The influence of ulnar nerve blockade on skin microvascular blood flow*

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Abstract. Microvascular research is seriously hampered by the great temporal and spatial variability of the measured skin blood flow and variation in sympathetic vasomotor reflexes within and between persons. Therefore skin vasomotor reflexes were studied before and after ulnar nerve blockade within the same person, resulting in a temporal complete denervation of the fifth finger and partial denervation of the fourth finger. Skin temperature and laser Doppler flux (LDF) were registrated to measure predominantly arteriovenous shuntflow. Measurements were performed on the palmar tip of the second and fifth finger in nine healthy volunteers, at baseline, and during a sympathetic reflex test (i.e. inspiratory gasp) and postural response test. Beat-to-beat digital blood pressure was recorded from the third and fourth finger by a Finapres device. Baseline capillary blood cell velocity (CBV) was measured at the nailfold of the second and the fifth finger. After ulnar blockade baseline skin temperature, LDF and CBV increased significantly, with respectively (mean ± SE) 3.2±0.9°C, 20.9 ±5.9 relative perfusion units and 0.79 ±0.40 mm s⁻¹. The percentage LDF decrease of the fifth finger during inspiratory gasp was 48.2 ±5.3% before and 3.1 ±0.9% after blockade. The postural response test showed a decrease in LDF of the fifth finger with no significant difference before and after blockade, respectively 12.3 ±14.7% and 8.0 ±2.7%, while no difference was found in the increase in digital blood pressure in the denervated fourth finger compared to both the same finger before blockade and to the third non-blocked finger. It is concluded that ulnar nerve blockade enables the study of sympathetic skin vasomotor reflexes by comparison of a denervated and a non-denervated vascular bed within the same person. After ulnar blockade arteriovenous shunt flow as well as nutritional capillary blood flow increased significantly. Postural vasoconstrictor response is not abolished by ulnar blockade, suggesting that local regulatory mechanisms are more important.

Keywords. Capillaroscopy, postural vasoconstrictor response, sympathetic vasomotor reflexes.

Introduction

The cutaneous microcirculation consists of terminal arteries (<30 μm in diameter) and a venous plexus. In between, two types of parallel situated blood vessels are located, i.e. surface capillary loops serving nutritional demands and arteriovenous anastomoses (AVA), essential for body temperature homeostasis [1]. At room temperature skin blood flow by far exceeds nutritional needs; 80–90% of total skin blood flow bypasses the nutritional through the AVA [2].

Cutaneous vasculature is predominantly under neural control from a dense sympathetic adrenergic nerve supply, especially in the acral regions. Vasoconstrictor sympathetic skin nerve fibres are the efferent arm of, respectively, thermoregulatory reflexes, baroreceptor reflexes, chemoreceptor reflexes and of the reflex response to upright position and exercise [3].

The massive increase in AVA skin blood flow in, for instance, diabetic patients with neuropathy, may compromise the nutritive circulation. This hypothesis, known as the capillary steal phenomenon, explains the simultaneous existence of an increased peripheral skin blood flow and trophic skin lesions [4]. Yet Flynn et al. found no difference in nutritive blood flow in the toe between patients with and without diabetic neuropathy [5].

A major problem with studies on sympathetic skin vasomotor reflexes is the great temporal and spatial variation of skin blood flow and technical problems of the methods used to study microcirculation. Therefore reproducibility of skin vasomotor tests is often troublesome [6–8].

Laser Doppler fluxmetry (LDF) has been widely used in microvascular research [9]. When the LDF probe is attached to an area richly supplied with AVA,
considerable evidence suggests that it partially measures AVA flow as well [10,11]. The laser Doppler signal is related to the relative blood flow in the vascular bed of a few square millimetres of exposed skin area. Therefore it can not be expressed in millimetres per 100 gram tissue per minute, but only in relative terms [12]. The technique that measures only capillary blood flow in undisturbed vascular beds is television microscopy [13]. Skin temperature, the diameter of the capillaries and limitations of the technique used to calculate capillary blood flow [7] influence the results of capillary microscopy [14].

