Acta Derm Venereol (Stockh) 1998; 78: 258–261

The Veno-arteriolar Reflex in Venous Leg Ulcer Patients Studied by Laser Doppler Imaging

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Posture-induced microcirculatory changes in the lower leg were studied in venous leg ulcer patients and in control subjects by means of laser Doppler imaging (LDI), a technique which allows almost real-time mapping of the perfusion from a distance, each perfusion value constituting the mean of a number of measurements at separate sites. LDI values for intact skin with the subject supine were 0.39 (0.32, 0.47) V [geometric mean (gm –SD, gm +SD)] and 0.32 (0.15, 0.70) V in two age groups of controls and 0.91 (0.66, 1.24) V in patients (NS). Values were 2.04 (1.25, 3.35) V for skin at the ulcer margin, and 1.44 (0.72, 2.88) V in the ulcer proper. With the lower leg passively dependent, lower LDI values were obtained at all sites in all groups, the reduction in intact skin value being 62 ± 11% (arithmetic mean ± SD) ($p < 0.01$) in the younger controls, 43 ± 24% ($p < 0.01$) in the older controls, and 62 ± 19% ($p < 0.001$) in the patient group, and the reduction in ulcer values being 45 ± 27% ($p < 0.05$) for the margin and 52 ± 23% ($p < 0.001$) for the ulcer proper. Thus, a high degree of postural vasoconstriction was present overall, even in the ulcer itself. Vasomotor tone in the skin of the lower leg was assessed by topical application of methyl nicotinate, a vasodilator. The skin perfusion value (supine position, no stimulus) was 71 ± 31% ($p < 0.01$) of the drug-induced (assumed peak) hyperaemia value [0.60 (0.30, 1.10) V] in patients and 24 ± 25% ($p < 0.001$) of the hyperaemia value (1.30 (0.64, 2.62) V] in the controls. It would appear that in ulcer patients the veno-arteriolar reflex, despite being comparable in magnitude to that in controls, may nonetheless be insufficient to reduce tone during dependency to a level similar to that in healthy controls. ***Key words: skin microcirculation; chronic venous insufficiency; veno-arteriolar reflex; skin microvascular tone.***

(Accepted December 22, 1997.)

Acta Derm Venereol (Stockh) 1998; 78: 258–261.

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The skin vasoconstriction that is activated by increased transmural venous pressure during limb dependency prevents the development of capillary hypertension (1–4). In patients with chronic venous insufficiency, local oedema is a consequence of hypertension at the capillary level (5) and structural derangement of the skin microcirculation (6, 7). In recent studies of patients with venous insufficiency, the veno-arteriolar reflex was found to be either intact (8, 9) or reduced (10, 11) and amenable to local treatment by compression. In these studies, the skin veno-arteriolar reflex was assessed by conventional fibre-optic laser Doppler flowmetry (LDF) at small, discrete skin sites, and the responses in intact skin, skin adjacent to a leg ulcer and the ulcer itself were not investigated simultaneously. The skin microcirculation is prone to manifest substantial temporal and spatial variation. Temporal changes can be assessed by continuous measurement using fibre-optic LDF. Spatial changes cannot be reliably assessed in this way, since a number of measurements at different sites need to be taken almost in real time. Laser Doppler perfusion imaging (LDI) (12, 13), a development of LDF, but performed from a distance, produces a perfusion image of a large area containing up to several thousand separate measuring sites almost in real time. This technology thus measures spatial skin perfusion in particular.

In this study, LDI was used to study posturally induced microcirculatory changes in the lower leg in patients with chronic venous insufficiency and venous leg ulcers and in control subjects; the assessments were made on intact skin, skin adjacent to the ulcer, as well as in the ulcer itself.

