13

Assessment of Human Skin Microcirculation and Its Endothelial Function Using Laser Doppler Flowmetry

Helena Lenasi
Institute of Physiology, Medical Faculty, University of Ljubljana
Slovenia

1. Introduction

Human skin is the largest organ of the body: it accounts for approximately 5% of the total body weight, extends over about 1.8 m^2 and has an average thickness of 1-2 mm. Besides providing the mechanical barrier to protect the surface of the body and to prevent water loss it has other important functions. It is engaged in sensory perception and vitamin D metabolism, in inflammation, hemostasis and wound healing. Its role in human thermoregulation is essential (Roddie, 1983; Rowell, 1993).

The major role of blood flow to the skin is therefore concerned with temperature regulation. Accordingly, it is not surprisingly that blood flow to the skin goes far beyond its nutritive demands. The nutritive blood flow is estimated to comprise only 20% of the skin blood flow (SkBF), whereas the rest represents the functional blood flow. On the whole, the SkBF has been estimated to amount to 0.3 l/min at rest in thermoneutral conditions. During exposure to cold it can be reduced to almost zero, whereas it can increase up to 8 l/min during strenous exercise in a hot environment. It is thus obvious that it plays an important role in hemodynamics, since during strenous exercise and in severe heat stress it can comprise over 50% of the cardiac output, as compared to only 5% in resting thermoneutral conditions (Johnson, 1996; Rowell, 1993). The range of flows is therefore wide, and, in extreme conditions, high SkBF also represents a burden on the working heart. Indeed, many persons with borderline cardiac failure develop severe failure in a hot weather because of the extra load on the heart and then revert from failure in cool weather. As one of the major functions of the skin is to eliminate excessive heat, its temperature is generally under 37°C.

In recent years, the cutaneous microcirculation has gained increasing interest. Its easy and noninvasive accessibility renders skin microcirculation an ideal site for measuring. Moreover, as a dynamic structure it may serve as a model for generalized microvascular function as studies have shown a correlation of vascular reactivity between different vascular beds over the body (coronary arteries, brachial artery and skin microcirculation to list a few of them) in health and disease, at least with regard to endothelial function (Holowatz et al., 2008).

There is a constant competition between thermoregulatory and non-thermoregulatory challenges. Many different mechanisms, ranging from systemic to local factors, play in

concert to balance these two demands and to eliminate redundant heat. This duality also explains the complex ultrastructure of the skin (Braverman, 1997). SkBF is regulated by centrally mediated neural mechanisms and by local humoral factors. Of local factors, the endothelium plays a pivotal role in the regulation of vascular tone. In spite of immense effort that was put in the investigation of endothelial function of skin microcirculation there are still many mysteries to be resolved. However, the term endothelial function is mostly used to refer to the ability of the endothelium to release substances that induce vasodilation by directly causing relaxation of the underlaying smooth muscle cells (SMC) (Crakowski et al., 2006).

Elucidating the role of endothelial vasodilators and their interactions as well as assessing endothelial function in human skin microcirculation is also important from the clinical point of view as early detectable endothelial dysfunction might precede clinical manifestation of the disease states.

One of the measures to improve endothelial dysfunction due to reduced nitric oxide (NO) bioavailability is regular physical activity. Nevertheless, the results on the impact of endurance training to the endothelium-dependent vasodilation, particularly in skin microcirculation of healthy persons, are controversial and the mechanisms induced by training remain to be elucidated.

Over the last years, new advanced techniques and strategies emerged in order to explain the function, the cell-to-cell communication and the complex interaction between pharmacological mediators in cutaneous microvasculature. Although different methods exist that tend to estimate skin blood flow none is optimal. A gold standard to evaluate skin microcirculation and its reactivity to various stimuli is laser Doppler flowmetry (LDF).

2. Skin microcirculation

2.1 Anatomy and physiology of skin microcirculation

The skin microcirculation is an anastomotic network of vessels with many crucial functions for the human as a whole. Namely, with its large capacity, it has to maintain nutrition of the epidermis and its adnexa, it is essential for human thermoregulation, in takes a major part in inflammation and wound healing, and, last but not least, it plays an important role in the determination of peripheral resistance. From all the above, it is obvious that the blood flow must be finely regulated and tuned in order to fulfill all the demands of the organism.

Because of its dimension, skin vasculature belongs to microcirculation only. It comprises arterioles, capillaries and venules (Braverman, 1997). The microvessels in the papillary dermis vary in diameter from 10 to 35 μm, but most range from 17 to 22 μm.

The cutaneous arteries arise from the subcutaneous tissue and enter the dermis to form the cutaneous arterial plexuses. The arterioles and venules of the cutaneous microcirculation form two important horizontal plexuses parallel to the skin surface: an upper horizontal plexus in the papillary dermis (subepidermal or subpapillary plexus) from which the nutritive capillary loops arise and a lower horizontal plexus at the dermal-subcutaneous border. These two plexuses communicate with each other with arterioles and venules and represent the physiologically important areas in the skin (Braverman, 1997).

Capillaries connect the arteriolar subpapillary plexus with the venous subpapillary plexus, and single capillary loops ascend to each papilla. They have a mean length of approximately 0.2 mm to 0.4 mm and each supplies on average 0.04 to 0.27 mm^2 of the skin surface.

Special feature of human skin microcirculation are arteriovenous anastomoses (AVAs). These are direct connections between arterioles and venules of the deep epidermal plexus and have thick muscular and richly innervated walls. They provide a low resistance short circuit for the passage of blood from arterioles to venules at high flow rates, as in response to body heating (Braverman, 1997; Roddie, 1983). They are found mainly in the apical regions of the skin (hands, feet, nose, lips and ears) and are most numerous in the nail beds, tips of digits, and palmar surfaces of digits, palms and soles, but they are almost absent from the dorsum of these areas.

The microvasculature of the skin varies significantly in different regions of the body, with respect to density and architecture of the vascular network as well as regarding blood flow. With respect to their structure and physiologic role, different skin parts could be divided into glabrous and nonglabrous areas: nonglabrous or hairy areas are areas over the skin of the trunk and extremities, whereas glabrous or nonhairy skin areas are found in palms, soles and lips. These regions differ with regard to the vascular control mechanisms. Nevertheless, there is not a discernible function that can be ascribed to one or another region.

2.2 Control of skin blood flow

As an important part of the cardiovascular system, SkBF undergoes periodic demands for increased and decreased blood flow in order to achieve cardiovascular adjustments during thermoregulatory challenges, during orthostasis and exercise.

Since SkBF takes part in the above-mentioned systemic reflexes its blood flow is mainly regulated by neural mechanisms rather than by local factors. However, local factors, in the first line factors released from the vascular endothelium and various substances co-transmitted from local nerve endings, can strongly modulate this general response to systemic challenges.

2.2.1 Neural control mechanisms

With respect to the anatomical organisation of skin microcirculation, there are principally two types of neural control mechanisms (Johnson & Kellogg, 2010; Kellog, 2006).

I. All skin areas (glabrous and nonglabrous) throughout the body are supplied with sympathetic vasoconstrictor fibers that secrete norepinephrine at their nerve endings and, under resting conditions, exhibit a tonic vasoconstrictor influence on skin blood vessels. The resting tone is maintained by a continuous nerve discharge of 1-3 impulses per second and can be varied in both directions to produce vasoconstriction and, without need for any other specialised vasodilatory fibers, also vasodilation. Indeed, under exposure to cold, there is an increased discharge from this part of the sympathetic system, causing further vasoconstriction. On the other hand, under heat stress, the tonic outflow through sympathetic vasoconstrictor system is reduced. This constrictor system is extremely powerful in glabrous areas with abundant AVAs – areas that are most frequently exposed to severe cold. Thermoregulatory as well as other reflexes in these regions are mediated by

changes in noradrenergic vasoconstrictor tone as well as direct effects of local temperatures on the skin (Johnson & Kellogg, 2010; Kellog, 2006).

II. In addition, nonglabrous skin areas also receive the so-called active (cholinergic) vasodilatory nerve fibers, most probably from the sympathetic origin too. Over the last years, many studies have been conducted to elucidate this cholinergic vasodilation mecanism, but the results are not conclusive. It is postulated that acetylcholine (ACh) is the most probable neurotrasmitter, possibly linked to sweating. The current hypothesis proposes a co-transmitter released by the nerve endings innervating either blood vessels directly or sweat glands and exerting its relaxant action on the vascular SMC. A putative co-transmitter has not been identified yet: the candidates include vasoactive intestinal peptide, substance P, histamine from mast cells acting on histamine H_1 receptors, prostanoids, and NO (Johnson & Kellogg, 2010; Kellog, 2006).

It should be mentioned that there are modulators of the neuraly mediated mechanisms involved in cutaneous vasoconstriction and vasodilation. In women, skin vascular reactivity depends on the phase of the menstrual cycle: progesteron resets the treshold for active cutaneous vasodilation (Charkoudian & Johnson, 2000). Furthermore, SkBF and the cutaneous microvascular reactivity are subject to diurnal rhytms (Aoki et al., 2003). Another modulator that resets the threshold for active cutaneous vasodilation is physical exercise (Johnson, 1996; Rowell, 1993) that is covered in more detail elsewhere in the chapter.

2.2.2 Local control mechanisms

Apart from neural mechanisms, local factors also impact SkBF. The standard meaning of the term applies to the mechanisms independent of nerves and hormones, by which organs and tissues alter their own arteriolar resistance. Included are classical vasodilators, such as decreased oxygen partial pressure, hydrogen ion, and locally released metabolites as adenosin, and increased concentrations of potassium. Their effect on the local vascular tone is more pronounced when inducing short-term hypoxia, such as a transient occlusion of the proximal artery. All things considered, little is know about these local mechanisms in controlling human skin microcirculation although their role seems to be of minor importance.

Much more important seems to be the role of vascular endothelium as a local regulator of vascular tone, also with respect to the skin microcirculation (Johnson, & Kellog, 2010; Kellog, 2006). The role of endothelium as a local regulator of vascular tone (in glabrous as well as in nonglabrous parts) has been assessed and confirmed by different independent studies.

Last but not least, local thermal factors, i.e. local heating and cooling of the skin, also play an important role in modulating vascular tone (Johnson, & Kellog, 2010; Kellog, 06).