These, and probably other unknown factors, make it difficult to compare microcirculatory measurements between healthy subjects and patients. Therefore in this study skin microcirculatory reactivity was studied within subjects, before and after proximal ulnar blockade, to compare microvascular changes in a denervated and in a non-denervated vascular bed. This model also enables the elucidation of the influence of the nervous system on the flow through the AVA and its consequences for the flow through superficial nutritive capillaries. Furthermore the vasoconstrictor response during postural changes can be studied in order to examine the role of the sympathetic nervous system in skin blood flow regulation.

Methods

Subjects

Eleven healthy volunteers gave written informed consent to the protocol, which was approved by the local ethics committee. None of them smoked or used any medication, except for oral contraceptives \((n = 3)\). There was no history of cardiovascular or pulmonary diseases. All subjects were normotensive, with a supine blood pressure below 140/80 mmHg. Diabetes was ruled out by a HbAlc below 6.4% and a fasting blood glucose below 5.6 mmol L\(^{-1}\). To exclude autonomic nervous system dysfunction, five standardized cardiovascular reflex tests were performed with an automated program using a Finapres device [15]. All the test parameters were above the 5th percentile of normal. Subjects were asked to refrain from caffeine- or alcohol-containing beverages for 12 h and from meals 2 h preceding the tests.

Instruments

In this study two Diodopp instruments (Applied Laser Technology, Maarheeze, the Netherlands) were used. This LDF device has the infrared laser source and the detectors integrated into the probe and has been shown to be a suitable instrument for microvascular research [7]. Both instruments were adjusted to an upper frequency limit of 12 kHz; gain and output circuit time constant were respectively 1× and 0.1 s. Skin temperature was measured using a Thermocouple (Ellab instruments, Copenhagen, Denmark). The digital blood pressure was registered by two Finapres devices (Finapres model 5; TNO, Amsterdam, the Netherlands). The capillary blood flow in the nailfold of the finger was measured using the technique of television capillary microscopy [14].

The measurement of capillary blood cell velocity (CBV) was performed in individual capillary loops using the video ‘dual window’ technique. This consisted of playing back the video tape through a video densitometer which electronically inserted two video cursors into the video screen viewed on the TV monitor. During the measurements, the densitometer provided analog signal linearly proportional to the light intensity of the area delimited by each video cursor. The video cursors were placed along the length of a capillary, at an upstream position, separated by a known distance. The temporal variations in light intensity measured by the densitometer were then subjected to a real time cross-correlation to determine the transit time of photometric events from one cursor to another. For these calculations a fully computerized system (CapiFlow AB, Kista, Sweden) was used [14].

Study protocol

The tests were performed in a climate room with a constant ambient temperature of 24.2 ± 0.4 (mean ± SD) °C and a relative humidity of 55.0 ± 0.7%. While the subjects acclimatized in a comfortable supine position for 1 h, the LDF probes were attached to the palmar side of the second and fifth fingertip of the non-dominant hand, by means of double-sided adhesive tape, after electrical zero calibration. The probes of the thermocouple were placed near the LDF probes. The Finapres cuffs were wrapped around the third and fourth finger of the same hand. The hand was fixed to the table alongside the body.

After at least 4 min baseline registration, an inspiratory gasp test was performed. The subjects were asked to take a deep breath as quickly as possible and hold it for 10 s. After at least 2 min of baseline recording a second inspiratory gasp test was performed. After 5 min of baseline registration, the subjects were tilted head-up with an automated tilt table to an angle of 60° from horizontal. After 5 min the table was turned back to the horizontal position and after 2 min of baseline registration all probes were removed. The nailfold capillaries of the second and fifth finger were visualized and video recordings were made during 1 min with a 140 × magnification and during 5 min with a 560 × magnification.

