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Disturbances of Cutaneous Microcirculation in Patients with Diabetic Legs: Additional Parameters for a New Therapeutic Concept?

S. BÜHLER-SINGER, D. HILLER, H.-P. ALBRECHT, C. SEIDEL and O. P. HORNSTEIN

Department of Dermatology, University of Erlangen-Nuernberg, Erlangen, Germany

To study disturbed microcirculation involved in the pathogenesis of diabetic neuropathic plantar ulcers (DNPU), we recorded dynamic changes in laser-Doppler flux (LDF) and cutaneous oxygen tension ($p_{cu}O_2$) caused by short-time arterial occlusion and local heating at three different sites (forefoot, ulcer edge, lower leg) in patients ($n = 14$) with DNPU and healthy controls ($n = 18$). Significantly reduced dynamic $p_{cu}O_2$ parameters coincided with a significant increase of flux in the patient group. This post-stimulatory "hypoxic hyperemia" indicates a shifting of blood flux, reducing circulation of the nutritive capillaries comparable to an internal "steal effect". This may predispose to the development of DNPU when additional stresses influence the initial borderline balance, characterized by similar $p_{cu}O_2$ and increased flux values compared to controls. Abolishment of normal vasoconstriction in the shunt vessels by diabetic polyneuropathy is the assumed cause of increased arteriovenous perfusion and therefore raised flux values. Non-invasive testing of microcirculatory functions demonstrates characteristic disturbances in DNPU patients and could be used as additional parameters for new therapeutic concepts as the intravenous retrograde perfusion (RVP). After RVP therapy, applied to a subgroup ($n = 7$) of the patients, some dynamic microcirculatory parameters improved, allowing a preliminary quantitative evaluation of a therapeutic regimen. ***Key words: oxygen tension; laser-Doppler fluxmetry; diabetic microangiopathia; neuropathic plantar ulcer; retrograde venous perfusion.***

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S. Bühler-Singer, Department of Dermatology, University of Erlangen-Nuernberg, Hartmannstraße 14, D-91052 Erlangen, Germany.

Currently, the average morbidity for manifest diabetes mellitus rates 2% in Europe. Ten per cent of the patients develop socalled diabetic legs, producing about 25% of the total expenses of hospital treatment in diabetics (1, 2). The socalled diabetic leg is in 60–70% due to diabetic neuropathy, while the rest is caused by arterial occlusive disease or a mixture of both (1). Solely neuropathic foot ulcers (DNPU) are characterized by indolent and deep plantar ulcers with surrounding hyperkeratosis and peripheral polyneuropathy with sensory loss combined with signs of a disturbed microcirculation. Infrared thermography often reveals additional plantar hyperkeratoses as "hot-spots" indicating imminent danger of ulceration (3).

For this reason, comprehensive investigations of the microvascular functions in these patients are important. However, most studies have used only one of the available non-invasive methods – laser-Doppler fluxmetry (LDF) or cutaneous oxygen tension measurement ($p_{cu}O_2$) – at one or two sites (4–10). Using $p_{cu}O_2$ and LDF simultaneously at three differently affected locations, one can pay special attention to the dynamic changes of the microcirculatory parameters caused by stimulus-response tests, e.g. short-time arterial occlusion and local heating.

Until now, the treatment of DNPU has been tedious and mostly unsatisfactory. A new therapeutic concept (11) uses the principle of Bier's arterial occlusion to get effective drugs in high concentration into the affected area and was reported to have impressive results in DNPU (12). Thus, the determination of the functional microcirculatory parameters seems necessary also in reference to the evaluation of new therapies.

PATIENTS AND METHODS

Patients, control group and therapeutic regimen

Cutaneous microcirculation was studied in 14 male patients (13 type II-diabetes, 1 type I-diabetes, age 48–78 years, mean 60 years) suffering from long-term diabetes (mean 17 years, range 4–55 years) treated with insulin ($n = 3$) or oral antidiabetics ($n = 11$). There was no intake of vasoactive drugs. All patients showed deep and sharply bordered, painless plantar ulcers of 1–4 cm diameter with surrounding hyperkeratosis, persisting for 0.5–120 months (mean = 17 months) and suffered from diabetic polyneuropathy, partly combined with osteolytic lesions at various metatarsal or phalangeal bones.

