

Microcirculatory Functions in Systemic Sclerosis: Additional Parameters for Therapeutic Concepts?

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To study the functional reactivity of the cutaneous microcirculation in progressive systemic sclerosis (PSS), hyperemic responses after arterial occlusion (3 min) and during local heating (42°C) were investigated with simultaneous measurements of red blood cell flux and cutaneous oxygen tension ($p_{cu}O_2$) of the skin in female patients ($n = 19$) with PSS and in healthy female controls ($n = 15$). Additionally, serum levels of 6-keto-prostaglandin 1α ($PGF_{1\alpha}$), a stable metabolite of prostacyclin, were compared to the microcirculatory data, and both were used to evaluate further a standardized therapy with 10-d intravenous calcitonin (100 IU/d) infusion in six PSS patients.

In PSS, the initial mean $p_{cu}O_2$ value was significantly reduced and was inversely proportional to flux and to $PGF_{1\alpha}$ levels, whereas the flux and $p_{cu}O_2$ responses to the above hyperemic stimuli showed significant reductions, revealing a pattern of "hyperemic hypoxia" probably due to exhausted

functional reserves of cutaneous perfusion. During calcitonin infusion significant rises in $p_{cu}O_2$ and temporarily in $PGF_{1\alpha}$ and flux were found. After 10 d of therapy, increased $p_{cu}O_2$ was associated significantly with decreased flux, indicating a shifting of blood from deeper regulatory vessels to the sub-epidermal capillaries.

Both clinical improvement and the results of microcirculatory measurements demonstrate a beneficial effect of calcitonin on the cutaneous microcirculation in PSS patients, possibly due in part to a short-term increase in release of endogenous prostacyclin from the vascular endothelium during the infusion. The disturbed reactivity of the dermal vessels in PSS is important for the evaluation of therapeutic concepts and stresses, together with the elevated $PGF_{1\alpha}$ plasma levels, vascular factors in the pathogenesis of PSS. *J Invest Dermatol* 101:211-215, 1993

The cause of progressive systemic sclerosis (PSS), characterized by fibrosclerosis of cutaneous and visceral connective tissues, diffuse vascular damage, and immunologic abnormalities, is still unknown. Typical microvascular abnormalities are focal telangiectasias [1], Raynaud's phenomenon, and stenosis of small arteries with entailing tissue damage. Thus, primary damage at the vascular and probably endothelial level is in one view involved in the pathogenesis of PSS [2,3], whereas others [4-6] hypothesize an alteration in collagen metabolism, possibly due to the abnormal release of cytokines from mononuclear and/or endothelial cells [7].

Recent microcirculatory studies of oxygen tension [8,9] and/or skin blood flow [10-12] demonstrated significant differences between PSS and healthy subjects. In addition, there are contrasting reports of reduced prostacyclin (PGI_2) levels in the skin [13] and of elevated PGI_2 levels in the peripheral venous blood of PSS patients [14], but without simultaneous data on microcirculation. A major compound in the prostaglandin pathway that can be determined in blood plasma by its stable metabolite 6-keto- $PGF_{1\alpha}$ [15], PGI_2 is a potent vasodilator acting on the muscle cells of precapillary vessels [16]. It modulates platelet actions and presumably, with several

other prostaglandin metabolites, also modulates inflammatory and immunologic reactions in early-phase PSS [17]. Accordingly, the action of PGI_2 could represent a link between the two opposing views of the pathophysiology of PSS.

A remarkable improvement of the deranged cutaneous microcirculation measured by thermography [16,18] was reported after short-term infusion of PGI_2 . Furthermore, recent laboratory data [19] with respect to pharmacologic effects of calcitonin agreed well with our experience of a favorable clinical course in PSS patients treated with short-term calcitonin infusions. This encouraged us to design a prospective study, firstly to correlate functional parameters of microcirculation devised by our group and simultaneous $PGF_{1\alpha}$ plasma levels in patients with PSS and healthy controls. Secondly, we wanted to determine the effect of calcitonin on microcirculatory parameters of the skin before, during, and after treatment. Plasma levels of $PGF_{1\alpha}$ were investigated simultaneously to test a possible effect of calcitonin on the metabolism of endogenous prostacyclin, as has been suggested by several authors [20,21].