Besides the above mentioned neural and local control mechanisms, skin microvessels also exhibit local and autonomous variations of blood diameter that cause corresponding flow variations and have been termed vasomotion. The contractions have a low frequency that can be obtained by transforming the signal of the laser Doppler flux (1-2 cycles/min) using spectral analysis. Vasomotion is suggested to facilitate blood flow through microvessels and can be amplified under conditions of circulatory and metabolic stress, as for examle by inducing a transient ischemia (Kvandal et al., 2006; Rossi et al., 2008). The origin of vasomotion remains to be fully elucidated: it has been proposed that the spontaneous muscular contractions are due to the intrinsic myogenic activity of vascular SMC.

2.3 Endothelium as a local regulator of vascular tone

In the last decades, a number of studies conducted in animals as well as in humans extensively expanded the knowledge about the pivotal role of endothelium in the regulation of vascular tone (Vanhoutte, 1989). In response to physical forces and neurohumoral mediators, endothelial cells secrete several vasoactive substances that affect vascular tone by inducing contraction and relaxation of the underlying smooth muscle. The most important endothelial vasodilators are NO, prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF), whereas vasoconstrictors include endothelin (ET), platelet activating factor (PAF), etc. We will focus on the role of vasodilators.

Physical stimuli are shear stress exerted on the vascular wall by the flowing blood, and pulsatility of the vessel wall, whereas pharmacological stimuli include a variety of endothelial agonists, such as ACh, bradykinin, thrombin, serotonin, estrogens etc. In general, endothelial function *in vivo* can be estimated by applying pharmacological agonists to induce endothelium-dependent vasodilation. When estimating endothelial function, the terms NO-dependent and nonNO-dependent vasodilation (Higashi & Joshizumi, 2003) are often used to discern between different mediators involved.

NO is constitutively secreted by endothelial cells upon the action of endothelial nitric oxide synthase (eNOS) that is constitutively expressed in all endothelial cells. eNOS can further be activated by increases of calcium ions or by different agonists that induce changes in the phosphorylation of specific aminoacid residues of the enzyme (Fleming, 2010). Its action depends on the bioavailability of substrats (such as L-arginine) and cofactors, such as tetrahydrobiopterin (BH_4). NO induces vasodilation by activating soluble guanilat cyclase in SMC that further catalyses cGMP, causing vasorelaxation of SMC.

As for the role of PGI_2 and other prostanoids, their importance in skin microcirculation is unequivocal. They are synthesised by the family of cyclooxygenase (COX) enzymes. PGI_2 acts principally to modulate the function of vascular SMC and platelets; it is suggested to play only minor role in the regulation of vascular tone in health. On the contrary, it may play a compensatory role in the settings of decreased NO bioavailability (McCord et al., 2006; Szerafin et al., 2006).

The vasodilatation that remains after the combined eNOS and COX blockade has been ascribed to a yet unidentified mechanism. As it induces hyperpolarization of endothelial and vascular SMC by activating different families of K^+ channels, it has been termed EDHF (Feletou & Vanhoutte, 2009). The potential mechanisms leading to the hyperpolarization of endothelial cells and SMC include arachidonic acid metabolites derived from cyclooxygenases, lipooxygenases and cytochrome P450 (CYP) pathways, as well as H_2O_2, CO and H_2S. Another putative mechanism associated with the hyperpolarization of both endothelial and vascular SMC is suggested to be electrical coupling through myoendothelial gap junctions following small and intermediate conductance Ca^{2+} activated K^+ channels (IK_{Ca} and SK_{Ca}, respectively.).

The contributions of NO, EDHF and PGI_2 to the endothelium-dependent vasodilation are difficult to define, as their importance may vary depending on the vessel type and size as well as on the agonist used to stimulate the endothelium (Feletou & Vanhoutte, 2009; Schrage et al., 2005). There is a redundancy in endothelial vasodilator mechanisms and in

the settings of compromised endothelial function, one vasodilator may overcome the deficiency of another one and in this way restore normal endothelial function. The same holds true when one or more pathways of vasodilator synthesis are inhibited, i.e. blocking eNOS and/or COX causes an increased production of the nonblocked substance(s). Also, a complex interaction and crosstalk between different mechanisms has been suggested (Feletou & Vanhoutte, 2009; Nishikawa et al., 2000; Osanai et al., 2000). It has been established that NO is involved predominantly in the control of vascular tone in larger conductance vessels, whereas in microcirculation, the role of EDHF seems to be more important (Pohl & deWit, 1999; Urakami-Harasawa et al., 1997). Most observations are based predominantly on animal studies and little is known about the impact of EDHF in humans *in vivo* (Yang et al., 2007). In spite of several investigations, the exact interaction of these mechanisms is still not well defined, specially not in human skin microcirculation.

3. Methods for assessing skin microcirculation

A number of methodologies have been used to provide indexes of SkBF. As SkBF is dynamically changing the technique employed must be able to detect these dynamic changes. Furthermore, the method has to be safe, easy to apply, accurate and reproducible, and should not affect SkBF. Usually it is not the basal flow that is of interest but rather vascular reactivity that can give insight into the vascular function. Thus, the measurement techniques are usually coupled to some provocation tests which can estimate vascular reactivity. The latter is usually tested to investigate the mechanisms involved in the regulation of SkBF, or to detect functional changes associated with the development of various diseases, and to evaluate the progression of a disease and efficiency of the disease treatment.

In general, there is no optimal method to assess SkBF; none of them is able to give accurate quantitative values of SkBF. Rather, the flow is presented in relative units. All of the methods have their advantages and disadvantages. Moreover, as they employ different principles, the direct comparison of the data obtained by different techniques is often not relevant, if at all possible.

Most frequently used methods to study the dynamics of the skin microcirculation include optical methods such as intravital dynamic capillaroscopy, photoplethysmography, venous occlusion plethysmography, laser Doppler flowmetry (LDF) and laser Doppler imaging (Serup et al., 2004). Alternative methods are based on thermographic assessment of blood flow or on measuring transcutaneous pO2 or on ^{133}xenon clearance. The most recently developed methods include orthogonal polarization spectral imaging technique, sidestream dark field imaging technique (Treu et al., 2011), and laser speckle contrast imaging (Turner et al., 2008).

3.1 Laser Doppler flowmetry

LDF is a noninvasive method that has been developed and accomplished over the past 20 years. It enables a sensitive, continuous, noninvasive and real-time assessment of blood flow, being uninfluenced by the underlying skeletal muscle blood flow (Saumet et al., 1988).

In research, it has been used to study reflex control of cutaneous blood flow, and, in conjunction with other techniques (iontophoresis, microdialysis, and local warming and heating) to address the involvement of the endothelium in the local control of SkBF. Furthermore, the responses to drugs can be evaluated with respect to their local effect on

skin microcirculation. Its use goes far beyond research purposes as it has currently also been used in clinical practice either as part of the diagnostic procedures or to follow the effectiveness of treatment.

3.1.1 Principles of laser Doppler flowmetry

The fundamental principles of LDF applied to the measurement of SkBF have been described in detail in several publications (Serup et al., 2006; Shepherd & Oberg, 1990).

The method is based on the Doppler shift of the emitted laser light when it travels through tissue and is reflected off the moving objects, such as the moving red blood cells. The Doppler effect is a physical phenomenon that occurs between two objects, a wave source and a wave receiver. The relative motion of the wave source and the wave detector causes a change in the wave frequency and wavelength. The difference between the frequencies of the emitted and received waves is called Doppler frequency shift.

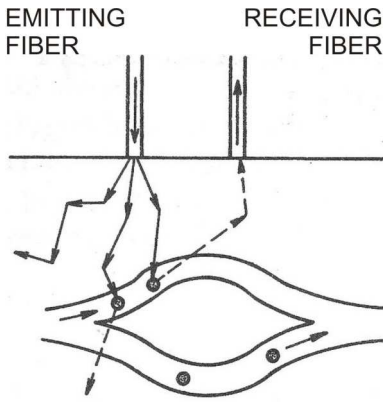


Fig. 1. A schematic diagram showing the detection of a red cell flux by laser Doppler flowmetry. Laser light is conducted to the skin via fiber optics. In the skin, a small fraction of the light is reflected by moving red cells with a shifting frequency (Doppler effect), whereas the rest is reflected by the same frequency. Both reflected beams are transmitted to the receiving optical fiber.

Prerequisites for LD technique are the characteristics of the laser beam, emitted to the tissue, such as monochromaticity and spatial and temporal coherence. The classical single-point probe consists of an emitting optical fiber with the source of laser light and usually, of two separate optical fibers that serve as receivers. The emitted light is guided through an optical fiber to the tissue where it is scattered, absorbed and only a small fraction is reflected (Fig.1). Stationary tissue reflects the light with the same frequency whereas moving cells reflect the light with a shifting frequency (optical Doppler effect). The frequency shift of the emitted light is proportional to the velocity of the moving erythrocytes. The reflected light is transmitted through one or more receiving optical fibers, and the nonshifted reference and Doppler-shifted beam are mixed on a photodetector and processed. The signal is then converted into electrical output that is fed through high-pass filters and amplifiers to finally obtain the signal as fluctuating voltage expressed in millivolts. The LDF signal is a stochastic

representation of the number of erythrocytes in the sample volume multiplied by their velocity and is referred to as flux; rather than flowmetry, LDF is termed LD fluxmetry. Since the red blood cell flux is linearly correlated with SkBF it is taken as an estimation of blood flow. That is why the flux signal obtained by LDF is usually expressed in arbitrary perfusion units (PU). Due to the complex structure and the random orientation of the cutaneous microcirculation the obtained measurements are only semiquantitative and relative.

Due to the scattering of the emitted light in the tissue, only a small portion of the light incident on a tissue surface will penetrate very deeply and return to the surface. This is one of the reasons why LDF is ideally suited for measuring superficial tissues such as skin and mucosa.

The penetration depth of laser light depends on its wavelength (shorter wavelengths penetrate superficially, whereas longer penetrate more deeply) and on the distance between the transmitting and receiving fibers. It has been estimated that at small fiber separations, the signal is obtained from more superficial layers, such as capillary loops whereas at greater separations the contribution of deeper venous plexuses may dominate. In brief, different fiber separations or the use of lasers with lights of different wavelengths may enable the inspection of different depths of the cutaneous microcirculation.

The depth of measurement by LDF varies among tissues: for skin, it is estimated to be 0.5-1 mm. Considering the anatomical organisation of skin microvessels, the LD devices capture signals predominantly from the deeper subpapillary layer – the site of subpapillary arterial and venous plexuses. Thus, it records the thermoregulatory blood flow rather than the nutritive blood flow from papillary loops and superficial plexuses (Yvonne-Tee et al., 2006).