Of all inpatients consecutively presenting themselves with DNPU, we excluded those with pronounced limb oedema and arterial occlusive vascular disease (AOD), using the following non-invasive parameters for determination: no symptoms of claudication, no rest pain, no undergone or planned vascular surgery, normal Doppler sonographic arterial pressure at the arm, the ankle and a normal ankle/arm index (ratio of leg systolic pressure to arm systolic pressure greater than 1 (29)). Additionally, patients with an ankle-arm pressure difference greater than 30 mmHg in supine position were excluded due to the possibility that stiff calcified vessels could mask peripheral AOD (28). Measurements of arterial pressure on the toes, alternatively angiography, duplex sonography or oscillometry, could also be used as parameters showing arterial insufficiency to be absent. However, these partly invasive methods were not available to our patients. The diabetic polyneuropathy was secured by neurologic consultation (typical sensory loss, diminished perception of vibration, cold and warmth, impaired nerve conduction velocity).

These criteria limited our patient group to only 14 patients in 2 years but also ensured that AOD or polyneuropathy of other origin did not influence the supposed disturbances of microcirculation in DNPU. Eighteen males (age 42–72 years, mean 60 years) suffering at the same time also as inpatients from other dermatologic diseases (e.g. melanoma), but without any vascular and/or metabolic alterations, served as a control group.

In a subgroup ($n = 7$) of the above patients – who had all given their informed consent – microcirculatory measurements were carried out before and after a 10-day therapy with retrograde transvenous perfusion (RVP) once a day, which is based on Bier's arterial occlusion, also termed "passive hyperemia". It was first employed in 1908 for regional anaesthesia in limbs (13). After aseptic puncture of a dorsal foot vein, arterial occlusion by a sphygmomanometer cuff above the knee was produced and an isotonic saline solution (120 ml) containing 120 mg gentamycin, 50 mg buflomedil, 4 mg dexamethasone, 4 mg lignocaine and 2500 IU of heparin (11) was injected. This occlusion was maintained for 20 min after injection (11, 12).

All measurements were carried out at a controlled room temperature (22°C) 4 h after breakfast, using the three sites positioned at heart level.

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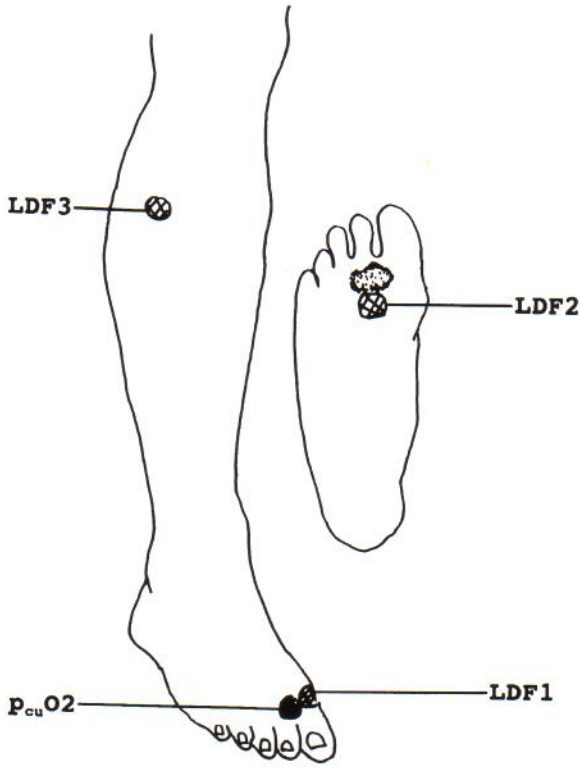

Fig. 1. Measurement sites of the laser-Doppler fluxmeters (LDF 1–3) and the transcutaneous oxygen electrode.

Measurements began after 30 min equilibration of patients and control subjects resting in supine position.

As illustrated in Fig. 1, the dorsal forefoot proximal hallux ($p_{cu}O_2$, LDF 1), the proximal edge outside the plantar ulcer (LDF 2) and the lateral lower leg 10 cm distal of the knee-joint level (LDF 3) were chosen as the three measuring sites, representing various clinically differently affected locations.

Methods used

Cutaneous oxygen tension was measured polarographically using a transcutaneous oxygen probe containing three platinum wire electrodes at a mutual distance of 2 mm (14) (Oxymonitor, Hellige, Germany). The measured pO_2 is the mean pO_2 in a skin area of approximately 4 mm diameter. The probe itself with the built-in heating system has a diameter of 2.0 cm. A probe temperature of 37°C was used. Depending more on local skin properties, including capillary flow, than on the arterial oxygen tension, pO_2 values at 37°C, termed cutaneous pO_2 ($p_{cu}O_2$), give information about the epidermis and the papillary layer (15).

