# Periwound Skin Microcirculation of Venous Leg Ulcers

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Chronic venous ulceration has an estimated prevalence of from 0.06 to 1.3% with about 57 to 80% of patients with leg ulcers having demonstrable venous disease. The sequence of events whereby chronic venous insufficiency leads to leg skin ulceration is not yet worked out. Venous hypertension may set the stage for subsequent ulcer development via linkages to observed changes in skin microvessel metrics, morphology, rheology, permeability, hemodynamics, and the interstitium. Periulcer measurements show decreased transcutaneous oxygen and elevated carbon dioxide tensions, yet the skin blood perfusion measured with laser Doppler fluxometry (LDF) is reported to be elevated. This elevated perfusion, $(Q)$, could be due to independent changes in blood velocity $(U)$, and volume $(V)$, with different mechanistic implications depending on the mode. Thus, we sought to determine the relative contributions of these two components with the aim of clarifying the mechanism responsible for the reported skin flow changes. Patients studied ($n = 16$) had unilateral venous ulcers, an ankle/brachial BP index >0.8, and venous pathology demonstrated by duplex imaging. Ulcer areas ranged from 0.6 to 76.9 cm$^2$ (mean = 13.7 cm$^2$) and were present for 2 to 144 months, mean = 32. With the patient supine, Q (ml/min/100 g), V (%), and U (mm/sec) were measured by LDF (Vasamedics, BPM403A) at two to three sites on periulcer skin and compared with measurements at corresponding sites on the nonulcerated limb at local skin temperatures of 35 and 44. Results confirm an elevation in basal periulcer flow (7.0 vs 1.8 ml/min/100 g, $P = 0.001$) and show this to be due to elevations in both circulating blood volume (1.24 vs 0.62%, $P < 0.001$) and velocity (1.23 vs 0.65 mm/sec, $P = .004$). Maximal Q, V, and U were also higher on the ulcer leg, being for Q, 11.2 vs 6.42 ml/min/100 g, $P = 0.03$; for V, 1.49 vs 1.13%, $P = .002$; and for U, 1.76 vs 1.33 mm/sec, $P = 0.020$. Expressing each leg's basal values as a percentage of its own maximal response shows the ulcerated leg to have higher values for Q, V, and U, with (ulcer leg/control leg) ratios being 2.5, 1.8, and 1.4, respectively. These findings show that the LDF perfusion increase is due to roughly equal increases in microvessel circulating blood volume and velocity. Based on the present functional data and the preponderance of *in vivo* microvessel changes reported in the literature, a plausible and consistent characterization of the periulcer tissue is one of a tissue in which the number of microvessels is reduced, with the remaining ones carrying a greater blood volume at an increased blood velocity.  © 1994 Academic Press, Inc.

## INTRODUCTION

The sequence of events whereby chronic venous insufficiency (CVI) leads to leg skin ulceration is not yet fully worked out. Chronic venous hypertension due to deep venous and/or ankle perforator incompetence (Fagrell, 1979) together with an increased reflux index (Raju and Fredericks, 1991) is thought to set the stage for subsequent ulcer development. Associated changes in skin microvessel metrics, morphology, rheology, permeability, hemodynamics, and changes in the

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interstitium have been observed and documented (Davis and Lawler, 1961; Winklemann et al., 1961; Fagrell, 1979; Ehrly and Partsh, 1989). One or more of these may provide the final link in the pathway that leads to chronic venous ulceration, a condition that has an estimated prevalence of from 0.06 to 1.3% with about 57 to 80% of patients with leg ulcers having demonstrable venous disease (The Alexander House Group, 1992). Various elements of the observed microangiopathies have been incorporated into several theories to account for the ulceration and these are presently being debated (Browse and Burnand, 1982; Franzec et al., 1984; Ehrly and Partsh, 1989). Bearing on this issue are functional measurements of skin near venous ulcers or on nonulcerated skin in patients with CVI at probable preulcer skin regions. These have demonstrated a decreased transcutaneous oxygen tension (Franzec et al., 1984; Sindrup et al., 1987), an elevated transcutaneous carbon dioxide tension, yet an increased skin blood perfusion as measured with laser Doppler methods (Sindrup et al., 1987). The significance of the elevated perfusion is controversial in part because it is unclear if the nutrient capillaries are adequately sampled by the method. Nonetheless, since the observed elevated tissue perfusion could be due to somewhat independent changes in blood velocity and volume, with different mechanistic implications depending on the mode, we sought to quantify the separate changes in these two components. Since laser Doppler-measured RBC flux depends on the separable product of the velocity and concentration of moving RBC in the measured tissue volume, this method suited the purpose and was used to measure perfusion in the periulcer skin of the ulcer-bearing leg and in the nonulcerated contralateral leg of 16 patients with venous ulcers.