From the above, it is obvious that the variables which influence the measurement in one individual are the structure of skin surface and skin thickness as well as hematocrit.

Many commercial devices from different manufacturers are available today (Fig.2). Most of the current devices use a two- or five mW-powered helium-neon lasers with a wavelength of 632,8 nm and small probes. The surface area captured by such devices is estimated to be about 1 mm^2, whereas the penetration depth is 1 mm, resulting in a theoretical total measured tissue volume of 1 mm^3. Other available instruments nowadays use infrared light with a wavelength of 780 nm, applied by a diode laser. It is a challenge to develop devices using lasers with wavelengths below 600 nm, which will be able to record signals from the epidermis-dermis border. This would enable a more selective distinction between the deeper lying thermoregulatory component of blood flow and the more superficial nutritional component of the blood flow (Yvonne-Tee et al., 2006).

3.1.2 Application in humans: Calibration, validation and precautions

Before any measurement, the device has to be calibrated to instrumental zero (standard or instrumental calibration) and later on, the biological zero has to be determined. Instrumental zero calibration is obtained by placing the probe against a white surface. To test the sensitivity and to calibrate the device the probe tip is placed into a suspension of latex particles undergoing Brownian motion and the output of the flowmeter is tested.

The term biological zero applies to the LD flux that remains after an occlusion of a proximal artery. Before any measurement, biological zero can be assessed. The relative ratio between the normal resting flux and the biological zero value varies from region to region: biological

zero LD flux can amount up to 20% of the resting flux (Tonneson, 2006, as cited in Serup et al., 2006). The sources of the biological zero seem to be blood cells that remained in the peripheral vessels during arterial occlusion and are still moving randomly and producing minor LD components recorded by the instrument as well as the Brownian movement of the interstitium (Kernick et al., 1999).

Before the measurement, the subject should rest for at least 20 minutes to acclimatize and calm down (Fig.2). Namely, a wide range of factors strongly influence the LDF signal and should be kept at minimum for a more relevant interpretation of the data. Such factors are subject-related: anatomical position of the probe, the subject's position, physical and mental activity, previous consumption of food, beverages containing caffeine or alcohol, smoking, taking drugs, menstrual cycle and temporal variations (circadian rhythms, inter-day variability, seasonal variations). Environment-related factors are first of all ambient temperature, air humidity and movement of the adjacent air. Factors that cannot be influenced and can have an impact on LDF are age, gender and, possibly, ethnic background. All these aspects must be taken into consideration and effort should be made to eliminate them or at least minimize to get the best possible results.

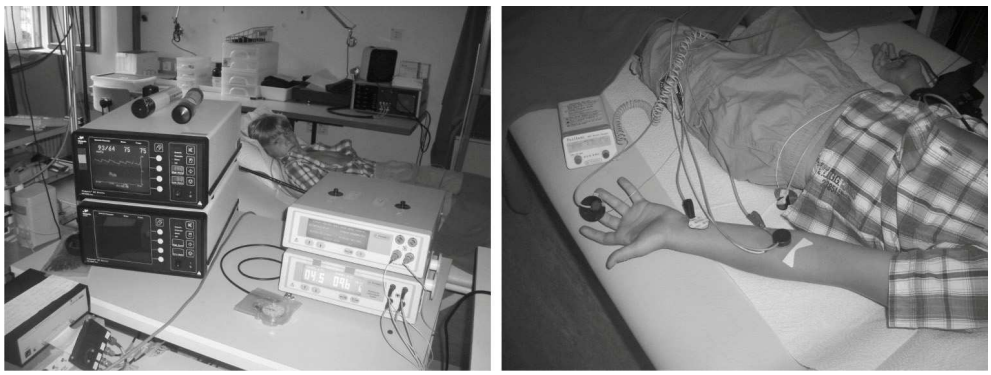


Fig. 2. A typical equipment for assessing skin microcirculation: Laser Doppler flowmeter (LDF, Perimed), the device to register blood pressure in the finger artery (Finapress, Ohmeda), and the transducer for a simultaneous transmission of the digital signals to the computer (left-hand). A typical position of the LDF probes: a standard LD probe (placed on the finger pulp, i.e. representative of glabrous site) and an iontophoretical delivery LD probe (placed on the volar forearm, i.e., representative of nonglabrous area) combined with the device for the application of iontophoresis (right-hand).

Debatable is also the temperature of the skin: while some authors recommend preheating the skin to a constant temperature and then performing a perturbation, others use the 'natural' skin without preheating.

Another problem are movement artifacts. To avoid it, person must lie completely still. Further, the probe must be in close contact with the measured skin; to do so, special two-sided adhesive tapes to fix the probe holder are available.

Due to the small measuring area and the anatomical architecture of the dermal microcirculation, there are considerable variations in blood flow (Braverman et al., 1990). To minimize the problem of spatial variability, several subsequent measurements have to be

repeated and the data obtained averaged. Furthermore, newer devices include multiple point probes that have been designed to overcome the problem of immense spatial variability.

When performing LDF measurement, usually multi-channel devices are used that enable a continuous measurement of LDF in many sites simultaneously (Fig.2). Usually, the measurements are performed in the representative sites of glabrous and nonglabrous areas. Mostly measured glabrous sites in the upper extremity are palms, finger pulps and nailfolds, whereas nonglabrous sites include volar forearm and dorsal aspect of the hand and fingers.

3.1.3 Interpretation of the results

Many methodological issues must be taken into consideration when interpreting the results.

First, the strong site- and time-dependence of the obtained LDF signal and a great inter-individual variability must be taken into account. An accurate measurement should include the assessment of reproducibility in terms of the intraindividual variability coefficient that, based on some reports, could come up to 40%.

It is also important to realize that SkBF is very dynamic and apart from known factors that influence the variations (vasomotion, neural and humoral activity, heart beat and respiration related changes, respectively) there are also factors that cannot be ascribed to any known factor and thus explained. As a consequence, a wandering baseline is usually obtained. The seemingly random variation is much lesser when SkBF is perturbed, e.g. increased by heating or decreased by cooling. This is the basis for the provocation tests to be applied. Accordingly, more information is obtained in assessing vascular reactivity as a response to these perturbations. Rather than in absolute values, changes in LDF after such a perturbation are usually expressed as a percent of the baseline value.

Furthermore, it is advised to apply perturbation to induce maximal vasodilation (i.e. heating the skin to 44^{o}C or applying an NO donor in excess) and thus achieve the highest (peak) flow and then express the quantity of the measured LDF relative to the peak flow. This would allow a comparison with the values assessed either by other devices or in other experimental conditions. Indeed, in many studies, the flow is expressed as a percent of the maximal flow (Crakowski et al., 2006; Turner et al., 2008).

Another recommendation is to express SkBF in terms of cutaneous vascular conductance (CVC) rather than in terms of flow. CVC is obtained by dividing the LD flux by mean arterial pressure. It is worth mentioning that all the measurements of LDF are usually performed with concomitant continual recordings of the blood pressure and the skin temperature.

The mostly exposed problem remains the standardization of the method as well as interpretation of the results that would enable a more accurate and relevant comparisons among studies using different devices and protocols. There is an urge to standardize the method as well as the protocols used (Crakowski et al., 2006; Turner et al., 2008; Yvonne-Tee et al., 2006).

3.1.4 Advantages and disadvantages

LDF enables a semiquantitative assessment of skin blood flow and is thus expressed in arbitrary PU. One of its disadvantages is thus inability to determine absolute blood flow.

Another disadvantage is great spatial and temporal variability. This must be taken into consideration when designing the measurements and interpreting the results. On the other hand, LDF has many advantages over some other methods: it is noninvasive, it can determine dynamic changes in skin microcirculation being uninfluenced by the underlying muscle blood flow (Saumet et al., 1988), it is easy applicable, reproducible, and, compared to many other methods, is relatively attractive regarding its price. As such, it remains the gold standard for assessing skin microcirculation and its reactivity in research purposes as well as in clinical practice.

3.2 How to study the endothelial function of the skin microcirculation

As with other techniques, there is no uniform technique that would separately evaluate only the endothelial function of skin microcirculation. Namely, the endothelium is not an entity per se but it is strongly associated with other structures, in terms of anatomy as well as physiology. There is a complex interaction between neural and other humoral mechanisms and the endothelium, so the methods used for evaluating separate mechanisms show considerable overlapping too. Therefore there is no single method that could give an insight into endothelial function but many different methods and tests have to be performed in order to get the clearest possible information.

Several methods to study endothelial function of the cutaneous microcirculation have been developed in recent years and are currently being used, each with their own advantages and disadvantages. By these methods, it is actually the vascular reactivity or vasodilator capacity that is being estimated: a part of this response also encompasses the endothelial component. So it is obvious that the results must be interpreted carefully when determining 'endothelial function'.

The usual approach to test endothelial function is to induce either a local or a systemic perturbation that will change the LDF. The stimuli used are pharmacological and physical ones. Of locally applied physical stimuli, flow mediated vasodilatation is important as well as locally induced changes in temperature, i.e. local heating and cooling (Johnson & Kellog, 2010; Kellogg, 2006). Flow mediated vasodilation is usually simulated by releasing a temporal occlusion of the proximal artery, and has thus been termed postocclusive reactive hyperemia (PRH).

The pharmacological stimuli are various agonists and antagonists that act directly on the endothelium or on the vascular SMC to induce changes in LDF. Often, they are coupled with the application of different blockers to inhibit the synthesis or release of the substance under investigation, as for example blockade of eNOS or COX or different adrenergic and cholinergic receptors as well as channels on the endothelial cells and SMC. There are various technique of application of these substances directly to the skin: their collective pitfall is that the exact concentration of the substance in the very tissue could not be determined accurately.

Let us briefly mention also commonly used systemic challenges that are often applied when studying skin microcirculation. These include systemic heating and cooling (more often the term whole body heating/cooling has been used), changing the oxygen partial pressure to induce hypo- or hyperoxia, and changes to perturb the baroreflexes: classic orthostatic test with a tilt-table or negative low body pressure and different mental tests. Namely, all the systemic changes also impact skin microcirculation and have been used to estimate the

involvement of skin in reflexes associated with thermoregulation, exercise and other cardiovascular adjustments.