MATERIALS AND METHODS

Patients, Control Group, and Therapeutic Regimen Cutaneous microcirculation was studied in 19 women suffering from PSS (type I and II [22], ages 45 to 65 years, mean 58 years) and in 15 healthy non-smoking age-matched women serving as a control group. Intake of any vasoactive drugs was discontinued 4 weeks prior to the study.

All measurements were carried out at a controlled room temperature (23°C) in the morning before breakfast, using the left hand positioned at heart level. Measurements began after 30-min equilibration of patients and control subjects resting in supine position (day 0).

On the next day (day 1) each patient was treated with 100 IU salmon calcitonin (Calcitonin L, Rorer Co., Germany) dissolved in 250 ml isotonic

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Abbreviations: LDF, laser-Doppler flux; $p_{cu}O_2$, cutaneous oxygen tension; PGI_2 , prostacyclin; PSS, progressive systemic sclerosis; $PGF_{1\alpha}$, 6-keto-prostaglandin 1α .

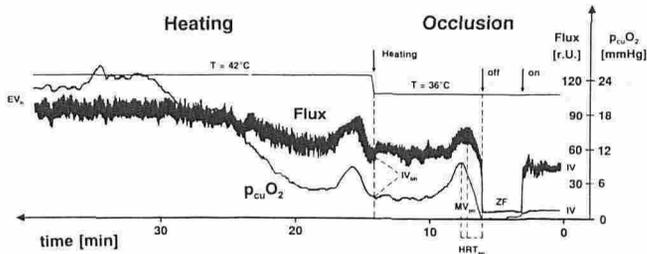


Figure 1. Original recordings of laser-Doppler flux (Flux) and cutaneous pO_2 ($p_{Cu}O_2$) in a healthy subject during the entire procedure. IV, initial values; ZF, zero flux; HRT_{po}, post-occlusive hyperemic recovery time; MV_{po}, maximum post-occlusive values; IV_{bh}, initial values before heating; EV_{bh}, end values after heating.

saline solution, by intravenous infusion of 2.5 h duration, once a day for 10 consecutive days. Plasma levels of PGF_{1 α} , red blood cell flux (flux), and cutaneous oxygen tension ($p_{Cu}O_2$) were determined at days 0 and 11 in all 19 patients. Additionally, flux and $p_{Cu}O_2$ were measured continuously and PGF_{1 α} serum levels were determined at 45-min intervals during the calcitonin infusion at days 1 and 10 in six patients. Furthermore, in this subgroup the initial measurements were repeated during the subsequent control infusion of isotonic saline solution (250 ml) over 2.5 h also on day 0.

Cutaneous Oxygen Tension Cutaneous oxygen tension was measured polarographically using a transcutaneous oxygen probe containing three platinum wire electrodes at a mutual distance of 2 mm [23] (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 had a diameter of 2.0 cm. A probe temperature of 37°C was used. The pO_2 values at 37°C, termed cutaneous pO_2 ($p_{Cu}O_2$), give information about the epidermis and the papillary body [8] and depend more on local skin properties including capillary flow than on the arterial oxygen tension. Because inter- and intraindividual differences in epidermal thickness and capillary density may reduce the comparability of individual data, the skin position of the probe and probe holder was marked exactly to ensure that measurements were comparable. Additional stimulatory tests are necessary to overcome the variability of pO_2 measurements at 37°C [24,25].

Red Blood Cell Flux Red blood cell flux was measured simultaneously beside the pO_2 electrode with a laser-Doppler fluxmeter [26,27] (Periflux Pf1d, Perimed, Sweden) having a tube-shaped probe of 5 mm outer diameter and a sampling window of 1 mm. Due to the deep skin penetration of the laser light, capillaries, dermal arterioles, and venules are within the measuring volume (1–2 mm in diameter). Depending on the measuring site, part or all of these vessels can contribute to the flux signal. Pulse synchronous flux oscillations can therefore be observed using a time constant of 0.2 seconds (Fig 1) and a frequency shift cut-off at 12 kHz, corresponding to a mean maximum detectable blood cell velocity of approximately 4 mm/second. However, it is not possible to determine which part of the laser-Doppler flux (LDF) signal arises from which precise anatomic structure within the measuring volume. Measurements at multiple sites, which would reduce this problem [28], could not be done in our study. To compensate for this we chose a single site with relatively high flux values in regard to the stimulus-response experiments (Table I).