Thereafter, 6 mL of xylocaine 1.5% was injected in the sulcus nervi ulnaris of the elbow. After a few minutes, anaesthesia of the fifth finger and the lateral part of the fourth finger was achieved for several hours. Anaesthesia was tested by determining whether there was absence of sensitivity to a pinprick or light touch. Subsequently the same protocol was repeated.
Before the inspiratory gasp tests and the postural response test skin temperature (in °C) of both fingers was noted. LDF was measured in relative perfusion units (RPU) [7]. During baseline registration the mean and variability (= maximum – minimum) flux was calculated every minute and averaged for 4 min. The parameters during the inspiratory gasp tests were mean LDF during the last minute of baseline registration, the lowest value during the gasp and the absolute and percentage decrease. During the postural response test the LDF was averaged for each minute of the 5 min baseline registration before the test, for 5 min in the upright position and for 2 min after the test. The percentage postural fall was calculated as mean 5 min baseline LDF before tilting—mean LDF in the 5 min in upright position, divided by the mean 5 min baseline LDF x 100%. Because the Diodopp instrument always records zero during suprasystolic occlusion [7], the LDF signal does not need to be corrected for a biological zero value [16].

The beat to beat digital blood pressure registration with the Finapres devices was recorded by a personal computer and automatically averaged for every 10 s during baseline registration and tilt test. The blood pressure change was calculated by the mean pressure during 5 min in the supine position minus the mean pressure during the upright position.

The video recordings of the nailfold capillaries in the same regions before and after ulnar blockade were mixed before analysis, to ‘blind’ the investigator. The number of capillaries were counted by using the 140 x magnification and expressed as number per 0.5 mm². On the TV screen a rectangle size 1 by 0.5 mm was placed parallel to the nailfold and all the visible capillaries were counted. The CBV was calculated by using the CapiFlow software and the 560 x magnification recordings during 5 min. The filter time constant was 1-0 s and the cross-correlation limit was above 0-5. The reported CBV is the mean of the 2–4 best visible capillaries. The 5 min video recordings of these capillaries were used to measure CBV. The measured CBV during 2 min with the lowest (at least less then 15%) artefact percentage was chosen as CBV (artefact excluded). The CBV of each capillary was calculated twice on different occasions and averaged (Coefficient of Variation (CV) in five normals was 4.2%). After ulnar blockade the same region of the nailfold was visualized using a striking capillary shape as landmark. In six subjects we succeeded in measuring the same capillaries before and after blockade, while in the others measurements were performed in the same region of the nailbed. (CV of CBV measurements of two video recordings of the same capillary in succession in five normals was 21.7%).

Statistics
The results are expressed as mean and its standard error (SE), unless stated otherwise. Statistical analysis was performed by Student’s t-test for paired samples and Wilcoxon signed rank test when appropriate. A P value below 0.05, two-sided, was regarded as statistically significant. Correlations were calculated with the Spearman rank correlation coefficient.

Results
In two of the eleven subjects the ulnar blockade was unsuccessful. In one man the second and third finger also showed hyposensitivity and in one woman anaesthesia was not present after two injections of 6 mL xylocaine. The mean age of the nine (five ♂) remaining volunteers was 25.4 ± 3.5 (SD) y.

Skin temperature
After blockade of the ulnar nerve the maximum temperature increase of the fifth finger was 3.2 ± 0.9°C. The temperature of the second finger showed a tendency to decrease (−1.9 ± 1.1°C). The skin temperature of the blocked finger (35.4 ± 0.2°C) was significantly higher compared both to the second finger after blockade (29.5 ± 0.8°C) and to the fifth finger before blockade (31.9 ± 0.9°C).

Baseline LDF and during inspiratory gasp
Baseline LDF of the fifth finger after blockade was significantly higher and the variation significantly smaller compared to the same finger before blockade and second finger after blockade (Table 1). After blockade the baseline LDF of the fifth finger increased by 20.9 ± 5.9 RPU (Fig. 1). The variability of the LDF signal decreased by 36.5 ± 3.6 RPU. The baseline LDF of the second finger showed a tendency to drop (−5.6 ± 6.0PU) after blockade (Table 1).

Before blockade the absolute and percentage LDF decrease during inspiratory gasp was nearly the same in both fingers. After blockade, LDF of the fifth finger decreased minimally during these sympathetic stimulation tests. The LDF decrease of the fifth finger was significantly smaller compared to the decrease before blockade and compared to the second finger after the blockade (Table 1).