## METHODS

### Patient Population

Sixteen consecutive patients presenting to our wound-healing center with venous ulcers were studied (11 males). Patient age was 61.1 ± 3.7 years with a range of 43–84 years. No patient had diabetes or intermittent claudication and all had an ankle-brachial systolic blood pressure index >0.8, and venous pathology on the ulcerated limb as demonstrated by duplex imaging. Of these 16 patients, 94% had incompetence of at least one deep vein (femoral, popliteal, or tibial), 47% had one-vessel involvement, 47% had two-vessel involvement, and 6% had three-vessel involvement. Ulcer location was on the medial gaiter in 14/16 patients, the anterior gaiter in 1 patient, and the lateral gaiter in 1 patient. Ulcers were traced and their areas evaluated. Areas ranged from 0.60 to 76.9 cm$^2$ and had been present for durations ranging from 2 to 144 months.

### Experimental

All measurements were begun after the patient had been resting in a supine position for 20 min. Using Laser Doppler fluxometry, LDF (Vasamedics, Model BPM403a) and an associated temperature control module, RBC flux was measured at two or three circumferential sites on periulcer skin close to the wound margins and also on the contralateral leg at corresponding anatomical sites. The order in which these measurements was made was randomized. The laser Doppler probe

(Model P435) was placed through a concentric hole in the heater module and the combination affixed to the skin with double-sided electrode tape. Two minutes after probe application at each site, LDF was first measured at a temperature setting of 35° for 2 min and then the temperature setting was increased to 44° and maintained for a period of 5 min. During this time the LDF analog signals representing the RBC perfusion (Qldf), volume (Vldf), and velocity (Uldf) were displayed on a chart recorder (Gould WindowGraf and also converted to digital form and acquired and stored in a microcomputer for later analysis. Room temperature during these studies was 23.6 ± 0.29°. Transcutaneous oxygen tension (TcPo2) was measured at periulcer and contralateral sites with a Novametrix Model 811 set at 45°. Steady-state values obtained after 20 min were recorded. Skin temperature at each LDF site prior to heating was measured with a surface temperature probe. As previously (Mayrovitz and Regan, 1993), data is presented in converted units as obtained directly from the LDF instrumentation in which ldf flow is expressed as ml/min/100 g, ldf volume as a percentage, and ldf velocity as mm/sec. It is recognized that there is controversy over the appropriate conversions, but data reporting in the converted units is at least as good as that reporting relative units.

### Analysis

Laser Doppler parameters at a particular site were determined as the time average of the basal (35°) 2-min recording and as a 2-min heated value (44°) centered ± 1 min around the maximal heated value. The periulcer and contralateral leg values were determined as the average of the basal and heated parameter values for the two to three sites per leg measured. Thus, each ulcer and each control leg is represented by a single average value for basal and heated conditions. Skin temperatures at each site were also averaged to yield a single skin temperature to represent the periulcer skin and the contralateral leg skin. Statistical comparisons were made using the nonparametric Wilcoxon test.

## RESULTS

### Blood Perfusion

Figure 1 shows the basal (35°) ldf flow values for each individual patient on the ulcer and the control leg. The mean values are respectively 7.0 and 1.8 ml/min/100 g with the periulcer values significantly greater ($P = 0.0004$). Figure 2 compares the mean values obtained at 35° and those after heating to 44°. The ldf flow at the maximally dilated heated condition is significantly greater on the ulcer leg (11.2 vs 6.4 ml/min/100 g, $P = 0.02$).