Since inter- and intraindividual differences of epidermal thickness and capillary density may restrict the comparability of the individual data, the exact marking of the skin position of the probe and probe holder is important to ensure approximately the same area within the measuring window of the probe. Additional stimulatory tests are necessary to overcome the variability of pO_2 measurements at 37°C (16–18).

Red blood cell flux (LDF) was measured simultaneously using two laser-Doppler fluxmeters (19, 20) (Periflux Pf 1d, Perimed, Sweden; blood-flow Monitor MBF 3D, Moore Instruments, U.K.) with tube-shaped probes of 5 mm, outer diameter, and a sampling window of 1 mm. Due to the deep skin penetration of the laser light used, capillaries as well as dermal arterioles and venules are within the measuring volume (1–2 mm in diameter). Depending on the measuring site, part of those or all can contribute to the flux signal. However, it is not possible to determine which part of the LDF signal arises from which precise anatomical structure within the measuring volume. Again, exact marking of the skin position of probe and probe holder is necessary to reduce the influence of the known intraindividual spatial heterogeneity of flux.

Transcutaneous measurements of pO_2 require skin/probe temperatures of at least 37°C. With the placement of the oxygen and flux probe

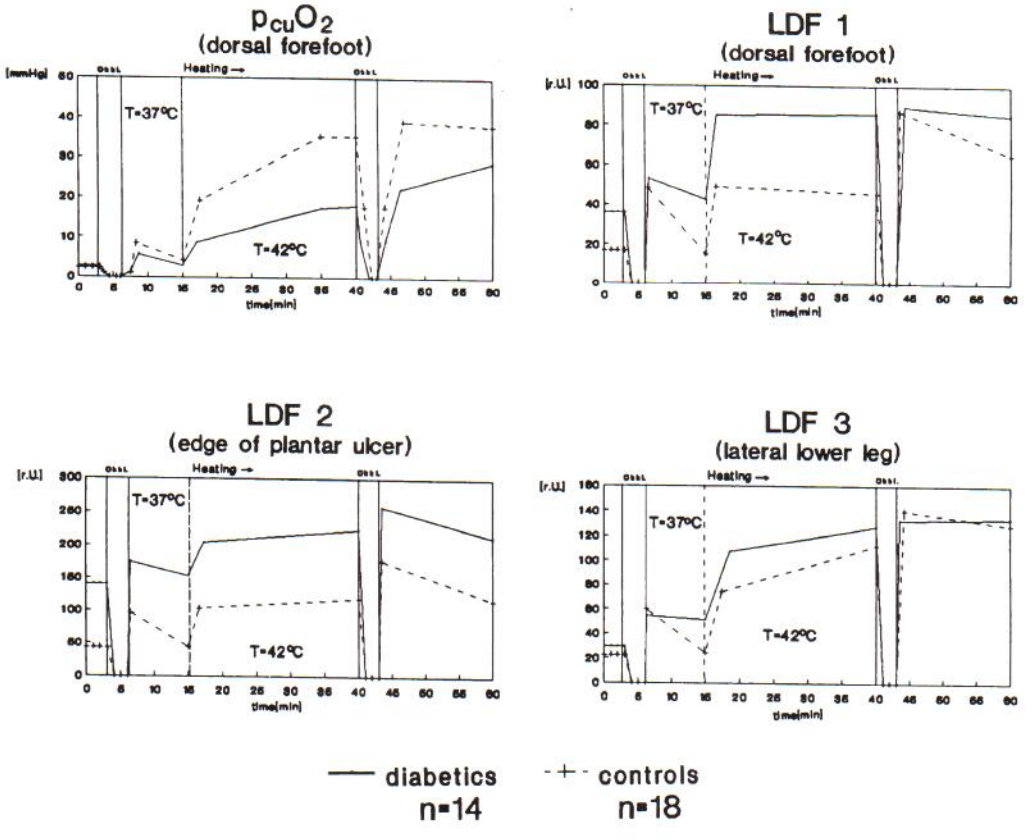

Fig. 2. Diabetic patients ($n = 14$) and controls ($n = 18$): mean values of $p_{cu}O_2$ and flux (LDF 1–3) continuously recorded before, during and after occlusion at 37°C (time 0–15 min), local heating to 42°C (15–40 min) and after occlusion at 42°C (40–60 min).

Table I. *Occlusion and heating parameters*

Static and dynamic parameters of cutaneous microcirculation (mean ± SEM) in patients with diabetic legs ($n = 14$) and in healthy controls ($n = 18$). Sites of $p_{cu}O_2$ and LDF 1: distal dorsal forefoot. LDF 2: plantar ulcer edge. LDF 3: lateral aspect of lower leg.