Postural vasoconstriction response
Passive tilting results in an increase in mean digital blood pressure of 42.5 ± 2.4 (SD) mmHg. After blockade no difference was found in baseline mean digital blood pressure, nor in blood pressure increase or the area under the curve during tilting between the third and partially denervated fourth finger (Fig. 2).

Before nerve blockade LDF of both fingers decreased in the same way during tilting (Fig. 3A); percentage fall in LDF of the second and fifth finger, was respectively 18.6 ± 10.8 and 12.7 ± 13.3%. After blockade baseline LDF of the fifth finger was higher as mentioned above and tilting still resulted in a significantly (P < 0.05) percentage decrease of LDF
Table 1. Laser Doppler flux (in RPU) measured from the second and fifth finger, baseline and during inspiratory gasp in duplicate and results of capillaroscopy of the nail-folds, before and after ulnar nerve blockade (mean ± SE)

<table>
<thead>
<tr>
<th>Laser Doppler Flux (RPU)</th>
<th>Before blockade</th>
<th>Digit II</th>
<th>Digit V</th>
<th>After blockade</th>
<th>Digit II</th>
<th>Digit V</th>
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<tbody>
<tr>
<td>Baseline (4 min)</td>
<td></td>
<td></td>
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<tr>
<td>mean</td>
<td>35.4 ± 5.4</td>
<td>46.7 ± 5.1</td>
<td>29.8 ± 4.0</td>
<td>67.7 ± 4.2†*</td>
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<tr>
<td>variability</td>
<td>47.3 ± 24.8</td>
<td>48.3 ± 11.3</td>
<td>43.8 ± 18.3</td>
<td>10.5 ± 6.0§</td>
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<tr>
<td>First inspiratory gasp</td>
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<tr>
<td>absolute decrease</td>
<td>18.4 ± 4.6</td>
<td>23.0 ± 3.4</td>
<td>17.4 ± 4.1</td>
<td>2.0 ± 0.6§</td>
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<tr>
<td>percentage decrease in %</td>
<td>50.2 ± 5.1</td>
<td>48.2 ± 5.3</td>
<td>53.9 ± 8.6</td>
<td>3.1 ± 0.9§</td>
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<td>Second inspiratory gasp</td>
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<tr>
<td>absolute decrease</td>
<td>21.3 ± 5.3</td>
<td>22.0 ± 3.5</td>
<td>19.0 ± 3.6</td>
<td>1.4 ± 0.4§</td>
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<td>percentage decrease</td>
<td>47.9 ± 6.6</td>
<td>47.0 ± 4.7</td>
<td>58.2 ± 7.1</td>
<td>2.1 ± 0.6§</td>
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<td>Capillaroscopy</td>
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<tr>
<td>Number of capillaries</td>
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<tr>
<td>(number per 0.5 mm²)</td>
<td>18.9 ± 1.2</td>
<td>16.0 ± 1.0</td>
<td>18.4 ± 0.9</td>
<td>19.0 ± 0.8§</td>
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<tr>
<td>Subpapillary plexus visible</td>
<td>142.2 ± 18.5</td>
<td>173.9 ± 27.4</td>
<td>110.5 ± 20.8</td>
<td>173.1 ± 38.9</td>
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<tr>
<td>Length (µm)</td>
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<tr>
<td>arteriolar limb</td>
<td>8.8 ± 0.7</td>
<td>9.0 ± 0.6</td>
<td>8.8 ± 0.4</td>
<td>8.4 ± 0.6</td>
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<tr>
<td>venular limb</td>
<td>13.2 ± 1.5</td>
<td>14.4 ± 1.4</td>
<td>11.8 ± 1.0</td>
<td>15.1 ± 1.5</td>
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<tr>
<td>Capillary blood cell velocity (mm s⁻¹)</td>
<td>0.26 ± 0.04</td>
<td>0.40 ± 0.07</td>
<td>0.25 ± 0.04</td>
<td>0.99 ± 0.20*‡</td>
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† P < 0.05; dig V vs. dig II, both after ulnar nerve blockade; * P < 0.05; dig V before vs. after ulnar nerve blockade; † P < 0.01; dig V vs. dig II, both after ulnar nerve blockade; § P < 0.01; dig V before vs. after ulnar nerve blockade.