### Blood Volume

Figure 3 shows the basal (35°) ldf volume values for each individual patient on the ulcer and the control leg. The mean values are, respectively, 1.24 and 0.62%, with the periulcer values significantly greater ($P = 0.0008$). Figure 4 compares the mean values obtained at 35° and those after heating to 44°. The ldf volume at the heated condition is significantly greater on the ulcer leg (1.49 vs 1.13%, $P = 0.007$).
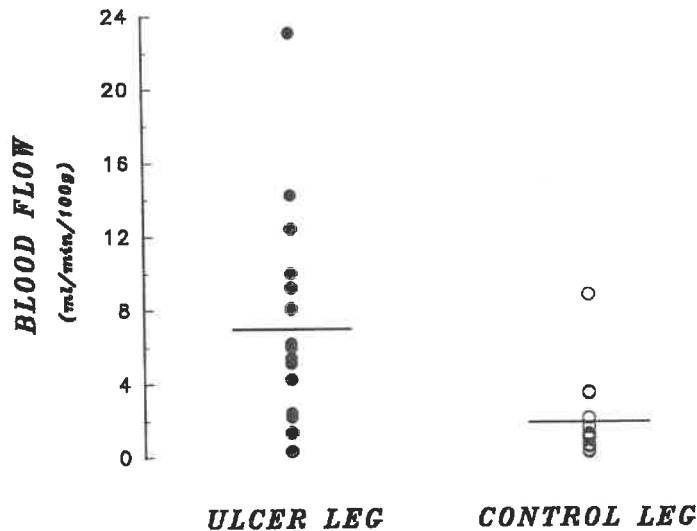
FIG. 1. Individual basal (35°) LDF-flow values for each patient. Horizontal line is the mean value.

## Blood Velocity

Figure 5 shows the basal (35°) ldf RBC velocity for each individual patient on the ulcer and the control leg. The mean values are, respectively, 1.23 and 0.65 mm/sec with the periulcer values significantly greater ($P = 0.004$). Figure 6 compares the mean values obtained at 35° and those after heating to 44°. The ldf velocity at 44° is significantly greater on the ulcer leg (1.76 vs 1.33 mm/sec, $P = 0.026$).

## Microvascular Reserve

The microvascular reserve (MVR) was assessed via the equation MVR = {1-($X35/X44$)}*100 where $X$ corresponds to the Qldf, Vldf or Uldf values as measured at 35 and 44°. The range on MVR is from 0 to 100%, with larger values indicating a larger reserve. This quantity reflects the percentage of the maximal available perfusion which is being utilized under basal conditions with lower values of MVR associated with greater basal perfusion percentages. Figure 7 shows the comparison for flow, volume, and velocity between ulcer and control legs which reveals a significant reduction in MVR for the ulcer leg in all three parameters, with significance levels of 0.0004, 0.006, and 0.036, respectively. The corresponding ratios of $X44/X35$ for control and ulcer legs were 5.57 vs 2.08 for Qldf; 2.44 vs 1.43 for Vldf; and 2.23 vs 1.08 for Uldf. Paired comparisons of basal ldf parameters in ulcer leg/control leg show that the overall ratio (mean $\pm$ SEM) is 2.5 $\pm$ 0.2, 1.8 $\pm$ 0.2, and 1.4 $\pm$ 0.1 for flow, volume, and velocity, respectively.

## Skin Temperatures and TcPo2

Skin temperatures in all patients was measured at the periulcer site and on the contralateral limb at corresponding sites. The periulcer skin temperature was elevated compared with that of the control limb (33.0 $\pm$ 0.28° vs 31.8 $\pm$ 0.34°, $P < 0.001$). In the last 10 patients studied, TcPo2 was measured on periulcer
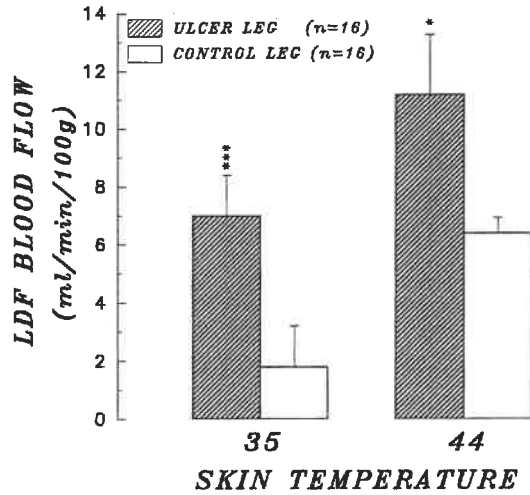
FIG. 2. Mean LDF-flow values for ulcer and control legs at local skin temperatures of 35° and 44°. Error bars are SEM. (*), $P < 0.05$; (***), $P < 0.001$ compared with control legs.