3.2.1 Pharmacological approach: Application of vasoactive substances

Endothelial function can be assessed by applying vasoactive substances locally and directly to the skin and recording the LDF response. In conjunction with LDF, this is performed most commonly by using iontophoresis and alternative methods, intradermal microinjection and microdialysis.

Iontophoresis

This is a method that enables application of soluble charged substances into the skin by means of an externaly applied direct electrical current (Kalia et al., 2004). Special iontophoretic drug-delivery electrodes have been designed that could be attached to the direct laser-Doppler probe (Figs.2,3). The quantity of the drug that penetrates into the skin is proportional to the magnitude of electrical current used. Usually, the quantity applied is expressed in milli Coulombs (mC), which are obtained as a product of the magnitude of electrical current (usually in order of μA) and the duration of the electrical current application. There are different modes of current application depending on the substance used and the protocol chosen: either protocol with a continuous application of current is used, or, more often, intermittent (interval) application of a constant or increasing current is accomplished. It must be taken into consideration that also curent alone causes vasodilation, referred to as 'galvanic response' (Abou-Elenin et al., 2002; Khan et al., 2004; Morris & Shore, 1996; Noon et al., 1998). The magnitude of the current-induced vasodilation depends on the vehicle used: a range of preparations have been used, including deionized water, tap water, sodium chloride and mannitol solutions, as well as different cellulose gels. The proposed mechanisms for the current-induced vasodilation are induction of an axon reflex (Noon et al., 1998), competition between ions of active substance and the vehicle (Khan et al., 2004), etc.

Another aspect that might influence drug delivery to the skin is skin resistance (Ramsay et al., 2002). It depends on the skin site, on the hydration status of the skin and also shows great inter-subject variability. To minimize the problem of skin resistance, skin should be cleaned with alcohol and gently rubbed mechanically to strip off the epidermis.

Nevertheless, iontophoresis has advantages over some other techniques. Compared to microinjection and microdialysis, it does not induce trauma affecting SkBF (Crakowski et al., 2007; Leslie et al., 2003). The quantity of drug is minimal and causes no systemic effects. Its disadvantage is the current-induced hyperemia, which can be minimalized by using an appropriate vehicle solution or by applying topical anesthesia (Crakowski et al., 2007; Morris & Shore, 1996); yet, the latter can impact the results in other way.

Microinjection and microdialysis

As alternatives to iontophoresis, microinjection or microdialysis have been used for the application of various pharmacological agents into the skin. By these techniques, either a solution containing a single substance or a mixture of different compounds can be applied. By microinjection, very small amounts (up to 10 μl) of agents of low concentrations are

applied intradermally (Leslie et al., 2003). The advantage of microdialysis over microinjection is that the skin can be continuously perfused with the solution containing the active substance and in this way enables a constant stimulus or blockade of a certain compound (Crakowski et al., 2007; Turner et al., 2008). Moreover, using microdialysis, it is also possible to remove effluent fluid from the tissue and to determine certain substances of interest in the dialysate. The major disadvantage of microdialysis is its invasiveness (Crakowski et al., 2007).

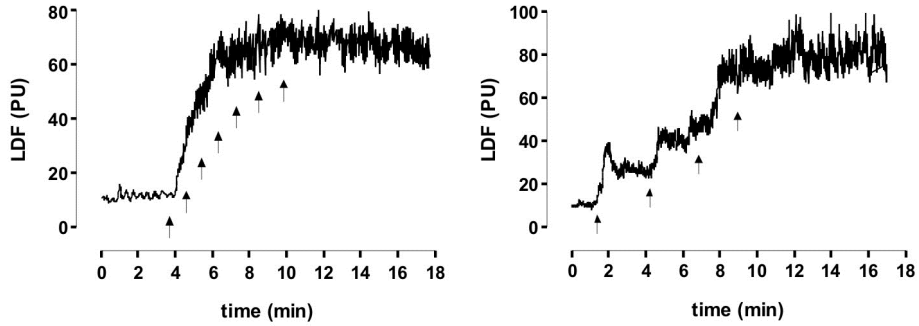


Fig. 3. A representative laser Doppler flux (LDF) response to pulsed iontophoretical application of acetylcholine, an endothelium-dependent agonist (left-hand) and sodium nitroprusside, an endothelium-independent agonist (right-hand) in the volar forearm of one subject. Arrows denote electrical pulses. PU, perfusion units.

Conventionally, ACh has been used as a standard drug to assess endothelial function (Fig.3, left-hand). It has been established, though, that apart from acting on muscarinic receptors expressed in endothelial cells it also affects other parts of the vessel wall: its potential action on the local nerve endings has also been proposed (Berghoff et al., 2002; Morris & Shore, 1996). In light of such observations, the data must be interpreted cautiously. Further, its action on the endothelium is debatable; multiple mechanisms have been proposed, including the involvement of NO, prostaglandins and EDHF (Holowatz et al., 2005; Kellogg et al., 2005; Khan et al., 1997; Morris & Shore, 1996; Noon et al., 1998; Turner et al., 2008).

Other commonly used drugs are metacholine, bradykinin, histamine, and substance P (Kalia et al., 2004). To test the reactivity of vascular SMC, sodium nitroprusside has been most commonly applied, usually by iontophoresis (Fig.3, right-hand).

3.2.2 Postocclusive reactive hyperemia

PRH refers to an increase in SkBF over the baseline following the release of a temporal occlusion of a proximal artery and has been used as an index of endothelial function (Fig.4), for research purposes as well as in clinical practice. It is characterised by an initial peak flux reached in a few seconds after the release that depends on the duration of the occlusion (Yvonne-Tee et al., 2008), and a subsequent return of the LDF to the baseline. However, PRH is a complex phenomenon that comprises metabolic and endothelial vasodilators, as well as a myogenic response and a sensory component (Lorenzo et al., 2007; Yvonne-Tee et al., 2008).

The endothelial component of the PRH response is believed to result from vasodilators (NO, PGI_2 and EDHF) released from the endothelium in response to augmented shear stress due to an increase in blood flow following the release of an occlusion. Yet, the results on the contribution of different endotehlial vasodilators are unequivocal (Bingelli et al., 2003; Dalle-Ave et al., 2004; Durand et al., 2004; ; Medow et al., 2007; Wong et al., 2003).

In the upper extremity, PRH has been performed by a compression of either brachial artery or diferent finger arteries, respectively to suprasystolic blood pressure. The most commonly used occlusion time is 3 or 5 min, but it can range from 1 and up to 15 minutes. Many parameters can be deduced from PRH; unfortunately, they are not standardised (Crakowski et al., 2006; Yvonne-Tee et al., 2008). Indices that are assessed most commonly are: the maximal (peak) LDF response after occlusion release (LDF_{peak}), the time to reach the peak response (t_{peak}), the half time in which the LDF returns to 50% of the baseline, the duration of hyperemia (time to recovery, t_{rec}) and the area under the curve (AUC) (Fig.4). The response of the microvasculature strongly dependes on the occlusion time (Wong et al., 2003; Yvonne-Tee et al., 2008).

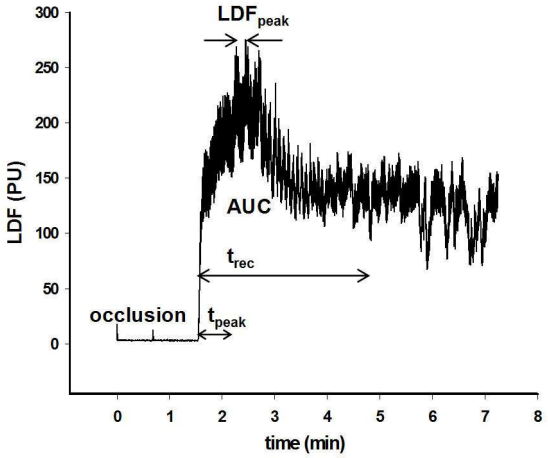


Fig. 4. A representative laser Doppler flux (LDF) response of postocclusive reactive hyperemia (PRH) obtained in the finger pulp after the release of a 3-min occlusion of the brachial artery. Typical indices of the PRH are indicated. PU, perfusion units.

PRH has also been used to induce or amplify the vasomotion in skin microcirculation (Rossi et al., 2008). It is proposed that amplified LDF oscillations are due to the local synchronicity of oscillatory blood flow in a group of cutaneous capillaries during ischemia. It is conceivable that these oscillations represent the local myogenic component of the PRH (Rossi et al., 2008).

3.2.3 Locally induced thermal changes: Heating and cooling

They are used to investigate the mechanisms involved in the local cooling and heating (Johnson & Kellog, 2010; Minson, 2010), as well as in clinical practice to determine microvascular (dys)function in different diseases.

Local heating is usually achieved by applying LD probes with a built-in heater; maximal vasodilation is obtained at a temperature between 42-44°C. As noted previously, maximal vasodilation is sometimes assessed to obtain the maximal vasodilating capacity; all other measurements are referred to this maximal vasodilation.

As for the cooling either LD probes with a built-in cooler or cold water of different degrees is used or flexible cold-gel packages are applied over the skin (Fig.5). Cooling by cold water or flexible packs also evoke a systemic response that can be tracked in other parts of the skin, as for example in the contralateral arm (Fig.5).

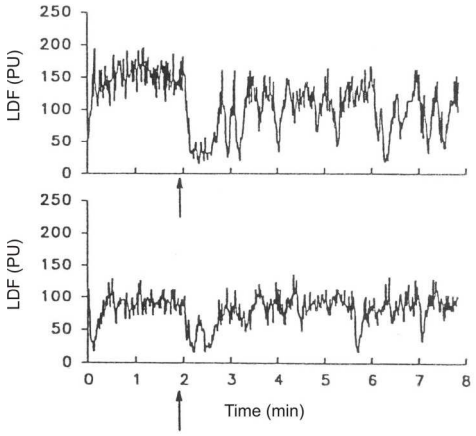


Fig. 5. A representative laser Doppler flux (LDF) response to local cooling obtained in the finger pulp of the ipsilateral hand (direct response, upper panel) and the contralateral hand (indirect response, lower panel), respectively. PU, perfusion units.

Both, heating and cooling, show typical patterns: an immediate response to the thermal stimulus as well as a later, sustained response. Part of the response is mediated by axon reflex, whereas there is also impact of NO and the endothelium. Cooling probably acts to inhibit NOS whereas heating causes activation of NOS (Johnson & Kellogg, 2010). Apart from eNOS, also involvement of neuronal NOS is proposed (Kellogg et al., 2009), which renders the interpretation of the results difficult. Additional studies are needed to clarify the exact contribution of different NOS isoforms (Kellogg et al., 2009).