MATERIAL AND METHODS

The study was undertaken in consenting volunteers after approval by the Central Oxford Research Ethics Committee and in accordance with the declaration of Helsinki. Twelve patients (5 women and 7 men, mean age 71 years, range 47–82 years) with chronic venous insufficiency and venous leg ulcers on the medial aspect of the leg were studied, as were 14 normal control subjects divided into two groups, a younger control group consisting of 6 subjects (4 women and 2 men, mean age 28 years, range 20–42 years) in whom 8 limbs were studied, and an older control group of 8 subjects (7 women and 1 man, mean age 53 years, range 48–61 years) in whom 11 limbs were studied. In those of the control subjects who were entered twice, both limbs were studied at an interval of at least 8 weeks. Smokers and diabetics were excluded. All patients had had venous ulceration, usually recurrent, some for 6 months, and others up to 18 years (mean: 14 years). The ulcers ranged in size from 0.5 cm^2 to 8.4 cm^2. Skin fibrosis was usually present at the ankle. Two of the patients had had their superficial veins removed surgically.

All subjects had ankle/brachial systolic blood pressure ratios > 0.9. Light reflecting rheography (LRR) showed all patients to have a recovery time of less than 15 s (normal: > 25 s). LRR in conjunction with occlusion of the superficial veins above and then below the knee showed 10 of the patients to suffer from deep venous insufficiency. In all the control subjects recovery time was more than 25 s.

During experimentation, room temperature was kept at 20–23°C. Conversation was kept to a minimum. Intake of tea and coffee was avoided during the 1.5 h period preceding the measurements. The ulcers were cleansed with 0.9% NaCl solution. If zinc paste had been used on the adjacent skin, the paste was swabbed off with gauze and dried paste was carefully removed with a pair of forceps. The ulcer was left exposed. The subjects were always allowed 20 min of rest, barefoot, in the supine position, before measurements were made.

Laser Doppler Perfusion Imaging (LDI). The technique has been described in detail elsewhere (12, 13). Briefly, the scanner consists of an He-Ne laser tube, a photodetector and a processing unit connected to a computer (Lisca Development AB, Linköping, Sweden). A data acquisition and analysis system generates, processes and displays images of tissue perfusion by sequentially moving the laser beam over the tissue from a distance through a maximum of 4096 measuring sites covering an area of approximately 144 cm^2 in about 5 min. The laser light penetrates the epidermis and diffuses into the dermis, where a

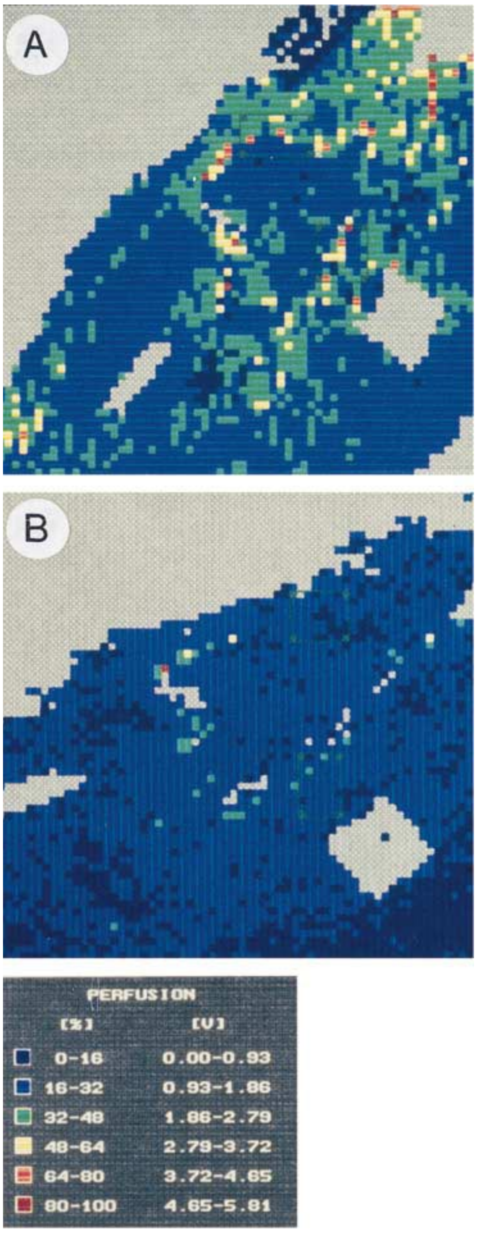

Fig. 1. Typical images made from LDI scans in supine (A) and dependent (B) positions in a patient with leg ulcer. Each square is related by its colour to the output from one measuring site. The image colour code is shown below.