skin and on the contralateral leg at a corresponding anatomical site. Ulcer leg TcPo2 was very low (4.6 ± 1.5 mm Hg) and significantly less than that measured on the control limb (39.1 ± 3.0 mm Hg, $P < 0.001$).

## DISCUSSION

The major new finding of the present study is that in patients with venous leg ulcers the laser Doppler skin blood perfusion in the periulcer region is elevated as a consequence of elevations in both the velocity and volume of moving RBCs. This is true at near-basal skin temperatures (35°) and during thermally induced
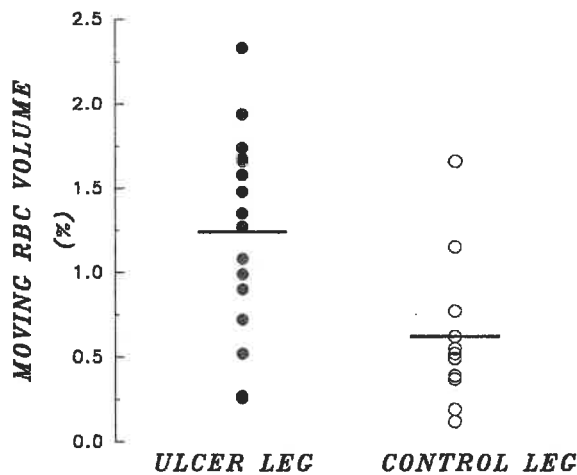


FIG. 3. Individual basal (35°) LDF-volume values for each patient. Horizontal line is the mean value.
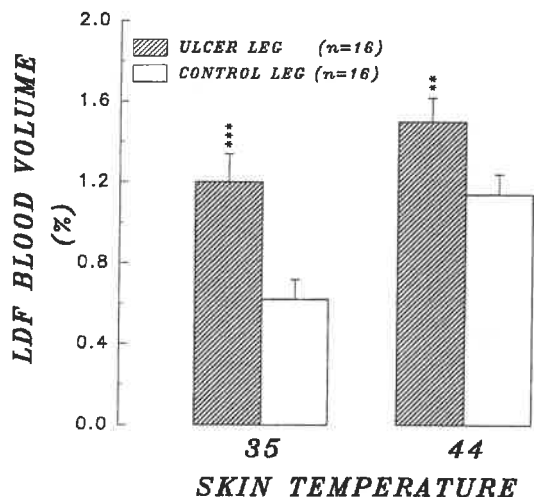
FIG. 4. Mean LDF-volume values for ulcer and control legs at local skin temperatures of 35° and 44°. Error bars are SEM. (**), $P < 0.01$; (***), $P < 0.002$ compared with control legs.

maximal vasodilation conditions (44°). Further, the findings indicate that the contribution of the volume and velocity components to the elevated perfusion are about equal. These hemodynamic differences are accompanied by elevations in the periulcer skin temperature and dramatically reduced levels of TcPo2.

