0.05, respectively; $P < 0.05$, ANOVA), as were ratios for sodium nitroprusside (0.14 ± 0.01, 0.25 ± 0.02, 0.32 ± 0.02 vs. 0.17 ± 0.03, 0.34 ± 0.03, 0.40 ± 0.03, respectively; $P < 0.05$, ANOVA).

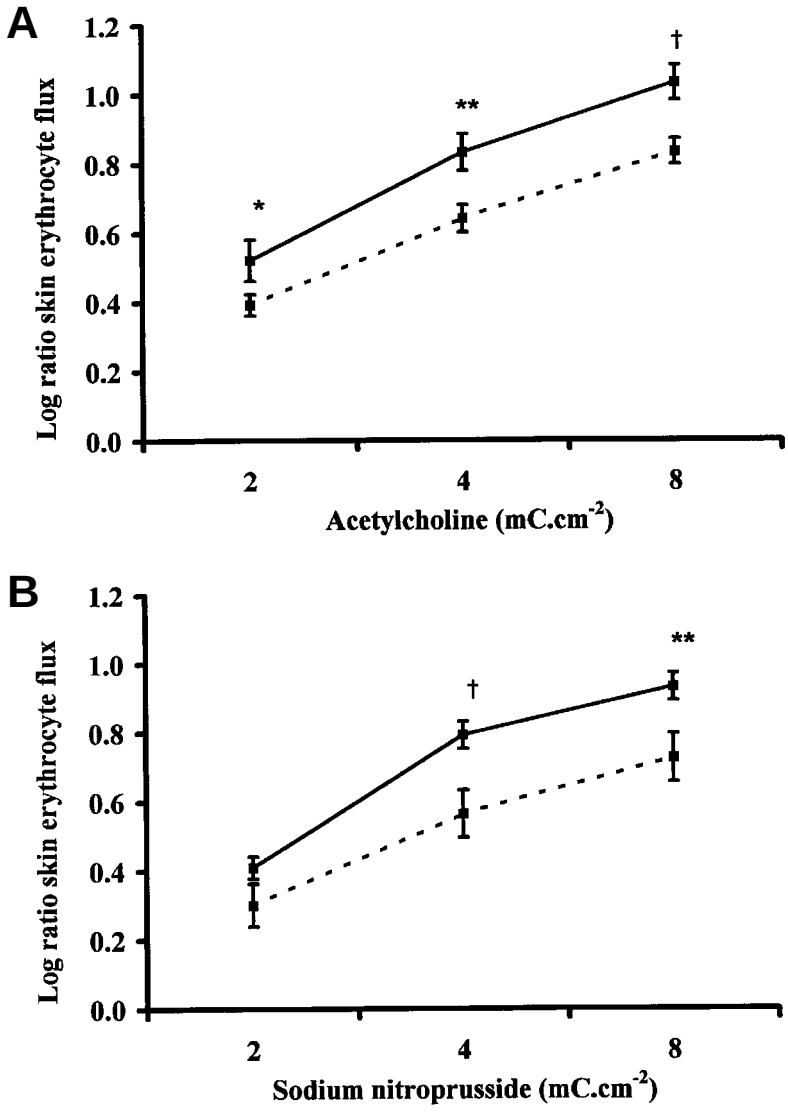

Figure 1—*Logarithmic ratios (response over baseline) of skin microvascular responses to iontophoresis of* (A) *acetylcholine and* (B) *sodium nitroprusside in patients with type 1 diabetes* (n = 55) *[- - -■- - -] and control subjects* (n = 25) *[—■—]. Responses were significantly reduced in patients with type 1 diabetes* ($P < 0.005$ *for acetylcholine and* $P < 0.001$ *for sodium nitroprusside, ANOVA).* *$P < 0.05$, **$P < 0.01$, †$P < 0.005$ *(post-hoc* t *tests).*

CONCLUSIONS — The results of this study show that endothelium-dependent and -independent vascular responses and maximal vasodilator capacity are significantly reduced in young people with type 1 diabetes who have no clinically detectable macrovascular or microvascular complications. Impairment of endothelium-dependent responses was related to the duration of diabetes and to glycemic control. The significant correlation between vascular responses to acetylcholine and sodium nitroprusside in the patient group suggests involvement of a common functional abnormality, perhaps related to impairment of smooth muscle function or defective NO-mediated vasodilation. However, the overall vascular dysfunction probably