3.2.4 Spectral analysis of the LDF signal

By means of spectral analysis, the LDF signal can be decomposed into components with different frequencies. It has been postulated that low frequencies around 0.01 Hz might reflect endothelial function, the frequencies at arround 0.04 Hz might reflect the neurogenic influence on the vessel wall, the ones around 0.1 Hz myogenic activity, whereas oscillations of higher frequencies at around 0.3 Hz and 1 Hz reffer to respiratory and heart beat activity, respectively (Kvandal et al., 2006). This is one of the tools used to assess endothelial function in physiologic (Kvernmo et al., 2003) and pathologic (Rossi et al., 2011) conditions. The most widely used spectral methods are fast Fourier transform, autoregressive modeling and wavelet analysis.

4. Human skin microcirculation and the endothelium: Impact of NO, prostaglandins and EDHF

The role of endothelium as a local regulator of vascular tone in skin microcirculation has been investigated in many studies. Furthermore, many studies have established an impairment of endothelium-dependent vasodilation in human skin microcirculation with aging (Black et al., 2008; Holowatz et al., 2007) and in disease (Colberg et al., 2005; Rossi et al., 2011; Taddei et al., 2006). Nevertheless, the exact contribution of various endothelial vasodilators to endothelium-mediated vasodilation has to be elucidated as the results are discrepant. The discrepancy may partly be due to different methods and agonists used to test endothelial function. It might be due to the use of different inhibitors of endothelial vasodilators or different mode of their application (oral, iontophoretical application, perfusion of the brachial artery). A mechanistic explanation would propose that inhibiting one pathway may lead to an upregulation of another one and thus blur the actual effect of the substance under investigation (Feletou & Vanhoutte, 2009; Nishikawa et al., 2000; Osanai et al., 2000). Furthermore, different endothelial vasodilators show a strong site-dependency (Schrage et al., 2005). All above must be taken into account when interpreting the results. It has not been extensively studied to what extent the endothelium contributes to the regulation of vascular tone and vasodilation in glabrous and nonglabrous skin area.

Although it is generally accepted that ACh mediates a NO-dependent response of endothelium (Kellogg et al., 2005; Turner et al., 2008), there are studies that did not confirm an involvement of NO (Hollowatz et al., 2005; Khan et al., 1997; Noon et al,1998). It has also been claimed that the contribution of NO to the ACh-induced vasodilation may be site-dependent: Noon et al (Noon et al.,1996) suggested that NO may be more important in the regions rich in AVAs (glabrous areas) than in areas with predominantly nutritive blood flow (dorsum of the hand). Even more debatable remains the role of prostaglandins in the ACh-mediated response in human skin microcirculation. While some studies have confirmed their role (Durand et al., 2004; Holowatz et al., 2005, Kellogg et al., 2005; Khan, 1997; Noon et al., 1998), others failed to do so (Abou-Elenin et al., 2002; Dalle-Ave et al., 2004; Morris& Shore, 1996).

A compelling study was performed by Hendry & Marshall: contrary to most other studies they showed that the inhibition of COX actually augmented the ACh- mediated increase in LDF (Hendry & Marshall, 2004). They speculate that in this setting, the vasoconstrictor products released by the endothelium (such as thromboxane A_2 and prostaglandin H_2) may limit the response to ACh, and not until the COX is blocked, could the maximal response to ACh be detected. In fact, it has been shown by other authors that thromboxane A_2 exerts its inhibitory effects on bioavailable NO by increasing oxidative stress (Tang et al., 2005) and inhibiting NOS (Yamada et al., 2003). Furthermore, Higashi et al. (2003) have shown that apart from NO, potassium-ATP channels, and cytochrome P-450, but not prostaglandins, may play a role in the ACh-induced vasodilation. Yet, their study was performed using a strain-gauge plethysmography (Higashi et al., 2003). In other studies, a portion of the ACh-mediated response, however, was attributed to an axon reflex (Berghoff et al., 2002;).

As for the role of NO and prostaglandins in the PRH, the results are controversial too (Dalle-Ave et al., 2004; Medow et al., 2007; Wong et al., 2003,). The role of NO seems less important than it was predicted. Namely, Wong et al. showed no effect of NOS inhibition on the PRH in the forearm skin (Wong et al., 2003). The results were substantiated by the study of Zhao et al., who assessed the concentration of NO in the microdyalisate using a NO selective

amperometric electrode (Zhao et al., 2004). Other studies have confirmed an involvement of NO in the PRH response (Bingelli et al., 2003; Medow et al., 2007), yet it seems only of minor importance compared to other mechanisms. A conceivable speculation has been made by Medow et al: they propose that the COX inhibition unmasks the NO dependence of PRH in human skin (Medow et al., 2007). The results could strengthen the hypothesis on the crosstalks between various endothelial vasodilators in human skin. The role of the cross-talk between angiotensin II and NO has also been proposed (Loot et al., 2009).

As part of the ACh- and PRH –induced vasodilation in human skin microcirulation persists after simultaneous eNOS and COX blockade, it is speculated that other endothelial mediators might also be involved. There are also cross-talks between endothelial and neural mechanisms: NO has been shown to attenuate cutaneous responsiveness to norepinephrine via postsynaptic mechanisms (Shibasaki et al., 2008); furthermore, it is the α_2-adrenergic receptor on the endothelium to mediate the NO-induced as well as prostaglandin-mediated vasodilation (Hermann et al., 2005). NO released from neuronal nNOS might be even more important than the one from eNOS (Kellogg et al., 2009). From above observations it is clear that different mechanisms contribute to the local regulation of vascular tone but their exact role has yet to be elucidated.

In the light of the aforementioned studies, we aimed at addressing the role of the nonNO-nonPGI$_2$-dependent mechanism in human skin in different measuring sites as well as using different provocation tests to stimulate the endothelium. We simultaneously applied eNOS and COX inhibitors to the volar forearm using intradermal micro-injection and performed iontophoresis of ACh. Our results have shown no effect of the combined inhibition on the baseline LDF. Furthermore, about 80% of the ACh-induced vasodilation persisted after combined NOS and COX blockade. We may only speculate about the mechanisms involved but according to other observations, it may be attributable to an endothelial mechanism other than NO and PGI$_2$, such as a putative EDHF. Other mechanisms, such as axon reflex, might also be involved; by applying an anesthetic cream to the measuring site we would be able to eliminate the potential axon reflex. This is one of the pitfalls of our study. Another pitfall is the microinjection tehnique; as mentioned previously, the quantity of a substance applied could not be determined. It might be that we were not able to achieve an adequate concentrations of the drugs *in situ*. In this regard, microdialysis would seem to be more suitable. In another set of experiments, we induced PRH after blockade of eNOS and COX to assess the role of nonNO-nonPGI$_2$-dependent mechanisms involved. After having obtained indices of PRH, the results have shown no differences in the L-NMMA and diclofenac treated sites as compared to the control sites, but there were differences in the AUC. Namely, AUC was slightly smaller in the treated sites (unpublished observation) which again points to only a minor role of NO and PGI$_2$ in the LDF response induced by PRH.

With respect to the role of EDHF in human skin *in vivo*, the studies are scarce. The existence of endothelium-dependent, NO/PGI$_2$-independent relaxations, potentially attributable to an EDHF, has been confirmed in humans *in vivo*. Based on studies in patients who exhibited a NO/PGI$_2$-independent response to an endothelial challenge, it has been suggested that EDHF might serve as a backup vasodilator in the settings of compromised endothelial function (Fichtlscherrer et al., 2004; Fischer et al., 2007; Taddei et al., 2006; Yang, 2007). Different putative mechanisms are proposed to mediate the EDHF-mediated vasodilation:

in humans, the most probable candidate is a CYP derived metabolite (Feletou & Vanhoutte, 2009). Indeed, a mechanism sensitive to CYP epoxygenase inhibition was confirmed (Bellien et al., 2005; Fischer et al., 2007; Fischtlscherer et al., 2004; Hillig et al., 2003; Taddei et al., 2006). Moreover, the CYP2C9 isoform was already confirmed on the endothelium of some human arteries where also a functional role of a CYP-derived EDHF was shown (Hillig et al., 2003; Larsen et al., 2006).

Based on the results from previous *in vivo* studies in humans our aim was to further characterize the nonNO-nonPGI$_2$-dependent vasodilation in the skin microcirculation in the forearm. Therefore, we assessed the LDF response to ACh and PRH in the presence and absence of the selective CYP 2C9 inhibitor, sulfaphenazole, respectively. Contrary to most other studies that mainly applied the CYP inhibitors by perfusion of the brachial artery, we applied it by an intradermal microinjection. Sulfaphenazole had no effect either on the baseline LDF or on the vasodilation induced by ACh (Lenasi, 2009) or PRH (unpublished results). This is in agreement with some studies using venous plethysmography performed in healthy humans (Passauer et al., 2003, 2005), whereas at odds with most studies performed in patients (Fischer et al., 2007; Fischtlscherer et al., 2003; Taddei et al., 2006). We may explain the discrepancy with the studies performed in patients by the fact that the expression of CYP might be upregulated in patients due to a decrased bioavailability of NO. Again, methodological limitations must be taken into consideration, such as microinjection and the selection of a proper CYP inhibitor. Also, to evaluate the impact of EDHF in the control of skin microcirculation, it would be suitable to apply another endothelial agonist besides ACh. Namely, it has been proposed that the nonNO-nonPGI-dependent vasodilation might be more sensitive to bradykinin than to ACh (Schrage et al., 2005). Furthermore, the assumption that the NO/PGI$_2$-independent relaxation is indeed due to an EDHF would be strengthened had we applied an inhibitor of Ca^{2+}-activated K-channels or a depolarizing agent to at least indirectly prove an endothelium-dependent hyperpolarization (Feletou and Vanhoutte, 2009).

Taken together, different endothelial vasodilators are involved in the control of vascular reactivity of the skin microcirculation. The exact mechanisms involved and the role of a putative EDHF remain to be established.

5. Exercise augments the endothelium dependent vasodilation in skin microcirculation

Beneficial effects of exercise on the vascular reactivity of human skin microcirculation have been reported in many studies (Hodges et al., 2010). In general, the collective finding is that regular physical exercise enhances endothelium-dependent vasodilation. Moreover, it has been shown that exercise also improves the decline of endothelium-dependent vasodilation of cutaneous microcirculation in age (Black et al., 2008) and disease (Colberg et al., 2005; Rossi et al., 2011; Taddei et al., 2006).

