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Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications

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Summary We have recently shown that the skin microcirculation of toes is significantly impaired in patients with diabetes and peripheral vascular disease, and this may be one major reason why these patients are highly susceptible to developing skin ulcers. The aim of the present study was to investigate whether the skin microcirculation is impaired also in diabetic patients free from macroangiopathy. One foot in each of 20 patients with insulin-dependent diabetes was investigated: 10 patients with and 10 patients without late complications. All patients had normal arterial circulation of their lower extremities. Two groups of age- and sex-matched healthy subjects served as controls. The capillary blood cell velocity in the nailfold of the great toe was investigated by computerised videophotometric capillaroscopy, and the total microcirculation within the same area evaluated by laser Doppler fluxmetry. The capillary blood cell velocity and the total skin microcirculation were studied during rest, and during postocclusive reactive hyperaemia. The total microcirculation was similar in patients and control subjects, whereas the capillary circulation was markedly reduced ($p < 0.01$) in the patients. The ratio between the capillary and total microcirculation was significantly decreased ($p < 0.05$–0.01) in the patients as compared to the control subjects, indicating a local maldistribution of blood in the skin microcirculation of the diabetic patients. The results of the present study show that in spite of a normal total skin microcirculation in the toes of insulin-dependent diabetic patients, both with and without late complications, the nutritional capillary circulation is severely impaired. These findings indicate that a chronic ischaemia is present in the skin capillaries of diabetic feet, and is related to the diabetic disease per se and not to late diabetic complications, and may be a cause for these complications. [Diabetologia (1995) 38: 474–480]

Key words Diabetes mellitus, skin microcirculation, capillary blood cell velocity, laser Doppler fluxmetry.

Several processes characteristic of the diabetic disease work together resulting in a condition called 'the diabetic foot'. This constellation consists of macroangiopathy, peripheral neuropathy, haemorheological and metabolic disturbances, which are combined to a varying extent and can result in severe nutritional disturbances leading to, e.g. bone degeneration and chronic ulcers in the foot [1]. A sufficient blood supply to the affected tissue is one essential factor for the healing process of ulcers. Normally, the main part of the skin blood flow is used for thermoregulation of the body, especially in the digits, and less than 10 % of the total skin blood flow passes through the nutritional skin capillaries [2–4]. However, a severely reduced blood flow through the nutritional skin capillaries has been found in patients with peripheral vascular disease (PVD), despite the fact that the total blood flow could be normal or even increased [5, 6]. We have recently shown that this mal-

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Abbreviations: PVD, Peripheral vascular disease; IDDM, insulin-dependent diabetes mellitus; CBV, capillary blood cell velocity; LDF, laser Doppler fluxmetry.

Table 1. Clinical details of the diabetic patients with (+ Compl) and without (No compl) complications and their respective control subjects

	Diabetes + no compl	Control group 1	Diabetes + compl	Control group 2
Sex (female : male)	5:5	5:5	2:8	2:8
Age (years)	29.9 ± 7.0	30.2 ± 7.9	46.1 ± 9.8	47.3 ± 9.2
BMI (kg/m^2)	22.8 ± 2.7	22.5 ± 2.9	23.5 ± 3.1	22.7 ± 2.3
Smokers *(n)*	5	3	6	3
Duration of diabetes (years)	4.8 ± 2.9		17.7 ± 9.6	
Non-healing foot ulcers *(n)*	0		6	
Retinopathy *(n)*	0		7	
Microalbuminuria *(n)*	0		3	

Results expressed as number *(n)*, or mean ± SD

Table 2. Peripheral blood pressure measurements in the diabetic patients and their respective control subjects

	Diabetes + no compl	Control group 1	Diabetes + compl	Control group 2
Arm systolic blood pressure (mmHg)	123 ± 11.4	121 ± 9.7	133 ± 15.1	128 ± 16.9
Arm diastolic blood pressure (mmHg)	77 ± 4.8	76 ± 5.5	81 ± 10.5	80 ± 11.5
Ankle blood pressure (mmHg)	117 ± 13.1	122 ± 7.5	141 ± 13.6	140 ± 23.1
Toe blood pressure (mmHg)	113 ± 13.5	115 ± 17.7	123 ± 19.6	122 ± 19.0
Toe/arm blood pressure index	0.90 ± 0.12	0.95 ± 0.12	0.94 ± 0.23	0.96 ± 0.13