Measurement of PGF_{1 α} Plasma levels of PGF_{1 α} were determined during infusion of calcitonin and saline by radioimmunoassay as described elsewhere [19] after stable values for flux and $p_{Cu}O_2$ had been reached, using 10-ml blood samples taken from the right cubital vein via a catheter placed at least 30 min before the first blood sample was obtained [29].

Placement of the Probes To find the best temperatures and sites for measurement, we determined flux and skin-surface temperature [same site, contact thermometer (TTW H11, Digimed, Germany)] on unheated skin at the dorsal aspect of the wrist, midhand and metacarpal bone of digit III, and on the fingertip of digit III. To compare the different sites, we studied mean flux values for 5 min after stable signals were reached following the application of the unheated laser probe to the marked sites. Because the highest flux values (in PSS), except for the fingertip, were found at the dorsal midhand (Table I) proximal to digit III, we chose this site, which was always sclerotic in our patients.

The different innervation control and the variety of vessels present at the fingertip make it an unsatisfactory site because both probes could not be

Table I. Flux and Skin-Surface Temperature at Different Sites on the Unheated Skin (Hand) in Patients with PSS (n = 19) and Controls (n = 15)^a

	Controls	PSS
Flux Measurements		
Site	Flux[r U]	Flux[r U]
Wrist	9.70 ± 2.48	19.95 ± 9.58 ^b
Dorsal hand	14.73 ± 5.45	29.85 ± 17.46 ^c
Digitus 3	19.73 ± 9.22	26.25 ± 18.63 ^c
Fingertip	124.40 ± 62.90	77.45 ± 47.20 ^d
Temperature Measurements		
Site	T [°C]	T [°C]
Wrist	29.21 ± 1.96	29.87 ± 1.64 ^c
Dorsal hand	30.02 ± 1.86	29.98 ± 1.72 ^c
Digitus 3	30.80 ± 2.36	28.76 ± 2.90 ^c
Fingertip	32.07 ± 2.24	26.98 ± 3.40 ^d

^a Mean ± standard deviation.

^b p < 0.01.

^c p < 0.05.

^d p < 0.001.

^e Not significant, p < 0.05.

placed on one finger. Transcutaneous measurement of pO_2 requires a skin/probe temperature of at least 37°C. With the oxygen and flux probe closely adjacent to each other, we chose to measure the red blood cell flux at a similar temperature (36°C due to the Periflux equipment), which, as compared to unheated skin, produces a moderate dilatation of the microvasculature. Because vessels in general were only partially dilated, stimulatory tests yielded satisfactory responses (Fig 1) and can considerably reduce the interindividual variability [25].

Functional reactivity of cutaneous microcirculation was tested by stimulus-response experiments. After stable initial values were obtained, abrupt arterial occlusion was produced by immediate automatic inflation of a standard-sized blood pressure cuff to 40 mm Hg above systolic pressure. Zero-perfusion was maintained for 3 min, and the cuff was then deflated. After 10 min recovery time, both probes were heated to 42°C (using the built-in electrical heating system with identical time pattern). All data were continuously recorded during occlusion, heating, and up to 25 min thereafter (Fig 1).

Statistics Statistical analysis was done by Student t test after correcting all flux values for the individual "0 flux" during occlusion to improve the interindividual comparability of the data [30]. The pre- and post-therapeutic data of the patients were compared. The data during calcitonin infusion were analyzed by Wilcoxon's rank sum test comparing values of day 1 and 10 to the data during saline infusion of the same individuals. p values of 0.05 were considered significant.

The data for flux and $p_{Cu}O_2$ were evaluated for each test separately with regard to their levels, time courses, and relation to each other. In this way, we could specify the following parameters as relevant to the microcirculatory response to defined stimuli.

Occlusion Parameters Studied for $p_{Cu}O_2$ and Flux IV, initial values for $p_{Cu}O_2$ (at 37°C) and flux (at 36°C); ZV, 0 flux; MV_{po}, maximum post-occlusive values; HRV_{po}, post-occlusive hyperemic response values (MV_{po} - IV); HRT_{po}, post-occlusive hyperemic recovery time up to MV_{po}; and OE, occlusion effect (HRV_{po} * 10 / HRT_{po}).

Heating Parameters Studied for $p_{Cu}O_2$ and Flux IV_{bh}, initial values before heating; EV_{bh}, end values after 25 min for flux and $p_{Cu}O_2$ at 42°C; and RW, regulation width (EV_{bh} - IV_{bh}).