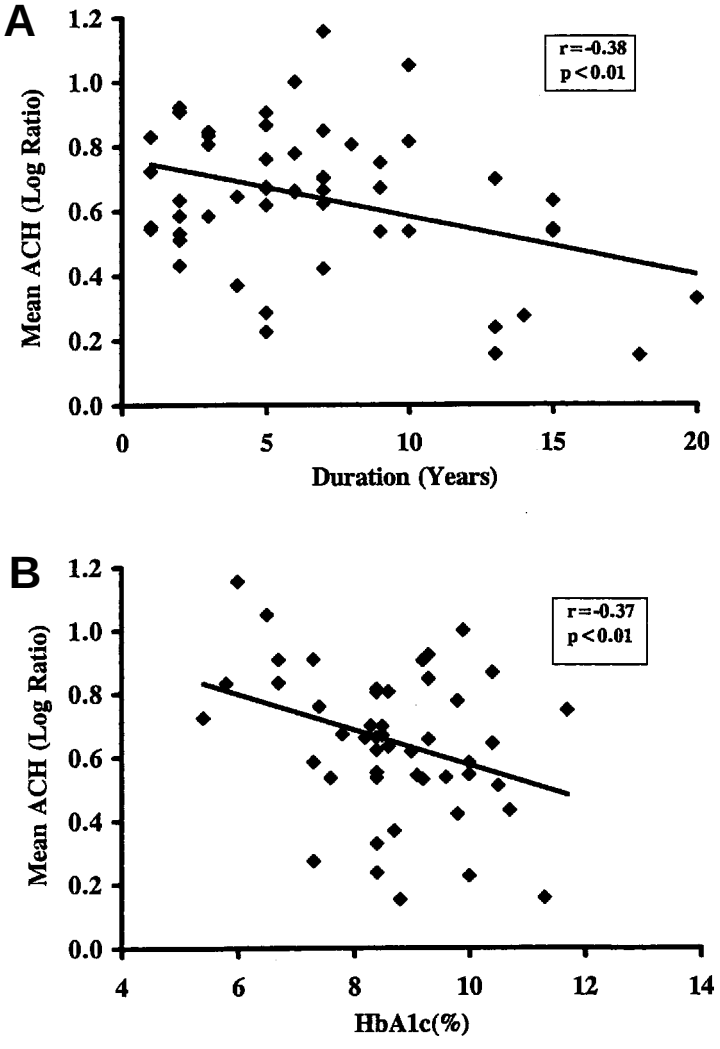

Figure 2—*Graphs showing the correlation between the mean response to acetylcholine (averaged over three doses) and (*A*) duration of diabetes and (*B*) HbA_{1c} levels in patients with type 1 diabetes.*

involves a complex interaction between functional and structural abnormalities in the microcirculation.

In the present study, we used iontophoresis and laser Doppler flowmetry, which have been used recently by others in patients with diabetes (mainly type 2) to show similar abnormalities to those in our study (17–19). Iontophoresis can alter skin perfusion through nonspecific electric effects, but at the low currents we used, these effects are negligible (16,17,19), and any vasodilator effect should be attributable directly to drug delivery.

Endothelial cell dysfunction

Several factors may be responsible for the reduction in vascular responses. One such factor may be endothelial dysfunction (20) with resultant decreased NO synthesis (8). Poor glycemic control may have promoted endothelial dysfunction, as shown by the negative correlation between HbA_{1c} and acetylcholine responses. These results suggest that it is important to maintain strict metabolic control (1,2) to achieve good microvascular function, and indeed, good glycemic control for 1 year has been shown to improve microvascular vasodilation (21).

Previously, we have shown that plasma levels of the endothelial marker von Willebrand factor are raised in children with diabetes (13), and more recently, we found elevated plasma levels of thrombomodulin and vascular endothelial growth factor in the same patients studied here (22). There were, however, no significant correlations between these elevated markers and skin microvascular function, suggesting that endothelial dysfunction affects these systems differently.

Elevated blood pressure may have contributed to endothelial dysfunction (23), but the lack of correlation between blood pressure and acetylcholine responses suggests otherwise. We cannot exclude the possibility that some of the patients had neuropathy, because we did not conduct electrophysiological tests. However, our protocol using low currents was designed to exclude any sensory, axon-mediated response, so any subtle neural changes are unlikely to affect our measurements.

Decreased NO activity

Endothelial cell damage cannot explain the reduced responses to sodium nitroprusside, and so other mechanisms must be involved. One possibility is decreased NO activity mediated via increased oxidative stress and free radical generation (24). Oxygen radicals are known to mediate the breakdown of endothelium-derived NO (25) and can be produced in diabetes by a number of reactions, including glucose auto-oxidation, nonenzymatic protein glycation, and cyclooxygenase catalysis.

Other factors that may affect NO activity are advanced glycosylation end products (26) and elevated HbA_{1c} levels (27). Bearing in mind the relatively short duration of diabetes in our patients, it is uncertain whether the elevated HbA_{1c} levels would have resulted in accumulation of advanced glycosylation end products or quenching of NO. Indeed, we found no correlation between HbA_{1c} levels and responses to sodium nitroprusside.

Structural abnormalities

Structural changes in the vasculature also probably contribute to impaired vasodilator ability, as demonstrated by the reduction in the maximal hyperemic response to heating. Basement membrane thickening may be a factor, although it has only been shown to affect the heat-induced hyperemia in adults with diabetes (28). Basement-membrane width has been shown to correlate with HbA_{1c} levels in postpubertal children (29), but these findings were made in the skeletal muscle of patients with HbA_{1c} levels that were higher than those in our patients (12.0 vs. 8.7%).

Other structural changes may include aortic stiffness, which is increased in adolescents with type 1 diabetes (30), although only in females. Although there was a greater proportion of females in our patient

group, we found no significant differences in vascular responses between the sexes. The patients studied by Hu et al. (30) were also older than ours (15–20 years, compared with 9–22 years), and all were postpubertal, which may increase the likelihood of vascular dysfunction because the passage through puberty and adolescence seems to accelerate the biophysical damage associated with type 1 diabetes (31). The presence of structural changes in the arteries, however, does not necessarily mean that the microcirculation will be affected, as demonstrated by the lack of correlation between macrovascular and microvascular abnormalities (30).

It could be argued that structural abnormalities are completely responsible for the reduced vasodilation to all stimuli used. However, if we assume that the response to local heating represents the maximal vasodilator capacity and express the responses to acetylcholine and sodium nitroprusside as ratios of this maximum, we still find significant reductions in the patient group compared with responses in control subjects. Thus, the relative responsiveness of the vasculature to endothelial cell stimulation and NO activity would appear to be reduced.

Early changes in microvascular function are present in young patients with type 1 diabetes, well before any clinical symptoms are present. We think that skin microvascular abnormalities may be related to a complex interaction between structural and functional changes in the endothelium and smooth muscle. Identifying possible causes for microvascular dysfunction is crucial, because premature vascular disease affects coronary, cerebral, carotid, and peripheral vessels and is potentially reversible, in contrast to established microangiopathy.

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