Figure 1. LDF registration (from right to left) of the second and fifth finger before and after ulnar nerve blockade. RPU = relative perfusion units.

Third finger  
Before ulnar nerve blockade  
Fourth finger  
After ulnar nerve blockade

Figure 2. Mean arterial pressure (mmHg) measured with a Finapres device from the third and fourth finger during the postural vasoconstriction response test, before and after ulnar blockade (mean ± SEM).

(Fig. 3B). The percentage fall in LDF after blockade of the second finger was 12.3 ± 14.7% and of the fifth finger 8.0 ± 2.7%, between the fingers there was no significant difference.

**Capillary microscopy**

Before blockade less capillaries were seen in the nailfold of the fifth finger compared to the second finger, respectively 16.0 ± 1.0 and 18.9 ± 1.2 capillaries/0.5 mm² (NS). The capillaries of the fifth finger could be followed over a longer distance, resulting in a difference in measurable length. After ulnar blockade the number of nailfold capillaries in the fifth finger increased significantly, from 16.0 ± 1.0 to 19.0 ± 0.8 capillaries/0.5 mm² (P < 0.01), as did the number of subpapillary plexuses (Table 1). No change was seen in diameter of both limbs or length of the capillaries of the fifth finger, after blockade. However ulnar blockade resulted in a pronounced increase in CBV (0.79 ± 0.40 mm/s⁻¹) of the fifth finger. CBV of the fifth finger after blockade was significantly higher compared to the second finger after blockade (P < 0.05) and to the fifth finger before blockade (P < 0.05) (Table 1).

**Correlations**

A significant correlation was found between the increase in baseline skin temperature and baseline LDF (r = 0.80, P < 0.01) and between the increase in skin temperature and the increase in CBV of the fifth finger (r = 0.72, P < 0.05), after ulnar blockade.

**Discussion**

Microcirculatory measurements before and after ulnar nerve blockade enable to establish a pathophysiological model for studying the skin blood flow in a denervated and a non-denervated skin area, within subjects. In this way the role of the sympathetic nervous system and the consequences of sympathetic failure on the regulation of skin blood flow can be studied.

After ulnar blockade skin temperature, LDF, the number of visible nailfold capillaries and CBV of the fifth finger increased. The variability in LDF expressed as range of the LDF signal decreased. No decrease in LDF was noticed during inspiratory gasp, but LDF still significantly decreased during the postural vasoconstrictor test. Ulnar nerve blockade did
not change digital blood pressure response during this test in the partially denervated fourth finger. These results show that the sympathetic nervous system plays a major role in determining baseline skin vascular tone and in skin vascular reactivity towards a deep breath. Its role is minor in skin vascular response towards postural changes.

After blockade of the ulnar nerve both skin tem-
Temperature and baseline LDF increased significantly and both changes were significantly correlated. A higher skin temperature after ulnar blockade was also found by Lewis & Pickering [17] and an increase in LDF was noticed by Saumet et al. after musculocutaneous and median nerve blockade [18]. Release of sympathetic vasoconstrictor tone after nerve blockade results in opening of the AVA and explains the increase of skin temperature and probably the increase in baseline LDF [19,20]. Sympathetic outflow to human skin nerves is increased by an inspiratory gasp [21], resulting in a decrease in skin blood flow [6]. After ulnar blockade LDF no longer decreased during this respiratory manoeuvre.

The LDF method relies both on the penetration and the return of laser light from moving elements within the skin. Only a limited theoretical basis is available for a prediction of the penetration depth of the laser light into the skin. It currently appears that the red and infrared wavelengths, coupled with an appropriate separation of transmitter and receiver provide a larger contribution from deeper dermal structures [22,23], as AVAs. By using this LD configuration LDF showed no change when the skin was visibly blanched by topical corticosteroids [11], but when blue laser light was used a detectable reduction in LDF was seen [24]. Synchronous assessment of human skin microcirculation by LDF and capillaroscopy showed discrepancies, which can be interpreted as evidence that LDF records blood flow in vessels in addition to the superficial, nutritional capillaries [10]. For these reasons it is suggested that LDF, used in areas richly supplied with AVAs partially measures AVA flow.