RESULTS

Table I shows the comparison of flux and temperature values on four different unheated skin sites in PSS patients and healthy controls. We found a significant twofold (p < 0.05) elevation of mean flux at the wrist and the dorsal midhand and a 100% (p < 0.001) lower flux at the fingertip in PSS patients compared to controls. Except for the temperature at the fingertip, which was significantly (p < 0.001) lower in patients than in controls, no significant differences in skin-surface temperatures could be found.

Details concerning static as well as dynamic parameters of cutaneous microcirculation are presented in Table II. Pronounced differences in the mean initial values for flux and $p_{Cu}O_2$ between the

Table II. Static and Functional Parameters (Occlusion and Heating) of Cutaneous Microcirculation in Patients with PSS^a

		Controls (n = 15)	PSS (n = 19)
Occlusion parameters			
Initial Values	p _{cu} O ₂ [mm Hg]	4.04 ± 2.05	2.62 ± 1.33 ^b
	Flux[r U]	15.83 ± 9.30	42.26 ± 20.96 ^c
Hyperemic recovery time	p _{cu} O ₂ [s]	101.33 ± 21.30	139.84 ± 22.04 ^c
	Flux[s]	16.93 ± 6.49	39.10 ± 18.25 ^c
Maximum post-occlusive value	p _{cu} O ₂ [mm Hg]	12.83 ± 3.05	7.66 ± 2.61 ^c
	Flux[r U]	51.40 ± 14.47	66.21 ± 21.18 ^b
Post-occlusive hyperemic response value	p _{cu} O ₂ [mm Hg]	8.85 ± 2.14	4.90 ± 2.61 ^c
	Flux[r U]	35.57 ± 10.26	25.72 ± 7.85 ^d
Occlusion effect	p _{cu} O ₂ [mm Hg/s]	0.88 ± 0.27	0.35 ± 0.18 ^c
	Flux[r U/s]	24.00 ± 10.24	7.28 ± 3.02 ^c
Heating Parameters			
Initial values before heating	p _{cu} O ₂ [mm Hg]	4.65 ± 3.42	6.35 ± 3.42 ^c
	Flux[r U]	15.52 ± 11.35	47.95 ± 23.99 ^c
Endvalues after heating	p _{cu} O ₂ [mm Hg]	45.50 ± 12.88	29.95 ± 11.94 ^d
	Flux[r U]	90.20 ± 31.60	89.11 ± 36.75 ^c
Regulation width	p _{cu} O ₂ [mm Hg]	38.05 ± 11.75	25.69 ± 10.29 ^c
	Flux[r U]	63.05 ± 24.65	42.25 ± 15.25 ^d

^a Mean ± standard deviation.^b p < 0.05.^c p < 0.001.^d p < 0.01.^e Not significant, p < 0.05.

controls and the patients are shown in Fig 2. The dynamic parameters (Table II) revealed highly significant lower mean values for the occlusion effect (p < 0.001) and regulation width (p < 0.01) in the patients. All the other dynamic parameters listed in Table II, especially the post-occlusive hyperemic response value and the post-occlusive hyperemic recovery time, which both reflect the microcirculatory functional performance, were also significantly reduced in the patients. These data indicate a diminished capability of the cutaneous microcirculation to respond adequately to functional demands in the altered tissue.

Concerning the effect of calcitonin infusion, we found before therapy a marked rise of flux (167%) combined with a decreased p_{cu}O₂ (65%) in the patients in comparison with the control group.

After therapy, the mean p_{cu}O₂ increased significantly up to the levels of healthy controls, and mean flux significantly decreased (30%, see Fig 2), but with no significant difference observed in the pre- and post-treatment PGF_{1α} levels. However, during calcitonin infusion a temporary (at 45 min) significant increase of PGF_{1α} level on days 1 and 10 coincided with a marked rise of p_{cu}O₂, whereas the mean flux increased significantly only at day 1. During saline infusion all parameters remained constant.

DISCUSSION

The methods used (LDF and p_{cu}O₂) have physiologic limits due to the inter- and intraindividual heterogeneity of microcirculation [27,31,32] but provide the advantage of non-invasiveness and continuous measurements. The restrictions have to be kept in mind even when minimizing external and internal influences on the microcirculation. Therefore, individual microcirculatory data should not simply be compared with each other, even those of a group of individuals may display great standard deviations. However, most investigators in this field regard the comparison of such groups as valid if the data from different groups show statistically significant differences. The close positioning of the flux and oxygen probes provides measurements of adjacent but not identical spots of microvasculature, as combined electrodes would do [33].