fraction of the beam becomes Doppler-shifted after scattering in moving red cells contained in capillaries as well as in larger dermal vessels. A small fraction of the frequency shifted light which is backscattered from the tissue is received as input to the photodetector and converted to an electrical output signal expressing tissue perfusion in terms of the product of the (mean) velocity and concentration of blood cells in the tissue volume of the measuring site. The output signals derived from each measuring site are stored and, after completion of a scanning procedure, a perfusion image of the underlying tissue can be generated. This map-like image is colour-coded, each colour corresponding to a certain level of perfusion defined as a fraction of the maximum perfusion level of a specific image. LDI values can also be retrieved as absolute values from a chosen number of measuring sites, and are expressed in volts (V). The laser beam was kept roughly perpendicular to the tissue, avoiding excessive convexity.

In experiment A (see below) an image of ulcer and adjacent skin was composed of 64×64 measuring sites, corresponding to a skin area covering approximately 64 cm^2. The ulcer was positioned centrally in the image (see Fig. 1). An image of intact skin on the narrower calf of healthy volunteers was composed of 40×40 sites–approximately 36 cm^2–with the centre 4 cm above the medial malleolus. In experiment B (see below) an image of intact skin was composed of 64×64 sites; each image was centred 4 cm proximal to the medial malleolus at the location used for drug delivery. Black circular markers (diameter, 5 mm) were used to locate the defined part of the leg in relation to its image, and at repeat scanning care was taken to maintain this relationship unchanged.

The LDI values were determined for different parts of each image; the value for the ulcer proper was based on >35 measuring sites, the value for the ulcer edge (including skin) on >70 measuring sites and the value for adjacent intact skin (>2 cm from the ulcer) on >35 measuring sites. At repeat measurements the same areas were sought. In order to avoid the influence of system off-set zero values, all LDI values are given after subtracting the value measured on a stationary grey scatterer.

Temperature measurements. Skin temperature was measured using a digital thermometer (ET 100/200). In patients, the probe was attached to the skin with surgical tape as close to the ulcer as possible, but outside the scanned area. In control subjects it was placed immediately above the medial malleolus. Deeper soft tissue temperature measurements were made with a digital thermometer placed on the skin of the calf under an insulating pad (Mon-a-therm thermometer and insulating pad, model 6510), also attached with surgical tape. The pad prevents the normal flow of heat outwards, allowing the temperature to equalize between the skin surface and deeper tissues. Once this stage is reached, heat flow from the deeper tissues is minimal and the temperature reading relates indirectly to the deeper tissue temperature (14). Readings were taken before and after scanning the leg.

Photography. Using a Polaroid camera (autofocus SX-70) and an automatic camera with a 28–80 mm zoom lens (P30, Pentax), photographs were taken of the limb prior to the experiment, with black markers in place.

Topical drug delivery. Methyl nicotinate (50 µl, 1.25 mM, aqueous solution, pH 5.5–5.7) was delivered transdermally by means of a reservoir (diameter: 12 mm) applied occlusively 4 cm proximal to the medial malleolus. Care was taken to avoid skin obviously afflicted by lipodermatosclerosis. Sixty seconds later the reservoir was rapidly removed and the skin dried with gauze. Preliminary experiments using conventional LDF (PF3, Perimed, Sweden) showed that the nicotinate hyperaemia had reached a stable level within 10–20 min following drug application.

Experimental series

(A) An image was first recorded with the leg supine at heart level and then while sitting with the ankle approximately 1 m below heart level 20 min after assuming dependency (patients: $n=8$; younger control subjects: $n=8$; older control subjects: $n=11$). Temperature readings were also taken.

(B) LDI images were made in the supine position before and 10, 15 and 20 min after topical application of methyl nicotinate. Eight of the patients and 8 of the control subjects (4 from the older age group) participated. There was an interval of 3 months beween experiments A and B.