After blockade capillary blood flow increased significantly. Both the number of functioning capillaries and CBV increased. The maximum measurable CBV using the CapiFlow software depends on the distance of the two video cursors on the TV screen (\(CBV_{\text{max}} = \text{cursor distance}/0.02\)). Therefore the length of the capillaries visible on the TV screen is important in measuring high CBV. Fortunately, the length of the capillaries of the fifth finger were visible over a longer distance than those of the second finger. As shown by others the second finger often has a steep nail wall reducing the visible length of the capillaries under study [25]. Another limitation of the technique is the fact that accurate measurements can easily be performed in capillaries in which plasma gaps are present. In this way bias is introduced in selecting capillaries for measurements of CBV. Capillaries with a high CBV show less or no clear plasma spaces and hence will not be measured accurately. Because of these limitations of the CBV measurements with the CapiFlow system the increase in CBV of the denervated finger, especially, is underestimated.

The increase in nailfold capillary blood flow after ulnar blockade may be important in understanding the changes in foot skin microcirculation in diabetic neuropathy. It is hypothesized that the increase in AVA skin blood flow may compromise capillary nutritive blood flow, and be responsible for the healing problems of neuropathic foot ulcers; a hypothesis known as the ‘capillary steal phenomenon’ [9]. The results in the present study are, however, in contradiction with a capillary steal hypothesis.

Flynn et al., using capillary microscopy, found no difference in CBV in the toe nailfold between patients with and without diabetic neuropathy. Because of the increase in erythrocyte column width, an increase in ‘erythrocyte flux’ (calculated from the measured values of CBV and erythrocyte column width, corrected for the duration of stop flow) was found [5]. They concluded therefore that foot skin capillary blood flow is increased in diabetic neuropathy. This increase in diameter of the capillaries is probably the result of long-standing increased capillary blood flow, cq capillary pressure. Recently Sandeman et al. found nail-fold capillary hypertension early in the course of diabetes [26]. Long-standing raised capillary pressure is supposed to induce late structural changes, which ultimately lead to loss of microvascular function and relative under-perfusion, which can result in reduced healing potential of skin following minor injury [27,28]. If the increase in capillary flow due to sympathetic denervation as found in our study, exist in diabetic neuropathic feet, it may in parallel accelerate and compound intrinsic microvascular functional abnormalities.

During postural change no difference in digital blood pressure was found in the partial denervated fourth finger. The postural vasoconstrictor response of the fifth finger was not abolished by ulnar blockade. This response is probably mediated by myogenic autoregulation at the precapillary level [29,30]. However evidence suggests that vasoconstriction in response to increased hydrostatic pressure is mediated by a local sympathetic axon reflex, which leads to vasoconstriction of the arterioles [31,32]. Local nerve blockade [33], in contrast to blockade at some distance [34] or sympathectomy [35], diminishes the postural vasoconstrictor response. However, the concomitant reduction in flow in both feet when only one foot is lowered below heart level, suggests that a central mechanism is involved too [36]. After lumbar sympathetic blockade, the flow in the horizontal foot remains virtually constant, indicating that the central component is mainly mediated via effenter sympathetic nerves. The present study once more shows that the vasoconstrictor response during tilting is mainly mediated by local neurogenic and/or myogenic mechanisms, partially supplemented by a central component [36].

In conclusion, microcirculatory measurements before and after ulnar nerve blockade enable the study of the regulation of the denervated and non-denervated skin microcirculation within persons. This is not only a model for studying the consequences of sympathetic failure, as it also creates the opportunity.
to study the role of the sympathetic nervous system in the regulation of skin microcirculation in syndromes with sympathetic dysfunction. After blockade the flow through AVA as well as nutritive capillaries increases significantly. In contrast the nervous system plays only a minor role in the vasoconstrictor response to postural changes, because LDF still decreased significantly after ulnar nerve blockade.

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