Results expressed as mean ± SD

Table 3. Cardiovascular autonomic function tests, and vibration perception threshold of the great toe in the diabetic patients with (+ Compl) and without (No compl) complications

	Diabetes + no compl	Diabetes + compl
Heart rate variation during deep breathing		
Exspiration/inspiration ratio	1.39 ± 0.12	1.08 ± 0.05
(reference value[a])	(> 1.26 ± 0.04)	(> 1.18 ± 0.05)
Heart rate response to tilt		
Acceleration index	26.2 ± 8.8	8.6 ± 6.9
(reference value[a])	(> 14.2 ± 2.3)	(> 9.2 ± 2.8)
Brake index	24.3 ± 9.9	5.4 ± 5.6
(reference value[a])	(> 11.7 ± 2.5)	(> 6.1 ± 3.1)
Vibration perception threshold (V)	9.5 ± 2.1	36.7 ± 9.5
(reference value[b])	(< 10.7 ± 1.8)	(< 17.0 ± 4.6)

Results expressed as mean ± SD.
[a] For age-related normal values see reference 13.
[b] For age-related normal values see reference 43

distribution of skin blood flow is even more pronounced in diabetic patients with PVD, and that the postocclusive reactive hyperaemia response is severely impaired [7]. A steal phenomenon, where the blood bypasses the nutritional skin capillaries [8], may be one of the most important factors for the development of tissue ischaemia in diabetic feet, but whether this maldistribution of blood is present also in diabetic patients without PVD is not fully known.

The aim of the present study was to investigate the total and the capillary skin microcirculation in toes of insulin-dependent diabetic patients clinically free from macroangiopathy in the lower extremities, in order to investigate whether also these diabetic patients, in spite of a normal macrocirculation, have a maldistribution of blood in the skin microcirculation, which could explain their increased rish for developing non-healing foot ulcers. The total skin microcirculation, mainly the circulation in the subpapillary vascular bed [9], was evaluated by laser Doppler fluxmetry in an area known to be rich in arteriovenous anastomoses, i. e. the toe nailfold [2]. Simultaneously, the capillary circulation was measured in the same area by a computerised technique of clinical capillaroscopy [3]. This combination makes it possible to investigate the distribution of blood flow between the non-nutritional, subpapillary microvascular compartments of the skin, and the nutritional skin capillaries.

Subjects and methods

Subjects. Two groups of IDDM patients, one with and one without late complications, were investigated. Clinical data are presented in Table 1. All patients were treated with intermittent doses of insulin (three to four times daily). The patients with foot ulcers were also on antibiotics, but otherwise no medication was given. All patients were clinically free from PVD, as evaluated by segmental blood pressure measurements [10, 11] (Table 2). The autonomic nerve function was evaluated by heart rate variation during deep breathing and the heart rate reaction to tilt [12, 13] (Table 3). Peripheral neuropathy was assessed by examining the ankle reflexes, and by measuring vibration perception thresholds of the great toe by biothesiometry [14, 15] (Bio-Medical Instrument Company, Newbury, Ohio, USA) (Table 3). Urine was collected in overnight samples for determination of microalbuminuria [16], and the eyes were examined by an ophthalmologist with ophthalmoscopy and fundus photography. In the patients without

late complications we also measured the nerve conduction velocity in the posterior tibial, the sural, and the median nerves.

Diabetic patients without late complications (Table 1). This group comprised one foot of each of 10 patients (5 males) with a mean age of 29.9 (range 21–35) years and a mean diabetes duration of 4.8 (range 2–10) years. All patients had normal cardiovascular autonomic nerve functions, elicitable ankle jerks, and normal vibration perception tests of the great toe (Table 3). Four patients had a slightly prolonged conduction velocity in some of the investigated nerves, most probably indicating a very early stage of neuropathy. None had microalbuminuria or retinopathy.

Diabetic patients with late complications (Table 1). This group consisted of one foot of each of 10 patients (8 males) with a mean age of 46.1 (range 30–54) years and a mean diabetes duration of 17.7 (range 6–33) years. All patients had evidence of autonomic neuropathy, and the vibration perception test of the great toe was abnormal (Table 3). None had elicitable ankle jerks, and six patients had non-healing foot ulcers for at least 2 (range 2–12) months. The ulcers were located under the metatarsal heads, and were classified as neuropathic ulcers. Three patients had microalbuminuria in overnight urine samples (> 20 mg/12 h), two patients proliferative retinopathy, and five patients background retinopathy.