The exact mechanisms of exercise-induced adaptations in human skin microcircultion remain unresolved. Various mechanisms have been proposed, the most likely one seems to be an increased production of NO by increased eNOS activity, probably due to repetitively increased shear stress on the endothelium (Green et al., 2004). Apart from endothelial adaptations, changes in the sensitivity of vascular SMC to endothelial vasodilators or alterations in neural vascular control may play a role. By inducing local heating as a

vasodilating stimulus, the study of Tew et al. has shown that aerobic fitness affects the contribution of noradrenergic sympathetic fibers to the heating-induced LDF response (Tew et al., 2011). Indeed, it has been shown that endurance trained athletes have a diminished temperature threshold for active vasodilation in skin compared to matched sedentary controls (Fritzsche & Coyle, 2000). It is thus obvious that apart from local, possibly endothelial mechanisms, neural mechanisms also are subject to adaptations.

We aimed at assessing vascular reactivity in highly endurance-trained athletes with a high maximal aerobic capacity (V_{O2max} 65 ml/min/kg) and comparing it with age-matched, sedentary controls (V_{O2max} 41 ml/min/kg). Vascular reactivity was assessed at two representative sites with different control mechanisms: glabrous and nonglabrous area, respectively. Namely, it has been shown that the hemodynamic changes associated with acute exercise differ between these two sites (Yamazaki & Sone, 2006). The amplitude of changes, expressed as CVC as well as the time in which the changes occur during exercise differ between these two sites which might point to different control mechanisms. We hypothesised that similar differences might also be observed regarding adaptation to chronic exercise. We determined vascular reactivity by an iontophoretical application of ACh and SNP as well as by inducing PRH. Unfortunately, we were able to aplly ACh and SNP only to nonglabrous skin sites. As for the glabrous areas, we assessed the response to a 4 min brachial artery occlusion on the finger pulp and compared it with the response in the volar forearm. As expected, we found an increased responsiveness, as assessed by peak response to ACh and the indices of PRH, respectively, in the trained. Yet, the differences in indices of PRH were observed only in the glabrous sites and not in the nonglabrous, respectively (Lenasi & Štrucl, 2010). This may prove that glabrous parts with abundant AVAs are indeed more strongly involved in thermoregulatory adaptations to exercise. The adaptation of skin microcirculation to exercise might be explained by a direct effect of exercise-induced shear stress on the vessels, as well as by adaptive changes attending thermoregulatory challenges. The discrimination between these two mechanisms is not possible as they overlap each other. Nevertheless, Lorenzo & Minson have shown that skin microcirculation also undergoes thermoadaptative changes as a part of acclimation to heat without eliciting reflexes engaged in exercise (Lorenzo & Minson, 2010). To do so, they trained subjects only at 50% of their V_{O2max}, intensity that assumingly does not induce changes in the reflexes affecting exercise. Another interesting approach was by Rossi et al.: by performing spectral analysis of the LDF signal, they showed an augmented amplitude of the low frequency band presumably reflecting endothelial function in the trained (Rossi et al., 2006). Similar observations were found in the study performed by Kvernmo et al. (Kvernmo et al., 2003).

Exercise training probably induces adaptations in all age groups. Namely, a recent study performed in adolescents showed an enhanced endothelium-dependent vasodilation in nonglabroous skin in the group of trained subjects (Roche et al., 2010). Also, in the group of postmenopausal women who underwent a 48-week regime of aerobic exercise training the endothelium-dependent and –independent vasodilation was enhanced as well as the response to local heating (Hodges et al., 2010). Black et al. have shown that age-related decline in endothelial function can be improved already after 24 weeks aerobic training (Black et al., 2008). Contrary to the observation by Hodges et al (Hodges et al., 2010) on an increased endothelium-independent vasodilation (induced by an application of SNP) we found a decreased response to SNP in the trained. The result is at odds with most other studies that either showed no differences in the SNP-induced vasodilation between the

sedentary and the trained or an increased response in the trained. Our observation is difficult to interpret; we suggested a decreased sensitivity of vascular SMC to a NO donor (Gori & Parker, 2002) which seems in certain respect illogical. The data on the vasodilator response to heating show that the LDF increase to the application of ACh occurs prior to the increase induced by SNP (Kellog et al., 2005). This might suggest that the endothelium is modified by exercise sooner then the vascular SMC. The observation would support the results of the studies that found no differences in endothelium-independent vasodilation between the trained and the sedentary.

Interestingly, most studies found no differences in the baseline LDF between the trained and the sedentary. The same holds true also for longitudinal studies that were performed in a cohort of subjects who underwent training and thus are more reliable (Black et al., 2008; Hodges et al., 2010). This seems logical as under resting conditions the nutritional needs of skin microcirculation are similar for the trained and the sedentary. When performing exercise, the trained exhibit a greater vasodilator capacity, probably of endothelial origin that may fulfil the increased demands for heat elimination in the trained.

It has been accepted that augmented endothelium vasodilator capacity induced by training are due to increased bioavailability of NO (Green et al., 2004). The speculative functional studies were confirmed by Wang, who has shown an increased response to ACh as well as an increased level of plasma NO metabolites after 8 weeks of training in healthy subjects. (Wang, 2005). Also, Vassalle et al. have shown an enhanced release of NO in the trained combined to an increased LDF response induced by PRH (Vassalle et al., 2003). Increased NO might be due to an upregulation of eNOS or its enhanced activity. Other mechanisms are proposed, such as an increase in plasma antioxidant activity (Franzoni et al., 2004) that prevent the scavenging of NO by free radicals and increase the bioavailability of its precursors (Doutreleau et al., 2010) and cofactors and thus increase its bioavailability. Apart from changes in the bioavailability of NO, an increase of other vasodilators, such as prostanoids (McCord et al., 2006) and EDHF (Taddei et al., 2006), may also be involved. It has been shown that prostanoids contribute to endothelium-mediated vasodilation in response to acute exercise (Duffy et al., 1999). Studies performed in animals have shown an increase in EDHF-mediated vasodilation induced by training (Minami et al., 2002). The same mechanisms are speculated to also play a role in humans. Additional studies in humans are needed to clarify this issue.

6. Conclusion

Skin microcirculation and the mechanisms controlling it remain an interesting area of research. As a dynamic structure, it is engaged in various thermoregulatory and non-thermoregulatory reflexes. Apart from neural control, the endothelium has been suggested to play an important role in the regulation of vascular tone and reactivity in skin microcirculation. However, glabrous and nonglabrous skin areas differ with respect to the control of vascular tone, also regarding the endothelium.

Laser Doppler flowmetry (LDF) represents an easy-to-apply, noninvasive and reproducible method to investigate skin microcirculation, yet with some disadvantages, such as great spatial and temporal variability. Coupled to LDF, many methods have been developed over the last 20 years in order to assess the endothelial function of human skin. Most frequently used are iontophoresis of ACh, postocclusive reactive hyperemia, and locally induced thermal changes. Apart from research purposes, they are often applied in clinical practice to

assess the endothelial (dys)function and its response to therapy. They are suggested to be applicable indices of global microvascular function. Nevertheless, none of them is able to detect specifically endothelial function: rather, they provide integrated indices of vascular reactivity. This must be taken into consideration when interpreting the results obtained.

As skin microcirculation is often compromised in disease, also due to endothelial dysfunction, strategies on how to improve it are being sought. One of the measures to improve endothelial function seems to be participation in regular aerobic exercise, as it has been shown to be associated with enhanced mirovascular reactivity, in particular endothelium-dependen vasodilation, in glabrous and nonglabrous areas over the body.

The mechanisms involved in adaptations to exercise training as well as the exact contribution of various endothelial vasodilators (NO, PGI_2 and EDHF) to the regulation of vascular tone in human skin microcirculation, remain to be resolved. Nevertheless, they represent putative therapeutical targets and are thus important also from the clinical point of view.

7. Acknowledgement

The author wish to thank Olga Shrestha for language editing.

8. References

Abou-Elenin, K., Xydakis, A., Hamdy, O., Economides, P.A., Horton, E.S., & Veves, A. (2002). The effect of aspirin and various iontophoresis solution vehicles on skin microvascular reactivity. *Microvasc Res*, 63, 1, 91-5.

Aoki, K., Stephens, D.P., Saad, A.R., & Johnson, J.M. (2003). Cutaneous vasoconstrictor response to whole body skin cooling is altered by time of day. *J Appl Physiol*, 94, 930-934.

Bellien, J., Joannides, R., Iacob, M., Arnaud, P., & Thuillez, C. (2005). Evidence for a basal release of a cytochrome-related endothelium-derived hyperpolarizing factor in the radial artery in humans. *Am J Physiol Heart Circ Physiol*, 290, 4, H1347-52.

Berghoff, M., Kathpal, M., Kilo, S., Hilz, M.J., & Freeman, R. (2002) Vascular and neural mechanisms of ACh-mediated vasodilation in the forearm cutaneous microcirculation. *J Appl Physiol,* 92, 780-788.

Binggeli, C., Spieker, L.E., Corti, R., Sudano, I., Stojanovic, V., Hayoz, D., Lüscher, T.F., & Noll, G. (2003). Statins enhance postischemic hyperemia in the skin circulation of hypercholesterolemic patients: a monitoring test of endothelial dysfunction for clinical practice? *J Am Coll Cardiol*, 42, 1, 71-7.

Black, M.A., Green, D.J., & Cable, N.T. (2008). Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. *J Physiol*, 586, 14, 3511-24.

Braverman, I., Keh, A., & Goldminz, D. (1990). Correlation of laser Doppler wave patterns with underlying microvascular anatomy. *J Invest Dermatol*, 95, 283-286.

Braverman, I.M. (1997). The cutaneous microcirculation: Ultrastructure and Microanatomical Organization. *Microcirc*, 4, 3, 329-340.

Charkoudian, N., & Johnson, J.M. (2000). Female reproductive hormones and thermoregulatory control of skin blood flow. *Exerc Sport Sci Rev*, 28, 108-112.

Colberg, S.R., Parson, H.K., Nunnold, T., Holton, D.R., Swain, D.P., & Vinik, A.I. (2005). Change in cutaneous perfusion following 10 weeks of aerobic training in type 2 diabetes. *J Diabetes Complications*, 19, 276-283.