Calculations and statistical analysis

Because of skewness in the distribution of the LDI values, they were logarithmically transformed and expressed as a geometrical means (gm–SD, gm+SD) (15). All other values are given as arithmetic means ± SD. Statistical significance of differences was assessed with the Wilcoxon paired rank-sum test or the Mann-Whitney U test. A p-level of 0.05 or less was considered significant.

Table I. *Summary of the LDI findings in supine and passive dependent positions. LDI values are expressed as geometric means (gm–SD gm+SD) and percentage decreases as arithmetic means ± SD.* *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

	LDI values (V)		
	Supine	Dependent	Decrease during dependency (%)
Control groups intact skin			
–younger subgroup	0.39 (0.32, 0.47)	0.14 (0.11, 0.19)**	62 ± 11
–older subgroup	0.32 (0.15, 0.70)	0.16 (0.10, 0.27)**	43 ± 24
Patient group			
–intact skin	0.91 (0.66, 1.24)	0.32 (0.19, 0.54)***	62 ± 19
–ulcer margin	2.04 (1.25, 3.35)	0.87 (0.72, 2.88)*	45 ± 27
–ulcer proper	1.44 (0.72, 2.88)	0.65 (0.30, 1.50)***	52 ± 23

RESULTS

In experiment A, superficial skin temperatures were 29.9 ± 1.0 (mean ± SD) versus 28.6 ± 1.0°C (dependent) for the patient group and 29.4 ± 1°C versus 28.1 ± 1.4°C for the younger control group, the corresponding values being 28.4 ± 2.0 versus 27.1 ± 2.1°C for the older control group. The corresponding deep tissue temperatures were 30.3 ± 2.9°C versus 28.9 ± 2.7°C for the patients and 32.0 ± 2.1°C versus 30.4 ± 1.7°C for the younger and 31.6 ± 0.9°C versus 29.2 ± 1.2°C for the older controls. Neither the group differences nor the differences between supine and dependent values were statistically significant.

Typical LDI images of a patient with leg ulcer are shown in Fig. 1A, B. Spatial differences in perfusion are clearly evident. Levels of perfusion in the ulcer and in the ulcer edge are higher than on intact skin. A marked, general decrease in perfusion levels is observed during dependency.

The results of experiment A are shown in Table I. Patient and control groups did not differ significantly (NS) in LDI values for intact skin, neither in the supine nor the dependent position. With the lower leg passively dependent, lower LDI values were obtained at all sites in all groups, and the differences were all statistically significant.

In experiment B, the LDI value in the patient group was 0.40 (0.21, 0.63)V [geometric mean (gm –SD, gm +SD)] before and 0.60 (0.30, 1.10)V after transdermal application of methyl nicotinate ($p < 0.01$). In the controls the corresponding values were 0.19 (0.06, 0.64)V versus 1.30 (0.64, 2.62)V ($p < 0.001$). Thus the LDI value before applying the drug was 71 ± 31% of that observed during the ensuing hyperaemia in the patients, the corresponding value in the control group being 24 ± 25% (of the hyperaemia value).

DISCUSSION

When a person changes posture from a supine to a sitting or standing position, the leg becomes exposed to a hydrostatic pressure corresponding to its vertical distance from the heart level. Among the events set in motion, the vascular network distends and an increased fraction of the blood volume tends to become accommodated mainly in the more distensible venous system in skin and muscle. Unchecked, this marked redistribution has consequences at a systemic as well as at a skin microcirculatory level. Capillary hypertension increases transcapillary filtration, and results in local oedema and tissue pressure changes. If not compensated for, these events may have detrimental effects on blood supply, nutrition and tissue integrity. The redistributional change is quickly corrected, however, by means of increased sympathetic activity, acting on the heart and the peripheral arterial and venous vascular networks and triggering cutaneous vasoconstriction (1–3, 16, 17). The relative importance of baroreceptor (cardiopulmonary and arterial) and positional (vestibular, exercise, veno-arteriolar and myogenic) reflexes in this response is still incompletely elucidated. Recently, a primary involvement of the veno-arteriolar reflex in the cutaneous vascular adjustments to postural change was reported (17). The reflex was found to relate to limb position rather than to body position or blood pressure, and to be peripherally rather than centrally mediated, and neurogenic rather than myogenic (17).