The ldf perfusion ratios (44/35°C) for ulcer and nonulcer legs found herein are similar to that reported by Sindrup and co-workers (1979) but the TcPO2 depression in the present case is much larger to that reported by these workers, although it is similar to values reported by others (Ehrly *et al.*, 1989). Discrepancies may
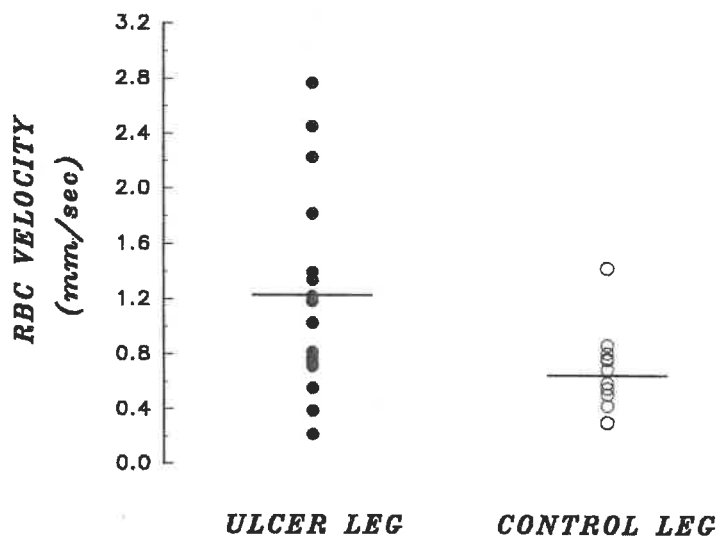


FIG. 5. Individual basal (35°) LDF-velocity values for each patient. Horizontal line is the mean value.
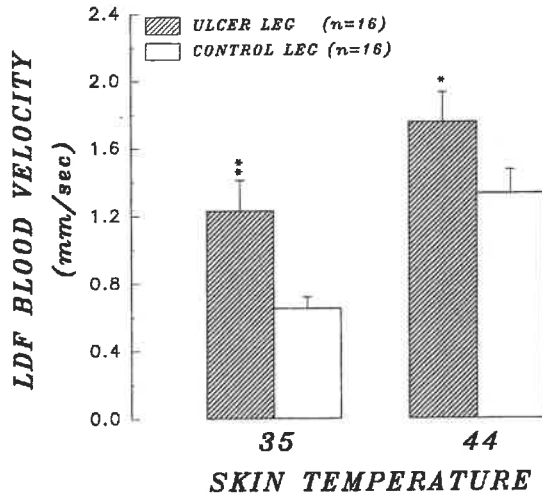
FIG. 6.   Mean LDF-velocity values for ulcer and control legs at local skin temperatures of 35° and 44°. Error bars are SEM. (**), $P < 0.01$; (***), $P < 0.002$ compared with control legs.

be related to the fact that the TcPo2 value is dependent on many factors, including the particular instrumentation used, the epidermal thickness at the site of measurement, the skin composition, the extent of edema present, and the temperature of measurement. Direct comparisons of absolute values may not be meaningful when these variables are dissimilar.

The control leg TcPo2 was found to have values lower than those found in normal legs, suggesting the possibility of some degree of otherwise undetected abnormality in this patient group. However, by the nature of the present study design in using the ulcer-bearing limb and the contrateral limb as comparison, the possibility that the perfusion differences are due to differences in hematocrit, systemic blood pressure, or other systemic variables is unlikely. The presence of a slight periulcer skin temperature elevation probably reflects the increased per-
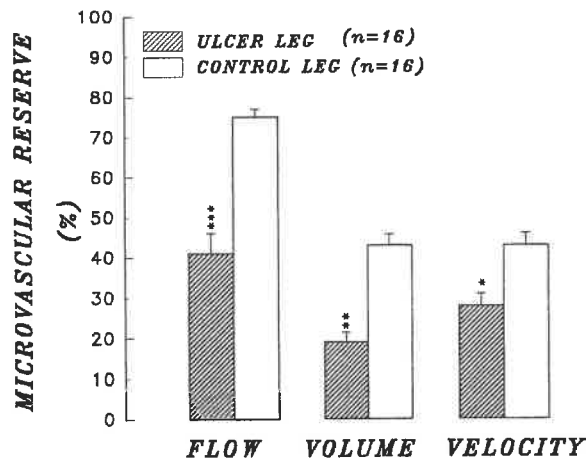


FIG. 7.   Microvascular reserve in response to heating from 35° to 45°.

fusion rather than being its cause. Thus, these results provide new functional data from which inferences regarding the mechanisms of the perfusion increase at the tissue level can be made. The two components of the RBC flux measured by LDF need to be considered separately.