Crakowski, J-L., Minson, C.T., Salvat-Melis, M., & Halliwill, J.R. (2006). Methodological issues in the assessment of skin microvascular endothelial function in humans. *TRENDS Pharmacol Sci*, 27, 9, 503-508.

Crakowski J.-L., Lorenzo, S., & Minson, C.T. (2007). Effects of local anaesthesia on subdermal needle insertion pain and subsequent tests of microvascular function in human. *Eur J Pharmacol*, 559, 150-154.

Dalle-Ave, A., Kubli, S., Golay, S., Delachaux, A., Liaudet, L., Waeber, B., & Feihl, F. (2004). Acetylcholine-induced vasodilation and reactive hyperemia are not affected by acute cyclo-oxygenase inhibition in human skin. *Microcirculation*, 11, 4, 327-36.

Doutreleau, S., Rouyer, O., Di Marco, P., Lonsdorfer, E., Richard, R., Piquard, F., & Geny, B. (2010). L-arginine supplementation improves exercise capacity after a heart transplant. *Am J Clin Nutr*, 91, 5, 1261-7.

Duffy, S.J., New, G., Tran, B.T., Harper, R.W., & Meredith, I. (1999). Relative contribution of vasodilator prostanoids and NO to metabolic vasodilation in the human forearm. *Am J Physiol*, 276, H663-H670.

Durand, S., Tartas, M., Bouye, P., Koitka, A., Saumet, J.L., & Abraham, P. (2004). Prostaglandins participate in the late phase of the vascular response to acetylcholine iontophoresis in human. *J Physiol*, 561, 3, 811-819.

Feletou, M., & Vanhoutte, P.M. (2009). EDHF: an update, *Clin Sci*, 117, 139-155.

Fichtlscherer, S., Dimmeler, S., Breuer, S., Busse, R., Zeiher, A.M., & Fleming, I. (2004). Inhibition of cytochrome P450 2C9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. *Circulation*, 109, 2, 178-83.

Fischer, D., Landmesser, U., Spiekermann, S., Hilfiker-Kleiner, D., Hospely, M., Müller, M., Busse, R., Fleming, I., & Drexler, H. (2007). Cytochrome P450 2C9 is involved in flow-dependent vasodilation of peripheral conduit arteries in healthy subjects and in patients with chronic heart failure. *Eur J Heart Fail*, 9, 8, 770-5.

Fleming, I. (2010). Molecular mechanisms underlying the activation of eNOS. *Pflugers Arch – Eur J Physiol*, 459, 6, 793-806.

Franzoni, F., Plantinga, Y., Femia, F.R., Bartolomucci, F., Gudio, C., Regoli, F., Carpi, A., Santoro, G., & Galetta, F. (2004). Plasma antioxidant activity and cutaneneous microvascular endothelila function in athletes and sedentary controls. *Biomed Pharmacotherap*, 58, 432-436.

Fritzsche, R.G., & Coyle, E.F. (2000). Cutaneous blood flow during exercise is higher in endurance-trained humans. *J Appl Physiol*, 88, 2, 738-44.

Gori, T., Parker, J.D. (2002). Nitrate Tolerance A Unifying Hypothesis. *Circulation*, 106: 2510-2513.

Green, D.J., Maiorana, A., O'Driscoll, G., & Taylor, R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol*, 561, 1, 1-25

Hendry, R,G,, & Marshall, J.M. (2004). Vasoconstrictor products of cyclo-oxygenase activity limit acetylcholine-induced cutaneous vasodilatation in young men. *Clin Sci (Lond)*, 107, 3, 323-30.

Hermann, D., Schlereth, T., Vogt, T., & Birklein, F. (2005). Clonidine induces nitric oxide- and prostaglandin-mediated vasodilation in healthy human skin. *J Appl Physiol,* 99, 6, 2266-70.

Higashi, Y., & Yoshizumi, M. (2003). New methods to evaluate endothelial function: Method for Assessing Endothelial Function in Humans Using a Strain-Gauge Plethysmography: Nitric oxide-dependent and –Independent Vasodilation. *J Pharmacol Sci*, 93, 399-404.

Hillig, T., Krustrup, P., Fleming, I., Osada, T., Saltin, B., & Hellsten, Y. (2003). Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J Physiol*, 546(Pt 1), 307-14.

Hodges, G.J., Sharp, L., Stephenson, C., Patwala, A.Y. George, K.P., Goldspink, D.F., & Cable, N.T. (2010). The effect of 48 weeks of aerobic exercise training on cutaneous vasodilator function in pst-menopausal females, *Eur J Appl Physiol*. 108, 1259-1267.

Holowatz, L.A., Thompson, C.S., Minson, C.T., & Kenney, W.L. (2005) Mechanisms of acetylcholine-mediated vasodilatation in young and aged human skin. *J Physiol*, 563, 965-973.

Holowatz, L.A., Thompson-Torgerson, C.S., & Kenney, W.L. (2007). Altered Mechanisms of Vasodilation in Aged Human Skin. *Exerc Sport Sci Rev*, 35, 3, 119-25.

Holowatz, L.A., Thompson-Torgerson, C.S., & Kenney, W.L. (2008). The human cutaneous circulation as a model of generalized microvascular function. *J Appl Physiol,* 105, 1, 370-2.

Johnson, J.M., & Proppe, D.W. (1996). Cardiovascular adjustments to heat stress. In: *Handbook of Physiology*. Environmental Physiology, 215-243, Am. Physiol. Soc., Bethesda.

Johnson, J.M., & Kellogg, D.L.Jr. (2010). Thermoregulatory and thermal control in the human cutaneous circulation. *Front Biosci*, S2: 825-853.

Kalia, Y.N., Naik, A., Garrison, J., & Guy, R.H. (2004). Iontophoretic drug delivery. *Adv Drug Deliv Rev*, 56, 619-658.

Kellogg, D.L.Jr., Zhao, J.L., Coey, U, & Green, J.V. (2005). Acetylcholine-induced vasodilation is mediated by nitric oxide and prostaglandins in human skin. *J Appl Physiol.* 98, 629-632.

Kellogg, D.L.Jr. (2006). In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *J Appl Physiol*. 100, 1709-1718.

Kellogg, D.L.Jr., Zhao, J.L., & Wu, Y. (2009). Roles of nitric oxide synthase isoforms in cutaneous vasodilation induced by local warming of the skin and whole body heat stress in humans. *J Appl Physiol*, 107, 5, 1438-44.

Kernick, D.P., Tooke, J.E., & Shore, A.C. (1999). The biological zero signal in laser Doppler fluximetry – origins and practical implications. *Pflugers arch Eur J Physiol,* 437, 624-631.

Khan, F., Davidson, N.C. , Littleford, R.C., Litchfield, S.J., Struthers, A.D., & Belch., J.J. (1997). Cutaneous vascular responses to acetylcholine are mediated by a prostanoid-dependent mechanism in man. *Vasc Med*, 2, 82-6.

Khan, F., Newton, D.J., Smyth, E.C., & Belch, J.J. (2004). Influence of vehicle resistance on transdermal iontophoretic delivery of acetylcholine and sodium nitroprusside in humans. *J Appl Physiol*, 97, 883-887.

Kvandal, P., Landsverk, S.A., Bernjak, A., Stefanovska, A., Kvernmo, H.D., & Kirkebøen, K.A. (2006). Low-frequency oscillations of the laser Doppler perfusion signal in human skin. *Microvasc Res*, 72, 120-127.

Kvernmo, H.D., Stefanowska, A., & Kirkeboen, K.A. (2003). Enhanced endothelial activity reflected in cutaneous blood flow oscillations of athletes. *Eur J Appl Physiol*, 90, 16-22.

Larsen, B.T., Miura, H., Hatoum, O.A., Campbell, W.B., Hammock, B.D., Zeldin, D.C., Falck, J.R., & Gutterman, D.D. (2006). Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK(Ca) channels: implications for soluble epoxide hydrolase inhibition. *Am J Physiol Heart Circ Physiol*, 290, 2, H491-9.

Lenasi, H. (2010). The role of nitric oxide- and prostacyclin-independent vasodilatation in the human cutaneous microcirculation: effect of cytochrome P450 2C9 inhibition. *Clin Physiol Funct Imaging*, 29, 4, 263-70.

Lenasi, H., & Štrucl, M. (2010). Regular physical activity alters the postocclusive reactive hyperemia of the cutaneous microcirculation. *Clin Hemorheol Microcirc*, 45, 365-374.

Leslie, S.J., Affolter, J., Denvir, M.A., & Webb, D.J. (2003). Validation of laser Doppler flowmetry coupled with intra-dermal injection for investigating effects of vasoactive agents on the skin microcirculation in man. *Eur J Clin Pharmacol*, 59, 99-102.

Loot, A.E., Schreiber, J.G., Fisslthaler, B., & Fleming, I. (2009). Angiotensin II impairs endothelial function via tyrosine phosphorylation of the endothelial nitric oxide synthase. *J Exp Med*, 206, 13, 2889-2896.

Lorenzo, S., & Minson, C.T. (2007). Human cutaneous reactive hyperaemia: role of BK_{Ca} channels and sensory nerves. *J Physiol*, 585, 1, 295-303.

Lorenzo, S., & Minson, C.T. (2010). Heat acclimation improves cutaneous vascular function and sweating in trained cyclists. *J Appl Physiol*, 109, 6, 1736-43.

McCord, G.R., Cracowski, J-L. & Minson, C.T. (2006). Prostanoids contribute to cutaneus active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol*, 291, R596-R602.

Medow, M.S., Taneja, I., & Stewart, J.M. (2007). Cyclooxygenase and nitric oxide synthase dependence of cutaneous reactive hyperemia in humans. *Am J Physiol Heart Circ Physiol*, 293, H425-H432.

Minami, A., Ishimura, N., Harada, N., Sakamoto, S., Niwa, Y., & Nakaya, Y. (2002). Exercise training improves acetylcholine-induced endothelium-dependent hyperpolarization in type 2 diabetic rats, Otsuka Long-Evans Tokushima fatty rats. *Atherosclerosis*, 162, 1, 85-92.

Minson, C.T. (2010). Thermal provocation to evaluate microvascular reactivity in human skin. *J Appl Physiol*, 109: 1239-1246.

Morris, S.J., & Shore, A.C. (1996). Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: Possible mechanisms. *J Physiol*, 496, 531-542.

Nishikawa, Y., Stepp, D.W., & Chilian, W.M. (2000). Nitric oxide exerts feedback inhibition on EDHF-induced coronary arteriolar dilation in vivo. *Am J Physiol Heart Circ Physiol*, 279, H459–H465.