In patients with venous insufficiency, the microcirculation of the leg becomes chronically exposed to an increase in hydrostatic pressure resulting in increased tissue pressure (18), a decrease in density of the most superficial dermal capillaries (7), dilatation and deformation of individual capillaries (6) with accumulation of leukocytes and platelets (19), and formation of a pericapillary halo (20) consisting mainly of fibrin (21). When the dependent position is assumed in venous insufficiency, the number of visible functioning capillaries decreases (22), as does the rate of movement of blood cells within these vessels–as can be observed after removing the overlying epidermis by suction (23).

The control groups in experiments A and B were not completely identical and there was an interval between the two studies. In the following it is assumed that these differences will not affect the results. Like conventional LDF, LDI relates to movement of particles–or rather, blood cells–in all directions within the measuring volume and does not permit quantitative measurement of skin blood flow in terms of volume flow through defined vessels. Neither the relative contribution of the arterial and venous dermal components of the microcirculation, nor the relative contributions from superficial papillary loops and deeper dermal vasculature can be discerned. LDI values in an ulcer cannot be directly compared with corresponding values in intact skin. The vascular networks differ structurally and, without interposition of (reflecting) epidermis, the LDF values are approximately 30% greater than those obtained on intact skin (24). Methyl nicotinate penetrates the epidermal barrier and reduces tone in the dermal microcirculation. It is assumed in the following that peak hyperaemic response to the drug is reached both in patients and in controls.

No significant difference in hyperaemic response to the drug has been observed between healthy young and old healthy individuals (25).

In patients, the vascular tone in the intact skin at rest was decreased compared with the controls. Expressed as a percentage of peak microvascular tone release during stimulation with methyl nicotinate, the baseline value at rest was 71% of the maximum in patients, while in the controls it was 24%. To some extent, these findings are consistent with those of a study where skin tone was reduced by local warming of the skin (26). Arterial inflow has been shown to be markedly increased in extremities with venous insufficiency (27), an observation that may in part be explained by a decrease in skin vascular tone.

Dependency of the lower leg resulted in vasoconstriction within the ulcer, at its edge and in adjacent skin, and the relative magnitude of the veno-arteriolar reflex, determined in percent of the respective baseline value, was fairly similar at all three sites and for patients and controls alike. The finding that the magnitude of the response in the ulcer bed and in the adjacent skin may be intact appears to be an original observation, while its undiminished presence in intact skin is consistent with previous findings by others (8, 9, 20, 22).

Taken together, the findings of a marked difference in baseline tone between patients and controls, and a veno-arteriolar reflex of the similar relative magnitude in patients and controls alike, suggest that an intact veno-arteriolar reflex in the patients may be insufficient to reduce tone during dependency to a level comparable with that in controls. The efficiency of the veno-arterial reflex has been assessed by means of percentage values alone (4). Since basal skin microcirculatory tone may vary markedly between patients and controls, such a procedure may yield misleading results.

The temperature measurements relate only indirectly to tissue volume blood flow; they do not allow quantitative assessment and are used only as a means of demonstrating a general circulatory change.

To sum up, the findings permit the following conclusions to be drawn: in venous leg ulcer patients, basal tone in intact skin at a distance from the ulcer was markedly decreased compared with controls; dependency of the lower leg resulted in vasoconstriction in the ulcer, at its edge and in adjacent skin, and the relative magnitude of the veno-arteriolar reflex, determined in percent of the respective baseline value, was similar at all three sites and for patients and controls alike; and, finally, it would appear that in ulcer patients the veno-arteriolar reflex, despite being comparable in magnitude to that in controls, may nonetheless be insufficient to reduce tone during dependency to a level similar to that in healthy controls.

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