	Sites		Diabetics before RVP	Controls
37°C:				
Initial values (IV 37°C)	Forefoot	$p_{cu}O_2$ (mmHg)	2.7± 1.7^{ns}	2.3± 1.6
	Forefoot	LDF 1 (r.U.)	36.1± 20.7**	17.0±10.2
	Plantar	LDF 2 (r.U.)	139.8± 86.4**	42.4±28.6
	Lower leg	LDF 3 (r.U.)	28.8± 26.0^{ns}	22.2±13.7
Maximum post-occlusive value (MV_{po})	Forefoot	$p_{cu}O_2$ (mmHg)	5.7± 3.5^{ns}	8.5± 3.2
	Forefoot	LDF 1 (r.U.)	53.1± 21.9^{ns}	48.2±22.2
	Plantar	LDF 2 (r.U.)	174.4± 97.4*	95.6±44.9
	Lower leg	LDF 3 (r.U.)	54.2± 43.3^{ns}	60.1±21.5
42°C:				
Initial values (IV 42°C)	Forefoot	$p_{cu}O_2$ (mmHg)	18.1± 8.4***	35.3± 9.8
	Forefoot	LDF 1 (r.U.)	85.9± 42.3**	45.9±28.9
	Plantar	LDF 2 (r.U.)	224.1±121.5*	108.3±53.3
	Lower leg	LDF 3 (r.U.)	127.4±105.1^{ns}	116.2±55.6
Maximum post-occlusive value (MV_{po})	Forefoot	$p_{cu}O_2$ (mmHg)	22.2± 6.2***	39.1± 7.9
	Forefoot	LDF 1 (r.U.)	99.6± 38.8^{ns}	87.1±43.1
	Plantar	LDF 2 (r.U.)	258.6±125.1^{ns}	176.9±59.3
	Lower leg	LDF 3 (r.U.)	132.6±140.3^{ns}	140.3±53.9
Heating:				
Initial values before heating (IV_{bh})	Forefoot	$p_{cu}O_2$ (mmHg)	2.9± 2.4^{ns}	4.2± 2.4
	Forefoot	LDF 1 (r.U.)	42.8± 23.5***	15.4± 6.7
	Plantar	LDF 2 (r.U.)	153.5± 89.5***	43.6±32.9
	Lower leg	LDF 3 (r.U.)	51.3± 68.9^{ns}	24.0±15.3
First maximum during heating (MV_{dh})	Forefoot	$p_{cu}O_2$ (mmHg)	8.6± 5.7***	19.2± 7.1
	Forefoot	LDF 1 (r.U.)	85.1± 48.2*	49.3±27.7
	Plantar	LDF 2 (r.U.)	205.2±112.9*	103.7±82.3
	Lower leg	LDF 3 (r.U.)	106.9±110.8^{ns}	74.2±48.4
End values after 20 min (EV_h)	Forefoot	$p_{cu}O_2$ (mmHg)	17.4± 8.3***	35.3± 9.4
	Forefoot	LDF 1 (r.U.)	85.9± 42.3**	45.0±22.6
	Plantar	LDF 2 (r.U.)	224.1±121.5^{ns}	118.7±44.4
	Lower leg	LDF 3 (r.U.)	127.4±105.1^{ns}	112.5±54.8

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns $p < 0.05$.

side by side at the dorsal forefoot we chose to measure the flux also at a similar temperature (36°C due to the Periflux equipment) which, as compared to unheated skin, produces a moderate dilatation of the microvasculature. Since at these temperatures vessels are far from being fully dilated, stimulatory tests yield good enough responses and can considerably reduce the interindividual heterogeneity of resting microcirculation together with assuring the same measuring temperature in each individual.

Functional reactivity of cutaneous microcirculation was tested by stimulus-response experiments. After reaching stable initial values (IV) for flux (LDF 1, LDF 2, LDF 3 at 36°C) and $p_{cu}O_2$ (at 37°C), which takes approximately 15 min, abrupt arterial occlusion was produced by immediate automatic inflation of a standard-sized thigh blood pressure cuff up to 40 mmHg above the systolic pressure. Zero-perfusion was maintained for 3 min and the cuff was then deflated. The subsequent reactive hyperemia was registered over 15 min. Then local hyperthermia (42°C) was produced in all probes using the built-in heating. After 20 min of continuous application of local heat, a further 3-min arterial occlusion was carried out. All the data were continuously recorded during the two occlusions, heating and up to 20 min afterwards with special determination of maximum post-occlusive value (MV_{po}), maximum value during heating (MV_{dh}) and end value after 20 min of heating (EV_h). No repeated investigations were made considering the duration of each measurement (approximately 2 h).