Control subjects (Table 1). Two control groups were investigated. Control group 1 consisted of 10 feet of 10 age- and sex-matched healthy subjects to the patients without complications, and control group 2 comprised 10 feet of 10 age- and sex-matched healthy subjects to the patients with late diabetic complications. None of the healthy subjects had a family history of diabetes.

Methods. The skin microcirculation in the nailfold of the great toe was investigated by computerised videophotometric capillaroscopy [3, 17, 18] and laser Doppler fluxmetry [9, 19]. All subjects were acclimatized for at least 30 min before the investigations started, and the room temperature was kept between 22–24 °C. All participants were asked to refrain from smoking and coffee drinking the day of the study. The subjects were investigated in the supine position with the knees slightly flexed and the legs comfortably resting in a special holder to avoid involuntary movements of the foot. A miniature cuff (20 mm wide) was applied at the proximal phalanx of the great toe so that arterial occlusions could be performed. The skin temperature of the investigated toe nailfold was continuously recorded with an electronic thermistor (Exacon, Copenhagen, Denmark).

Videophotometric capillaroscopy. Nailfold capillaries of the great toe were visualized on a TV-monitor by a Leitz Laborlux microscope (Leica (Leitz), Wetzlar, Germany) on which a CCD video camera (ICD-44 DC, Ikegami, Tokyo, Japan) was mounted. The image was stored on videotape for subsequent analysis. The capillary blood cell velocity (CBV) was determined by a computerised, videophotometric, cross-correlation technique [3, 17, 18] (Capiflow AB, Stockholm, Sweden). The CBV was measured in a suitable capillary with good contrast and visible signals. This has been shown to be relevant for studying skin microvascular reactivity [20]. The following variables were determined: resting CBV (mm/s); peak CBV (mm/s) and time to peak CBV (s) following release of a 1-min arterial occlusion at the proximal phalanx of the great toe with a cuff pressure of 200 mm Hg; per cent increase of resting CBV (CBV %) during postocclusive reactive hyperaemia.

The reproducibility of the capillaroscopic technique used was tested in the present study. Duplicate measurements of post-occlusive reactive hyperaemia with 2–3 min separation were performed in 30 subjects. There was a strong linear association for peak CBV ($r = 0.98$, $p < 0.001$), and a significant correlation for time to peak CBV between the two measurements ($r = 0.92$, $p < 0.001$). No systematic difference was observed between the two measurements (paired t-test), either for peak CBV ($p = 0.501$), or for time to peak CBV ($p = 0.814$).

Laser Doppler fluxmetry. The total skin microcirculation was measured by laser Doppler fluxmetry (LDF) (Periflux, Pf 1d, Perimed, Stockholm, Sweden) simultaneously with videophotometric capillaroscopy [19]. The laser Doppler output signal, which to more than 90 % is generated by flow in subpapillary vessels [9], was continuously recorded on a pen recorder and full scale deflection was 10 Volt. A bandwidth of 4 khz and a gain of 10 times were used. The laser Doppler probe was placed within the skin area immediately adjacent to the microscopic field of view, and the following variables were measured: resting LDF (V); peak LDF (V), time to peak LDF (s), and per cent increase of resting LDF (LDF %) after a one minute arterial occlusion at the toe base. The remaining flux signal during the arterial occlusion was considered to be the biological zero value (V), which was subtracted from the total laser Doppler signal [9, 21].

The reproducibility was also tested in the present study for the LDF method. Duplicated measurements of postocclusive reactive hyperaemia with 2–3 min intervals were performed in 26 subjects. There was a strong linear association for peak LDF ($r = 0.98$, $p < 0.001$), and no systematic difference between the two measurements was observed ($p = 0.464$). The time to peak LDF showed also a significant correlation between the two measurements ($r = 0.92$, $p < 0.001$), and no systematic difference was noticed ($p = 0.645$) between the two measurements with paired t-test.