Although a number of studies on microcirculation in PSS using different single methods have been done [8–11], results of the simultaneous application of several non-invasive methods have not been published. A very recent study [12] on PSS used LDF and oxygen tension measurement consecutively, yet no stimulatory tests with time responses were carried out.

Hyperemia due to arterial occlusion can well be reproduced showing a typical pattern of hyperemic response [33,34]. However, the physiologic mechanisms involved in reactive hyperemia are not fully elucidated, despite the frequent use of dynamic tests [11,33,35,36]. Different views on the cause of post-occlusive hyperemia focus on accumulation of vasodilating metabolites in the tissue or intravascular changes of pressure and so-called compliance, i.e., elasticity and distensibility, of the vessels [37].

The microcirculatory response to local heating always revealed a biphasic pattern with a first and second maximum (Fig 1) indicating a sensitive vascular mechanism that controls the interaction of thermoregulation, local perfusion, oxygen demand, and supply of tissue [38]. After the first peak, the declines of flux and p_{cu}O₂ reflect the vascular adjustment toward the new level of temperature and the

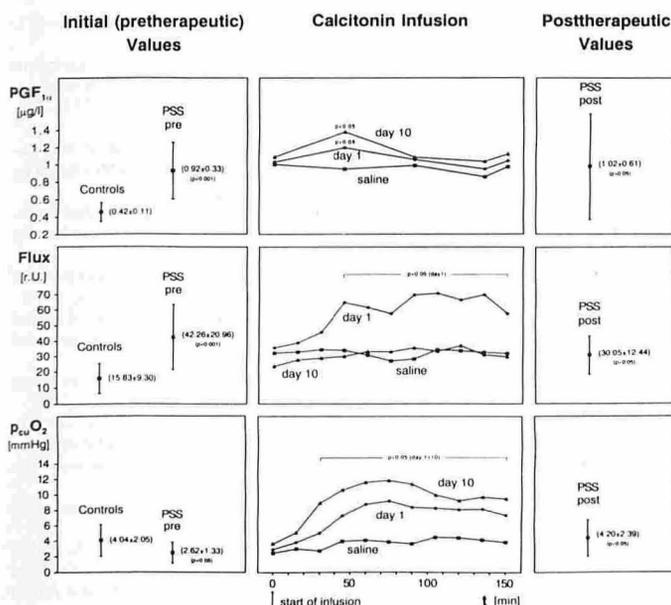


Figure 2. Baseline values of PGF_{1α}, flux, and p_{cu}O₂ (mean ± SD) in patients with PSS (n = 19) and in healthy controls (n = 15). Same parameters (mean) during infusion of calcitonin (days 1 and 10) and saline in patients with PSS (n = 6).

changed perfusion. Further penetration of warmth into the skin mobilizes additional blood reserves for perfusion from the deeper vessels, leading to a slower but steady increase of $p_{cu}O_2$ and flux. Therefore, their final values at 42°C (EV_h) provide, with respect to the initial values (IV_{bh}), the so-called regulation width ($RW = EV_h - IV_{bh}$) as a partial measure for what has been termed cutaneous vascular reserve [33].

The differences found in flux on unheated skin between the PSS and control groups (Table I) do not correlate with the measured skin surface temperatures except for the fingertip. The latter can hardly be compared to the dorsal midhand owing to different innervation and microvasculature [10,11]. The inverse relationship between $p_{cu}O_2$ level and flux for the initial values (Table II) is partly consistent with a known decreased capillary density in sclerotic skin. However, this may not be the sole reason because the relationship changes after therapy (Fig 2), and capillary density is unlikely to increase significantly just as a result of a 10 d infusion. Therefore, the lowered $p_{cu}O_2$ levels, apart from reduced capillary network, may also be due to impaired oxygen diffusion from the capillaries to the skin surface as well as to perivascular fibrosclerosis and partial thickening of the dermis [39]. The simultaneously observed elevation in flux seems to reflect a compensatory increased perfusion of the pre-capillary vasculature, thereby maintaining a minimal circulation in the nutritive-capillary area [40]. Furthermore, the elevated plasma levels of $PGF_{1\alpha}$ (Fig 2) could indicate a vascular mechanism reactive to peripheral hypoxia.