Comparing the data between diabetics and controls and between the subgroup ($n = 7$) of diabetics prior to and after RVP, statistical analysis was performed using the Wilcoxon's rank test after correcting all flux values against the individual "zero-flux" during occlusion to improve the interindividual comparability of the data (21).

RESULTS

The mean values of $p_{cu}O_2$ and flux calculated for selected points during the continuous recording of data are shown for all three measuring sites in Fig. 2. Details of the patients and the controls are shown in Table I.

Similar initial values (IV 37°C) for $p_{cu}O_2$ (dorsal forefoot proximal hallux) could be found in the patients (2.7±1.7 mmHg) and control group (2.3±1.6 mmHg). However, after the first occlusion (MV_{po} 37°C) a trend (not yet significant) to lower $p_{cu}O_2$ values for the diabetics (5.7±3.5 mmHg) in comparison to the controls (8.5±3.2 mmHg) became evident, and a highly significant ($p < 0.001$) $p_{cu}O_2$ decrease in the patient group during the following heating test (MV_{dh}, EV_{dh}) and after the second occlusion (MV_{po} 42°C) (Table I) was found.

At first view (Fig. 2) of the flux values (LDF 1, 2, 3), an

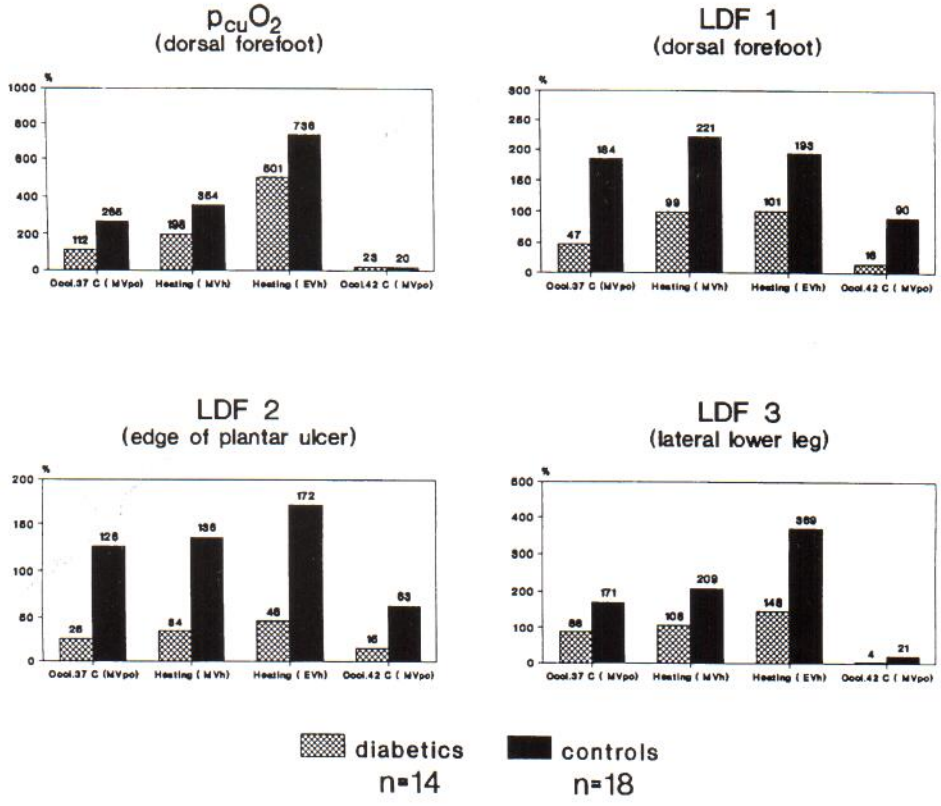

Fig. 3. Diabetic patients ($n = 14$) and controls ($n = 18$): percentages of increases of the mean values after defined stimuli based on the values before stimuli; MV_{po}: maximum post-occlusive values; MV_{dh}: first maximum during heating; EV_h: end values after 20 min of heating.

overall increase of most of the LDF values in the diabetics, compared to the controls, is notable. Especially at the edge of the plantar ulcer (LDF 2), significantly increased flux values were found.