Blood tests. Venous blood was taken for determination of haemoglobin, haematocrit, blood glucose, glycated haemoglobin (HbA_{1c}), plasma fibrinogen, serum cholesterol and serum triglyceride. Blood glucose was measured by the glucosoxidase/peroxidase method using dry chemistry (Kodak Ektachem Clin Chem, Rochester, Mn., USA); HbA_{1c} by the ELISA-method using monoclonal antibodies (Dakopatts, DAKO Diagnostics Ltd. Cambridge, UK); plasma fibrinogen by a fibrin-polymerization rate assay (Fibri-Prest Automate, Stago, Asnielres, France); serum cholesterol and serum triglyceride were measured by dry chemistry (Kodak Ektachem Clin Chem); microalbuminuria by a nephelometric assay (Array, Beckman Instruments, Brea, Calif., USA).

Statistical analysis

Data are given as mean ± SD. The Mann-Whitney U test was used to test differences between the groups. A value of $p < 0.05$ was considered statistically significant. The study was approved by the ethics committee of the Karolinska Hospital.

Results

Skin temperature (Table 4). The skin temperature was similar in all four groups, and did not change during the investigation.

Table 4. Microcirculatory data in the diabetic patients and their respective control subjects

	Diabetes + no compl	Control group 1	Diabetes + compl	Control group 2
Skin temperature (°C)	27.3 ± 2.0	28.7 ± 1.4	29.0 ± 2.3	28.9 ± 2.8
Resting CBV (mm/s)	0.09 ± 0.05[b]	0.33 ± 0.24	0.17 ± 0.12	0.35 ± 0.42
Peak CBV (mm/s)	0.18 ± 0.14[b]	0.61 ± 0.39	0.14 ± 0.07[c]	0.63 ± 0.40
Time to peak CBV (s)	19.7 ± 9.1	14.5 ± 6.3	19.9 ± 9.7	14.5 ± 5.7
CBV %	102 ± 157	93 ± 52	31 ± 112[a]	153 ± 122
Resting LDF (V)	1.5 ± 1.3	1.7 ± 1.6	2.8 ± 2.5	1.2 ± 0.8
Peak LDF (V)	2.7 ± 2.0	2.8 ± 1.7	3.8 ± 2.8	3.2 ± 2.2
Time to peak LDF (s)	9.1 ± 3.1	8.8 ± 3.0	9.7 ± 6.4	8.1 ± 4.7
LDF %	171 ± 194	127 ± 109	85 ± 96	175 ± 125
Biological zero (V)	0.10 ± 0.12	0.21 ± 0.11	0.12 ± 0.08	0.19 ± 0.12
Resting CBV/resting LDF	0.12 ± 0.11[a]	0.35 ± 0.30	0.11 ± 0.10[a]	0.37 ± 0.43
Peak CBV/peak LDF	0.13 ± 0.19[a]	0.32 ± 0.28	0.07 ± 0.09[b]	0.24 ± 0.14

Values are given as mean ± SD.
[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ as compared to healthy control subjects

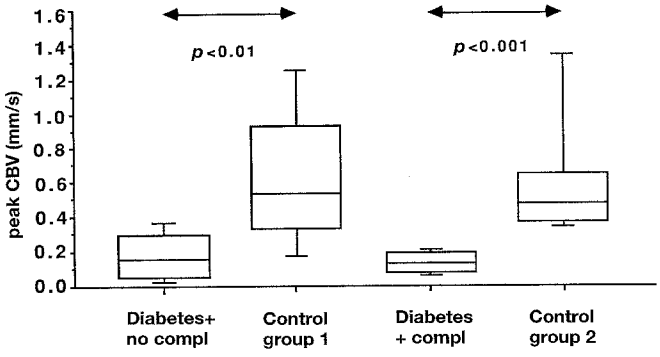

Fig. 1. Peak capillary blood cell velocity (CBV) in diabetic patients with (+ Compl) and without (No compl) complications and their respective control subjects. Box-plot values of peak CBV showing median values and the 10th, 25th, 75th and 90th percentiles