Our results confirm those obtained by Belch *et al* [14] and seem to contradict reduced $PGF_{1\alpha}$ levels found by Hensby *et al* [13] in PSS skin with a suction-blistering technique. The disparity, however, may be due to disturbances of local prostaglandin metabolism in the affected skin that are possibly counteracted through a compensatory increase of PGI_2 production by endothelial cells of still uninvolved larger vessels. Of course, this hypothesis requires comparative investigations of the PGI_2 levels, both in skin and venous blood, also with regard to the suction procedure. Furthermore, it should be studied whether the elevation of PGI_2 plasma levels reflects a compensatory reaction to peripheral hypoxia or inflammatory metabolic changes in the tissue.

Both the post-occlusive hyperemic response value (HRV_{po}) and the speed of post-occlusive recovery (HRT_{po}) yield appropriate measures for the limited reactivity of the dermal vasculature to hypoxia and ischemia in PSS [11,35]. Additional dynamic parameters such as occlusion effect also indicate a significantly reduced ability for post-occlusive compensation through the peripheral vessels in the patients unlike the healthy controls.

Former studies using the $^{133}Xenon$ washout method on subcutaneous tissue in PSS suggested a lowered skin compliance of the fingertips [35] and found unchanged distensibility of the vascular bed on the dorsum of the hand [41]. Similarly, our findings of strongly reduced vascular reactivity do not suggest a diminished distensibility of the cutaneous vessel walls within the measuring volume of LDF. Instead, the decreased reactivity of the dermal vessels in our studies reflects mainly exhaustion of their functional perfusion reserves. This view is based on the observation that the post-occlusive hyperemic flux responses (HRV_{po}) are limited by the elevated initial value (IV) and not by a restriction of the maximum post-occlusive values (MV_{po}) (Table II). In contrast, for $p_{cu}O_2$, the reduction of HRV_{po} mainly depends on a restricted MV_{po} even despite a reduced IV. These data indicate that increased blood flow in the deeper vessels insufficiently compensates for reduced capillary perfusion and its consequently lowered epidermal oxygen supply. A similar type of "hyperemic hypoxia" has been found in the surrounding tissue of leg ulcers by chronic venous insufficiency [42].

In contrast to the control group, which showed the same $p_{cu}O_2$ values before and after occlusion (IV and IV_{bh}), there was a marked increase of IV_{bh} compared to IV for $p_{cu}O_2$ in PSS patients. This could indicate a longer lasting reactive hyperemia in the capillaries to cover the post-ischemic oxygen demand. Additionally, a possible protective reduction of local epidermal oxygen consumption could

lead to higher $p_{cu}O_2$ values on the skin surface. However, for valid comparison with the healthy controls, the same experimental set-up, i.e., 10 min recovery time, that proved sufficient in the controls had to be used in the patients group as well.

The prolongation of HRT_{po} detected in our PSS patients confirms similar results of Goodfield *et al* [11] and points to an altered compliance of the dermal vessels due to their disturbed reactivity and/or to the fibrosclerotic perivascular tissue. Moreover, the marked reduction of the dynamic parameter regulation width for $p_{cu}O_2$ supports the theory of an insufficient epidermal oxygen supply by severely restricted oxygen diffusion and capillary perfusion despite mobilization of blood from deeper dermal vessels. The markedly restricted regulation width also for flux clearly demonstrates a reduced cutaneous vascular reserve [33], allowing only small additional flux increases owing to the elevated initial value.

The significant therapeutic rise of $p_{cu}O_2$ up to the level of healthy controls combined with a significant decrease in flux after calcitonin therapy may be caused by a beneficial shifting of blood from deeper regulatory vessels to the subepidermal nutritional capillaries. Our data, together with an improvement in the patients' general state, may be partly due to a short-term release of endogenous PGI_2 from the endothelial cells during the infusion. However, the reason for the prolonged favorable therapeutic effects of the therapy on $p_{cu}O_2$ and flux despite a change in the post-therapeutic $PGF_{1\alpha}$ levels is still unknown. Further follow-up studies, especially of the reactivity of the dermal vessels after calcitonin infusion, may elucidate this matter.

In conclusion, the present study yields ample evidence for a severely disturbed reactivity of the dermal vessels in PSS patients and demonstrates that non-invasive testing of microcirculatory functions provides important and reliable additional parameters for the evaluation of therapeutic agents such as calcitonin.

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