In order to test the capacity of post-stimulatory vasodilatation, short-time arterial occlusion and local heating were carried out and the percentages of the subsequent post-stimulatory increases

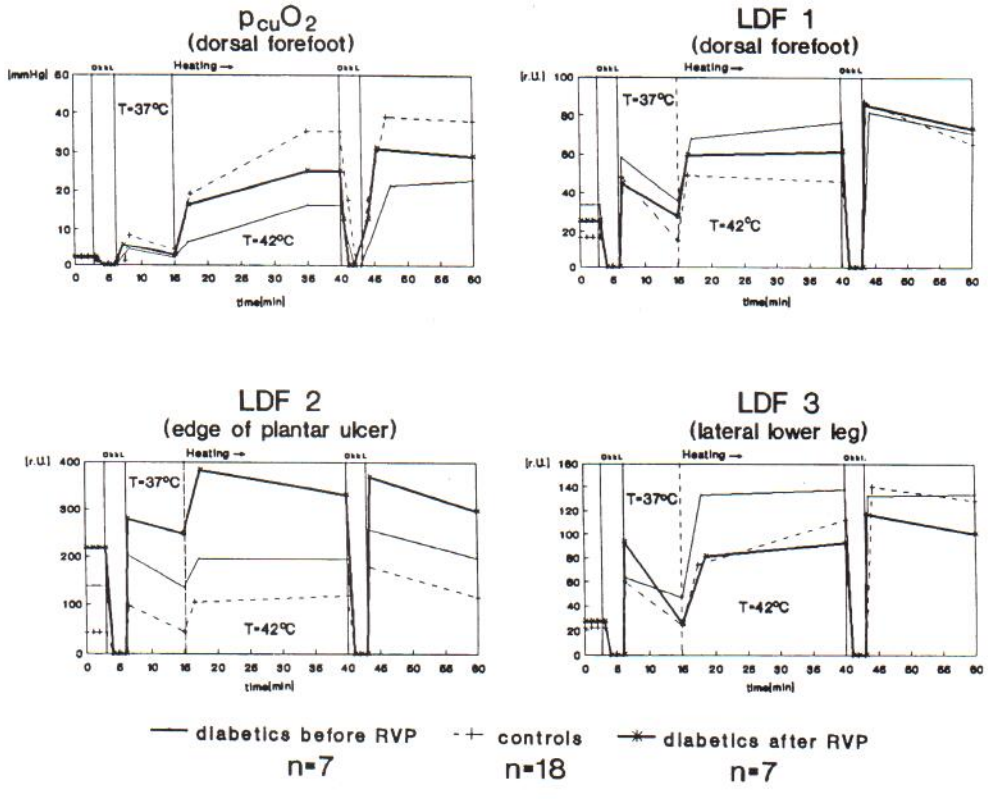

Fig. 4. Subgroup ($n = 7$) of diabetic patients before and after RVP: schematic time course drawing.

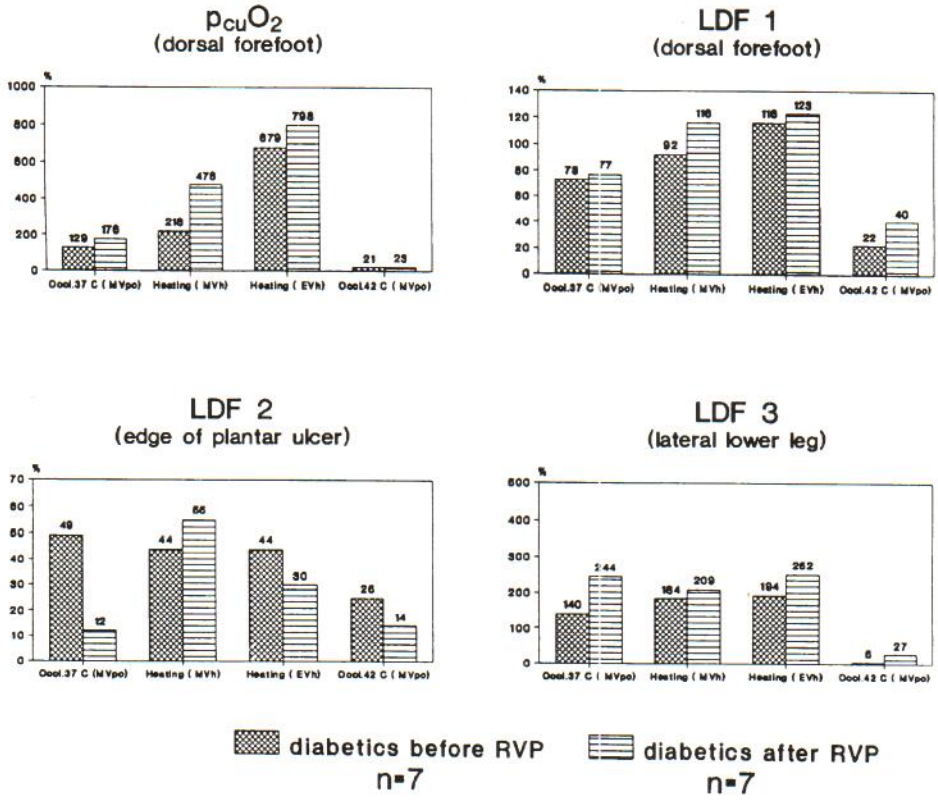

Fig. 5. Subgroup ($n=7$) of diabetic patients before and after RVP: percentages of increases of the mean values after defined stimuli based on the values before stimuli.