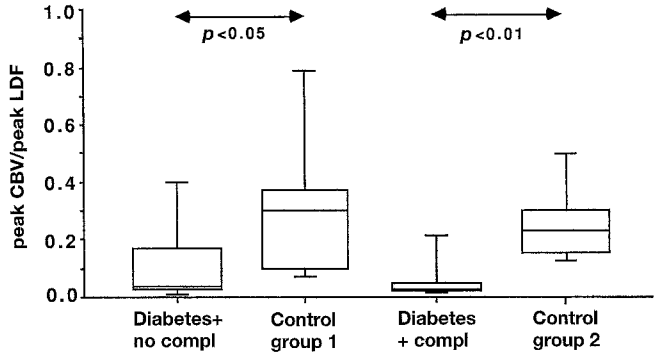

Fig. 2. Ratio between peak blood flow in nutritional (CBV) and non-nutritional (LDF) microvascular compartments in diabetic patients with (+ Compl) and without (No compl) complications and their respective control subjects. Boxplots, see Figure 1

Total skin microcirculation (Table 4). Resting LDF, peak LDF; time to peak LDF and LDF % were similar in both patients and control subjects although there was a tendency for higher LDF-values in the patients with complications. The biological zero value was small and similar in all groups. No differences were found between patients with and without foot ulcerations regarding the LDF variables.

Capillary circulation (Table 4). Resting CBV was lower ($p < 0.01$) in the diabetic patients without complications, as compared to the control subjects, while resting CBV in the diabetic patients with complications did not differ from the control subjects. Peak CBV was markedly decreased in both patient groups ($p < 0.01$), as compared to the control subjects (Fig. 1), while time to peak CBV was not significantly different from the control subjects. CBV % was significantly lower in the diabetic patients with complications ($p < 0.05$) as compared to the control subjects, while it was similar to control subjects in the diabetic patients without complications.

The ratio between CBV and LDF, representing the distribution of blood between nutritional and non-nutritional microvascular compartments, was significantly lower ($p < 0.05$) in both patient groups as compared to the control subjects, both during rest and reactive hyperaemia (Fig. 2).

No differences were found in the CBV variables between patients with and without foot ulcerations.

Blood tests (Table 5). Blood glucose was similar in both patient groups and significantly higher ($p < 0.001$) as compared to control subjects. HbA_{1c} was equally elevated in the patient groups. No significant correlations were found between the microvascular variables and the values of blood glucose and HbA_{1c} respectively. Haemoglobin, haematocrit, plasma fibrinogen, serum cholesterol and serum triglyceride were similar in patients and control subjects.

Table 5. Blood tests in the diabetic patients and their respective control subjects

	Diabetes + no compl	Control group 1	Diabetes + compl	Control group 2
Haemoglobin (120–150 g/l)	144 ± 10	139 ± 10	138 ± 17	145 ± 10
Haematocrit (37–43 %)	42 ± 3.0	41 ± 2.9	40 ± 4.8	42 + 2.7
Blood glucose (3–6 mmol/l)	11.3 ± 6.5[a]	4.5 ± 0.4	9.7 ± 1.3[a]	4.7 ± 0.6
HbA_{1c} (2.5–4.0 %)	6.8 ± 1.0		7.6 ± 1.5	
Plasma fibrinogen (2.1–4.2 g/l)	3.1 ± 0.6	2.8 ± 0.7	3.4 ± 1.1	2.7 ± 0.4
Serum cholesterol (< 5.5 mmol/l)	4.9 ± 0.9	4.4 ± 0.7	5.2 ± 1.0	5.1 ± 1.1
Serum triglycerides (0.6–2.2 mmol/l)	1.0 ± 0.4	1.0 ± 0.3	0.9 ± 0.2	1.2 ± 0.4

Normal reference values in parentheses. Values are given as mean ± SD. [a] $p < 0.001$ as compared to healthy control subjects

Discussion

A common observation in diabetic patients, particularly those with peripheral neuropathy, is a hot and red foot with easily palpable pulses. Despite this apparently adequate blood supply these patients sometimes develop non-healing foot ulcers. In agreement with these observations several studies have shown that diabetic patients have an increased blood flow in the extremities [22–24], and also an increase of the total skin microcirculation [25, 26]. Peripheral neuropathy, in combination with repeated mechanical stress, is considered the main reason why chronic foot ulcers develop in skin areas with an increased or normal blood flow, but another contributing factor may be a maldistribution of blood between the nutritional capillaries and the deeper subpapillary vessels. Peripheral sympathetic denervation of precapillary vessels most probably leads to an opening of arteriovenous connections [27], resulting in blood bypassing the nutritional skin capillaries. This hypothesis is strengthened by the finding of an increased venous oxygenation in the diabetic foot [8], but how this maldistribution influences the circulation in the nutritional capillaries is not fully known.