Noon, J.P., Haynes, W.G., Webb, D., & Shore, A.C. (1996). Local inhibition of nitric oxide generation in man reduces blood flow in finger pulp but not in hand dorsum skin. *J Physiol,* 490, 501-8.

Noon, J.P., Walker, B.R., Hand, M.F., & Webb, D.J. (1998). Studies with iontophoretic administration of drugs to human dermal vessels in vivo: cholinergic vasodilatation is mediated by dilator prostanoids rather than nitric oxide. *Br J Clin Pharmacol*, 45, 545-550.

Osanai, T., Fujita, N., Fujiwara, N., Nakano, T., Takahashi, K., Guan, W., & Okomura, K. (2000). Cross talk of shear-induced production of prostacyclin and nitric oxide in endothelial cells. *Am J Physiol Heart Circ Physiol*, 278, H233–H238.

Passauer, J., Pistrosch, F., Lässig, G., Herbrig, K., Büssemaker, E., Gross, P., & Fleming, I. (2005). Nitric oxide- and EDHF-mediated arteriolar tone in uremia is unaffected by selective inhibition of vascular cytochrome P450 2C9. *Kidney Int,* 67, 5, 1907-12.

Passauer, J., Bussemaker, E., Lassig, G., Pistrosch, F., Fauler, J., Gross, P., & Fleming, I. (2003). Baseline blood flow and bradykinin-induced vasodilator responses in the human forearm are insensitive to the cytochrome P450 2C9 (CYP2C9) inhibitor sulphaphenazole. *Clin Sci (Lond)*, 105(4), 513-8.

Pohl, U., & deWit, C. (1999). A Unique Role of NO in the Control of Blood Flow. *News Physiol Sci*, 14, 74-80.

Ramsay, J.E., Ferrell, W.R., Greer, I.A., & Sattar, N. (2002). Factors critical to iontophoretic assessment of vascular reactivity: implications for clinical studies of endothelial dysfunction. *J Cardiovasc Pharmacol*, 39, 9-17.

Roche, D.M., Rowland, T.W., Garrard, M., Marwood, S., & Unnithan, V.B. (2010). Skin microvascular reactivity in trained adolescents. *Eur J Appl Physiol*, 108, 1201-1208.

Roddie, I.C. Circulation to skin and adipose tissue. In: *Handbook of Physiology.* J. T. Shepherd, and S. R. Geiger (Eds.). Bethesda: American Physiology Society, 1983, pp: 285-309.

Rossi, M., Santoro, G., Maurizio, S., & Carpi, A. (2006). Spectral analysis of skin blood flowmotion before and after exercise in healthy trained subjects. *Int J Sports Med*, 27: 540-545.

Rossi, M., Carpi, A., Galetta, F., Franzoni, F., & Santoro, G., (2008). Skin vasomotion investigation: A useful tool for clinical evaluation of microvascular endothelial function? *Biomed Pharmacother*, 62: 541-545.

Rossi, M., Bradbury, A., Magagna, A., Pesce, M., Taddei, S., & Stefanovska A. (2011). Investigation of skin vasoreactivity and blood flow oscillations in hypertensive patients: effect of short-term antihypertensive treatment. *J Hypertens,* 29, 8, 1569-76.

Rowell, LB. (1993). *Human cardiovascular control*. Oxford University Press, New York, Oxford.

Saumet, J.L., Kellogg, D.L.Jr., Taylor, W.F., & Johnson, J.M. (1988). Cutaneous laser-Doppler flowmetry: influence of underlying muscle blod flow. *J Appl Physiol*, 65, 478-481.

Schrage, W.G., Dietz, N.M., Eisenach, J.H., & Joyner, M.J. (2005). Agonist-dependent variablity of contributions of nitric oxide and prostaglandins in human skeletal muscle. *J Appl Physiol*, 98, 4, 1251-7.

Serup, J., Jemec, G.B.E., & Grove, G.L. (eds.) (2006). *Handbook of Noninvasive Methods and the Skin*, CRC Taylor & Francis Group, ISBN 0-8493-1437-2, Boca Raton, London, New York.

Shepherd, A.P., & Oberg, P.A. (Eds.) (1990). *Laser-Doppler blood flowmetry*, Kluwer Academic Publishers, ISBN 0-7923-0508-6, Boston Dordrecht London.

Shibasaki, M., Low, D.A., Davis, S.L., & Crandall, C.G. (2008). Nitric oxide inhibits cutaneous vasoconstriction to exogenous norepinephrine. *J Appl Physiol*, 105, 5, 1504-8.

Szerafin, T., Erdei, N., Fulop, T., Pasztor, E.T., Edes, I., Koller, A., & Bagi, Z. (2006). Indcreased cyclo-oxygenase-2 expression and prostaglandin-mediated dilation in coronary arteries of patients with diabetes mellitus. *Circ Res*, 99, 112-117.

Taddei, S., Versari, D., Cipriano, A., Ghiadoni, L., Galetta, F., Franzoni, F., Magagna, A., Virdis, A., & Salvetti, A. (2006). Identification of a cytochrome P450 2C9-derived endothelium-derived hyperpolarizing factor in essential hypertensive patients. *J Am Coll Cardiol*, 48, 508-15.

Tang, M., Cyrus, T., Yao, Y., Vocun, L., & Pratico, D. (2005). Involvement of thromboxane receptor in the proatherogenic effect of isoprostane F2alphaIII: evidence from apolipoprotein E- and LDL receptor-deficient mice. *Circulation,* 112: 2867-2874.

Tew, G.A., Saxton, J.M., Klonizakis, M., Moss, J., Ruddock, A.D., & Hodges, G.J. (2011). Aging and aerobic fitness affect the contribution of noradrenergic sympathetic nerves to the rapid cutaneous vasodilator response to local heating. *J Appl Physiol*, 110, 1264-1270.

Treu, C.M., Lupi, O., Bottino, D.A., & Bouskela, E. (2011). Sidestream dark field imaging: the evolution of real-time visualization of cutaneous microcirculation and its potential application in dermatology. *Arch Dermatol Res*, 303, 69-78.

Turner, J., Belch, J.J.F., & Khan, F. (2008). Current Concepts in Assessment of Microvascular Endotehlial Function Using Laser Doppler Imaging and Iontophoresis. *Trends Cardiovasc Med*, 18, 109-116.

Urakami-Harasawa, L., Shimokawa, H., Nakashima, M., Egashira K, & Takeshita A. (1997). Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest*, 100, 2793-2799.

Vanhoutte, P.M. (1989). Endothelium and control of vascular function. *Hypertension*, 13, 658-667.

Vassalle, C., Lubrano, V., Domenici, C., & L'Abbate, A. (2003). Influence of chronic aerobic exercise on microcirculatory flow and nitric oxide in humans. *Int J Sports Med*, 24, 1, 30-5.

Wang, J.S. (2005). Effects of exercise training and detraining on cutaneous microvascular function in man: the regulatory role of endothelium-dependent dilation in skin vasculature. *Eur J Appl Physiol*, 93, 429-34.

Wong, B.J., Wilkins, B.W., Holowatz, L.A., & Minson, C.T. (2003). Nitric oxide inhibitions does not alter the reactive hyperemic response in the cutaneous circulation. *J Appl Physiol*, 95, 504-510.

Yamada, T., Fujino, T., Yuhki, K., Hara, A., Karibe, H., Takahata, O., Okada, Y., Xiao, C.Y., Takayama, K., Taniguchi, T., Shiokoshi, T., Ohsaki, Y., Kikuchi, K., Narumyia, S., & Ushikubi, F. (2003). Thromboxane A_2 regulates vascular tone via its inhibitory effect on the expression of inducible nitric oxide synthase. *Circulation*, 108, 2381-2386.

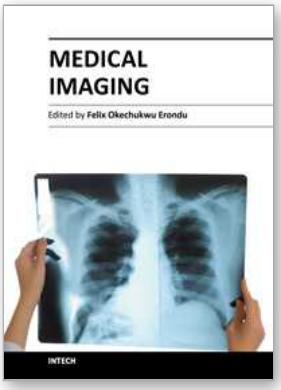
Yamazaki, F., & Sone, R. (2006). Different vascular responses in glabrous and nonglabrous skin with increasing core temperature during exercise. *Eur J Appl Physiol,* 97, 5, 582-90.

Yang, Q., Yim, A.P.C., & He, G.-W. (2007). The Significance of Endothelium-Derived Hyperpolarizing Human Circulation. *Curr Vasc Pharmacol*, 5, 85-92.

Yvonne-Tee, G.B., Rasool, A.H.G., Halim, A.S., & Rahman, A.R.A. (2006). Noninvasive assessment of cutaneous vascular function in vivo using capillaroscopy, plethysmography and laser-Doppler instruments: Its strengths and weaknesses. *Clin Hemorheol Microcirc*, 34, 457-473.

Yvonne-Tee, G.B., Rasool, A.H.G., Halim, A.S., Wong, A.R., & Rahman, A.R.A. (2008). Method optimization on the use of postoclussive hyperemia model to assess microvascular function. *Clin Hemorheol Microcirc*, 38, 119-133.

Zhao, J.L., Pergola, P.E., Roman, L.J., & Kellogg, D.L.Jr. (2004). Bioactive nitric oxide concentration does not increase during reactive hyperemia in human skin. *J Appl Physiol*, 96, 628-632.



Medical Imaging

Edited by Dr. Okechukwu Felix Erondu

ISBN 978-953-307-774-1
Hard cover, 412 pages
Publisher InTech
Published online 22, December, 2011
Published in print edition December, 2011

What we know about and do with medical imaging has changed rapidly during the past decade, beginning with the basics, following with the breakthroughs, and moving on to the abstract. This book demonstrates the wider horizon that has become the mainstay of medical imaging sciences; capturing the concept of medical diagnosis, digital information management and research. It is an invaluable tool for radiologists and imaging specialists, physicists and researchers interested in various aspects of imaging.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Helena Lenasi (2011). Assessment of Human Skin Microcirculation and Its Endothelial Function Using Laser Doppler Flowmetry, Medical Imaging, Dr. Okechukwu Felix Erondu (Ed.), ISBN: 978-953-307-774-1, InTech, Available from: http://www.intechopen.com/books/medical-imaging/assessment-of-human-skin-microcirculation-and-its-endothelial-function-using-laser-doppler-flowmetry

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821