(I_{ps} = [post-stimulatory maximum value/pre-stimulatory initial value] × 100) are shown in Fig. 3.

The percentages of post-stimulatory increases for mean $p_{cu}O_2$ and LDF values after occlusion 1 (37°C), local heating and occlusion 2 (42°C) were found to be lowered in the patients by 1.5 to 5.5 times in comparison to controls.

Furthermore, in a subgroup ($n=7$) of the above patients, measurements were repeated after 10 days of RVP therapy. After RVP the mean of the initial $p_{cu}O_2$ values (1.8±1.2 versus 2±1.5 mmHg) did not improve significantly; yet maximum post-occlusive (MV_{po} 37°C) $p_{cu}O_2$ values (4.2±2.3 versus 5.6±2.7 mmHg) showed significant improvement ($p<0.05$). The $p_{cu}O_2$ reactions to local heating (MV_{dh} = 16.1±5.1 mmHg) and to the second occlusion (MV_{po} 42°C = 31±6.3 mmHg) showed a significant increase ($p<0.05$) of approximately 50% and 100%, respectively. In the schematic plot (Fig. 4) of the mean values versus time a marked decrease of previously increased flux values (compared to the controls) can be seen for LDF 1 and LDF 3 (LDF 1 before/after RVP: IV 37°C = 33.7±20.5/25.1±17.7 r.U., LDF 3 before/after RVP: IV 37°C = 26.5±24.1/27±14 r.U.). However, it was not significant due to the large standard deviation and small number of patients. In addition, with the exception of LDF 2 all post-stimulatory reactions (Fig. 5) improved up to two fold.

For the plantar location (LDF 2) a further decrease of post-stimulatory reactions compared to pre-therapeutic values and controls could be found, because the flux at the edge of plantar ulcer (LDF 2) increased further after RVP, even becoming significant at 37°C (LDF 2 before/after RVP: IV 37°C = 137.9±59.7/205±74.3).

DISCUSSION

The methods used (LDF, $p_{cu}O_2$) have physiological limits due to the inter- and intraindividual heterogeneity of microcirculation (20, 22, 23) but have the advantage of quantitative non-invasive and continuous measurements. Their restrictions have to be kept in mind even when minimizing external and internal influences on the microcirculation. As published before (18), the following factors, which influence the heterogeneity of microcirculation, should be observed:

1. Control of intraindividual spatial heterogeneity by exact marking of probe positions on the skin.
2. Control of interindividual spatial heterogeneity by using always the same anatomically defined and marked position, comparing only groups of subjects, performing stimulatory tests (14, 16, 22, 23) and correcting all flux values against the individual zero flux.
3. Control of temporal heterogeneity by using always the same standard procedure in the stimulatory tests, measuring always at the same time of day and under the same standardized environmental conditions.

Measurements of cutaneous hyperemia reactive to short-time arterial occlusion or local thermic stimulation enable investigations of the socalled vascular reserve of skin (10). In particular, the capacity of post-stimulatory vasodilation can be tested (24). The physiological mechanisms involved in the post-occlusive hyperemia of skin vessels are not yet fully understood. On one side, accumulation of vasodilating metabolites in the tissue is the assumed major cause, whereas changes of intravasal pres-

sure and socalled compliance of the blood vessels, i.e. their elasticity and distensibility, are thought to be the other reason (24–26).

In a group of diabetic patients with DNPU we found, with different degrees of severity, an increased flux at all three measurement sites and a reduced post-stimulatory $p_{cu}O_2$ at the dorsal forefoot (Fig. 2). This indicates a "shifting" of blood from nutritional capillaries to the deeper vessels, which could be explained by the enhanced perfusion of arteriovenous (a.-v.) shunts. Furthermore, the percentages of the poststimulatory increase (I_{ps}) after short-time arterial occlusion and after local heating were markedly lowered, for $p_{cu}O_2$ and LDF (Fig. 3). The same initial $p_{cu}O_2$ values in patients and controls together with a significantly increased flux suggest a just compensated "borderline" situation of cutaneous oxygen supply. After defined additional stresses, the delicate regulation of blood flow between the superficial capillaries (nutritional) and deeper vessels (thermoregulatory) breaks down, leading to insufficient O_2 supply and in the long run to ulcer formation. This would explain the clinically known fact that such patients develop ulcers even after small trauma or insufficient footwear. Healing of such ulcers can be achieved sometimes by long-time bedrest and corrective orthopedic shoes eliminating plantar pressure, which "acts" as additional stress.