The results of the present study clearly show that a marked maldistribution of blood between the nutritional capillary and subpapillary microvascular compartments actually exists in the skin microcirculation of diabetic feet, with a striking reduction of maximal blood flow in the nutritional skin capillaries. This impairment of capillary blood flow may lead to an impairment of the regional exchange of nutrients and oxygen, which has also been indicated in other investigations [28]. The fact that the reduction in capillary blood flow was more pronounced during the postocclusive reactive hyperaemia than during basal conditions indicates that the capillary ischaemia is more severe during stress situations when the nutritional demand is increased, e. g. during walking and increased pressure from shoes. Flynn et al. [29] have recently investigated the resting CBV in the toe nailfolds of diabetic patients with neuropathy, and could not find any differences as compared to healthy subjects. By measurements of capillary diameter they also estimated the capillary blood flow to be increased in the patients. However, the skin temperature in their diabetic patients was 5 °C higher than in the control subjects, and as CBV is positively correlated to skin temperature [4, 30], CBV in their patients should have been significantly increased compared to the control subjects. As this was not the case, CBV must have been significantly reduced in the diabetic patients. In our study skin temperature was similar in all groups and this is most probably the reason why our results differ from theirs.

One of the most interesting observations in the present study is that the capillary ischaemia in skin of diabetic feet seems to be present already at an early stage of diabetes, as the patients with only 5 years' diabetes duration also showed this maldistribution. Similar findings of impaired microvascular reactivity in forearm skin have been demonstrasted by transcutaneous oxygen tension measurements in children with short-term diabetes [31]. This indicates that the impaired skin capillary circulation is related to the metabolic disturbances and is not a secondary phenomenon to the diabetic complications. A decreased capillary circulation and an increased arterio-venous shunting has been found also in other tissues of the lower extremity in diabetic patients, e. g. the vasa nervorum of the sural nerve [32], and these findings also support the suggestion that microvascular disturbances contribute to the diabetic foot complications. A successive deterioration of the nerve function with neuropathy as the final state could be the consequence of ischaemia also in nutritional capillaries to the nerves [32, 33]. This is further supported by the finding that the four patients in the present study who had a slightly prolonged nerve conduction velocity also had a severely impaired capillary circulation despite no other evidence of diabetic complications. The extremely high blood flow seen in diabetic patients with osteoarthropathy has been explained by sympathetic denervation of arterioles, leading to an increase in blood flow and rarefacation of bone [1, 34, 35]. Our findings of the present study suggest that another contributing factor to the degeneration of bone tissue in these patients may also be an im-

paired circulation in the nutritional capillaries of the bone tissue.

The exact mechanism behind the maldistribution of blood between nutritional and non-nutritional microvessels is not fully known, but several factors are most probably involved. Long-term hyperglycaemia causes endothelial and metabolic disturbances leading to an elevated blood circulation, capillary hypertension and increased vascular leakage [24, 36, 37, 26, 38], which may progress to irreversible structural changes [39], such as a thickening of the capillary basement membrane [40]. An increased capillary blood pressure, which has been demonstrated in both the toe and finger nailfolds of diabetic patients [26, 38], may be a consequence of an opening of arteriovenous shunt vessels with a transformation of the arteriolar pressure out into the subpapillary venular plexus, resulting in a decreased arteriovenous pressure difference. Since the skin capillaries are rigid tubes [2, 41], such a reduced pressure difference will result in a reduced capillary blood flow, leading to hampered tissue nutrition. Haemorheological disturbances, such as a reduced erythrocyte deformability [42], endothelial cellular dysfunction [37], increased blood viscosity and fibrinogen levels [43, 44], are additional factors which may further enhance the impaired capillary function.

In conclusion, we have shown that a local microvascular dysfunction with a maldistribution of blood between non-nutritional and nutritional skin vessels is present in the feet of IDDM patients. This maldistribution seems to be more related to the diabetic disease per se than to diabetic complications. As the skin of the toes and feet is exposed to great stress, the disturbed circulation in the nutritional capillaries may generate a regional ischaemia contributing to the syndrome known as 'the diabetic foot' [1]. If the described capillary dysfunction is a primary cause for the diabetic foot complications new therapeutic modalities may improve the prognosis for these patients.

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