There are at least two possible explanations which can account for the increased blood velocity component measured in the periulcer tissue. One could account for it strictly on the basis of a vasodilated arteriolar network supplying the skin tissue in this region. The vasodilatory stimulus is unknown but based on other studies could be metabolic in origin as a consequence of either increased demand of the wound tissue and/or related to the increased levels of tissue $CO_2$ as has been demonstrated via transcutaneous carbon dioxide tension measurements (Burnand et al., 1982; Belcaro et al., 1988, 1989, 1991). Further evidence for this concept is that total blood flow in the region of the ulcer has been shown to be elevated using positron emission tomography (Hopkins et al., 1983) and more recently using magnetic resonance flowmetry (Mayrovitz and Larsen, 1994).

Alternatively, it may be possible to account for the elevated RBC velocity based on changes in the microvessel features themselves. One feature that might be able to account for an increased velocity would be a change in the number density of microvessels within the periulcer tissue. The predominant observation is that the density of vessels decreases, but a few workers have reported increases. Thus, in patients with chronic venous insufficiency (with or without skin ulceration), capillary density was reported to be reduced by at least a factor of 4.5 in hyperpigmented skin. Contrastingly, in skin with or without trophic changes, capillary density in normal skin ($45/mm^2$) and patient skin ($40/mm^2$) was not significantly different (Franzec et al., 1984). Other workers have reported an increase in capillary density in the ulcer region and an increase associated with chronic venous hypertension (Burnand et al., 1981, 1982). Independent of whether an increase or decrease in density was reported, these observers and others (Fagrell, 1979, 1982; Ryan, 1983) report an increase in vessel cross-sectional area (dilatation) of the visualized microvessels. If, as is likely, these observations reflect changes which include those in the intrapapillary capillary loops and the postcapillary venules (Braverman and Yen, 1977), then the more widely observed changes in topography and metrics would tend to have oppositely directed effects on the measured RBC velocity. A reduction in vessel number would tend to increase velocity in those vessels which remained, whereas vessel dilatation would tend to cause a decrease in the linear RBC velocity in the remaining vessels. Each of these tendencies is dependent on the net effect their combined change has on microvascular resistance and the prevailing pressure gradient. Nonetheless, one can conceive of a set of conditions in which the laser Doppler-determined velocity could increase solely on the basis of oppositely directed changes in vessel size (increase) and number density (decrease). This possible explanation hinges on the concept of microvessel number density reduction being present. As discussed subsequently there is controversy on this point.

The laser Doppler volume component, which is related to the number of circulating RBCs within the measured tissue volume, may theoretically be increased due to several causes. A greater functional microvessel density in the periulcer skin of these patients could by itself account for this finding. Although, as noted previously, some early histological reports indicated the presence of a greater

number of microvessels in patients with chronic venous insufficiency more recent findings suggest that this may have been a false interpretation attributable to the changed microvascular topology. Indeed, direct *in vivo* microscopic observations of the skin have provided compelling evidence to indicate a reduction in vessel number and the presence of both vessel dilatation and significant tortuosity in the remaining vessels. Although it has been reported that capillary coiling is a feature of the cutaneous vasculature of the foot dorsum and ankle [9] the corkscrew and spiral vessel structures observed in severe CVI are much more extensive and would contribute to an increase in the effective RBC path length in the remaining vessels. Since the laser Doppler perfusion measurements include contributions from the skin capillaries and venules, there is the possibility that the elevated volume component arises due to increased vessel diameter and total vessel length in the periulcer tissue. If one accepts the *in vivo* microscopic observations as being representative of the metric and topographical changes in these patients, then the present functional data would indicate that the reduction in vessel number is overcompensated for by the combined dilation and elongation of the micro-vascular network. This results in the net increase in the circulating RBC volume within the periulcer skin, as evidenced by the presently measured elevated volume component.

The portrait that emerges after combining the anatomical and functional data remains somewhat ambiguous. However, one plausible portrait, consistent with both the preponderance of *in vivo* vessel data and the present functional measurements, is that of a tissue in which the number of microvessels is reduced, with the remaining ones carrying a greater blood volume at an increased blood velocity. The available functional information is insufficient to determine the absolute differences in vessel number between periulcer and nonulcer skin.

## ACKNOWLEDGMENTS

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