Earlier studies have already demonstrated distinct alterations of the cutaneous microcirculation in diabetic patients. However, most measurements using only one method were carried out in adults or children with diabetes without skin lesions and at sites such as forefoot, ankle and forearm (4, 5, 8, 9, 16). Former measurements of elevated pO_2 levels in the venous blood of diabetics (23) support our findings of increased a.-v. shunt perfusion (5, 27–29, 31). Partsch (30) has also demonstrated an increased a.-v. shunt volume in neuropathic limbs using labelled human albumin microspheres. Again, this increased perfusion of a.-v. shunts can be explained by abolishment of normal regional vasoconstriction caused by diabetic neuropathic denervation. In animal experiments, an opening of a.-v. shunts could be induced by lumbal sympathectomy (31). Skin biopsies taken from humans with alcoholic polyneuropathy revealed a loss of sensory nerves and nerve endings with denervation of the cutaneous vessels (32).

For diabetic polyneuropathy, an analogous degeneration of sympathetic nerve fibres innervating the cutaneous a.-v. shunts can be assumed. A further hint for the enhanced perfusion of a.-v. shunts at the expense of the capillaries is provided by the socalled "hot spots" measured by infrared thermography, which may indicate imminent plantar ulceration (3). Accordingly, an additional local stress (e.g. heating, occlusion) may further reduce the bypassed capillary circulation, leading to an internal "steal effect". The significantly decreased post-stimulatory $p_{cu}O_2$ values (IV 42°C, MV_{po} 42°C, MV_{dh}, EV_h) measured in the diabetics confirm this hypothesis. However, MV_{po} 37°C was not significantly decreased compared to the controls. Thus, the occlusion at almost physiological temperature may not exhibit a strong enough provocation.

One has to keep in mind that our patients were a selected group with polyneuropathy and without macroangiopathy, oedema and vasoactive drugs. Aside from objectively assessing the microcirculatory disturbances in DNPU patients caused by diabetic microangiopathy, the described techniques can be used to quantitatively assess therapy also in other dermatologic diseases with known microangiopathy, as has already been done in progressive systemic sclerosis (18) and necrobiosis lipoidica (33). They also make possible a first evaluation of the new therapeutic concept of RVP in DNPU.

The local intravenous application of drugs during a 20-min arterial occlusion of the limb is most effective to achieve high tissue concentrations. This was shown in experiments with 99 mTc pertechnetate and 99 mTc labelled human serum albumin in patients with DNPU (34). Encouraged by the clinical results of Acevedo & Schoop (35) and Ferreira et al. (11), we introduced RVP for treatment of DNPU in our hospital some time ago (12). To our knowledge, studies of cutaneous microcirculation using both, $p_{cu}O_2$ and LDF measurements in DNPU patients also referring to RVP treatment have not yet been performed. Up to now, only 7 patients have been included in this study due to our strict exclusion criteria (e.g. no evidence of large vessel disease). Additional measurements are necessary. The impact of RVP therapy on the microcirculatory parameters showed an improvement for post-stimulatory values of $p_{cu}O_2$, LDF 1 and LDF 3 that coincided with the beginning of the healing of the ulcers. The changes found in local blood flux and $p_{cu}O_2$ (reduction of the initially hyperemic flux LDF 1 and LDF 3, increase of $p_{cu}O_2$) can be explained by a beneficial shifting of blood flow from deeper vessels to nutritional capillaries and a reduction of inflammatory elevated O_2 consumption leading to an increased $p_{cu}O_2$. For the plantar location (LDF 2), the decrease is poststimulatory reactions due to the further flux increase after RVP could be due to an abacterial inflammatory reaction (e.g. "cellular necrectomy") in the phase of tissue repair. The beginning of a clinical healing process at this location makes a drug-induced impairment less likely; however, further investigations are necessary.

In conclusion, the present study yields ample evidence for a characteristically disturbed microcirculatory status and functional reactivity in DNPU patients, showing that non-invasive testing of microcirculatory functions provides important and reliable additional parameters for the evaluation of therapeutic regimens such as RVP.

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