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Assessment of the Microcirculation: Laser Doppler and Transcutaneous Oxygen

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Introduction

The recent increased recognition that alterations in the microvasculature can play an important role in the etiology and outcome of vascular disease processes and complications has led to intensified efforts to deal with these at the clinical level. Disease targets include diabetes, hypertension, lower extremity arterial disease (LEAD), and processes involved in chronic skin ulcer development and healing potential. Important goals are to improve our understanding of the nature of the functional microvascular deficits and to develop tests to aid in diagnosis and treatment. Laser Doppler flowmetry (LDF) provides a quantitative method for the evaluation of skin red blood cell perfusion, which together with the more established measurement of transcutaneous oxygen tension ($TcPo_2$) makes available powerful tools to probe the human microcirculation. Singly and in combination they may be used in a clinical setting to (1) provide indirect information on the generalized microvasculature status of the patient, (2) provide direct information about the skin microvascular status as it relates to the underlying pathophysiology being studied, and (3) provide microvascular test data useful for specific assessments of the limb vascular status.

Transcutaneous Oxygen Tension

Physiological Basis

Red blood cells (RBC's) moving through the minute blood vessels carry and subsequently release oxygen (O_2) to supply the O_2 needs of the tissue cells. The released O_2 diffuses through the vascular wall and interstitial spaces, whereupon its concentration is diminished roughly in proportion to its utilization by cellular metabolic processes. The partial pressure of O_2 is the quantity expressed by the transcutaneous oxygen ($TcPo_2$) measurement and is related to the O_2 concentration and solubility in the region of the measurement. Under normal circumstances the skin oxygen consumption is low and the O_2 delivery is physiologically regulated to match, but not significantly exceed, the O_2 demand. The net RBC blood flow and hence the rate of O_2 delivery depend on the product of the number of capillaries (Nc) that are actively perfused with flowing RBC's and the speed (URBC) with which the RBC's move through the capillaries. If, in a subject with normal limb circulation, heat is used to increase microvascular blood flow, then a mismatch between O_2 delivered and consumed results, and a $TcPo_2$ measurement would record increased values proportional to the intravascular Po_2. If the same skin heating maneuver were done in a patient with a compromised limb circulation then lower values of $TcPo_2$ would generally be recorded. The amount of the reduction depends on many factors. At one extreme is the patient with severe LEAD in whom the arterioles are near maximally dilated under non-heated basal conditions. Heating would cause less vasodilation and less change in the O_2 delivery; a lower recorded $TcPo_2$ reflects the reduced microvascular reserve of severe LEAD.

Physical Principles

Most commercial devices measure Po_2 using the polarographic technique. The sensing electrode is made of platinum (cathode) and the reference electrode is made of silver/silver chloride (anode), both immersed in an electrolyte and covered by a thin stabilizing membrane. Oxygen diffuses through the membrane, and at the cathode a chemical reaction occurs resulting in the reduction of the oxygen and an associated generation of electrical current, which forms the basis of the $TcPo_2$ measurement. At normal temperatures the stratum corneum has a very high diffusion resistance and thereby restricts the O_2 passing across the epidermis to the $TcPo_2$ sensing electrode. However, heating of the skin to above 40°C causes a reversible structural change, which results in an increase in O_2 diffusivity by about a factor of 100, thereby minimizing this effect. At a given temperature setting, the measured $TcPo_2$ depends on the arterial Po_2 and the nutritional skin blood flow in the region of the measurement. Technical and mathematical details may be found in an abundant literature.[1,2]

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Clinical Technique

A low $TcPO_2$ attributable to a decreased arterial P_{O_2} (cardiopulmonary dysfunction) can usually be detected by comparing values obtained on the chest at the infraclavicular level with those made on the limb site. Values at the limb site significantly lower than the chest suggest limb perfusion as the source of the low $TcPO_2$. A normal level of $TcPO_2$ is about 70% of the arterial O_2 value. A frequent standard measurement site is on the foot dorsum, but the choice of site is dictated by the clinical problem. Various skin properties affect the measured $TcPO_2$, including its composition, thickness, metabolism, capillary density, interstitial constituents, and the presence of edema. The initial $TcPO_2$ measurement combined with the amount of increase in $TcPO_2$ upon breathing 100% oxygen may enhance the accuracy of healing prediction in lower extremity amputations.[3] Limb elevation may also be useful.[4,5]

Clinical Applications

One application of $TcPO_2$ is in the assessment of the adequacy of local tissue perfusion to support lower extremity ulcer healing or as an aid to amputation-level selection.[3-8] Specific levels of $TcPO_2$ thresholds for healing vary from laboratory to laboratory. It is widely agreed that values less than 20 mmHg portend absence of healing in most patients without significant interventions to improve local blood perfusion or oxygenation, though there are numerous reports of healing in patients with very low values of $TcPO_2$.[6] Values $\geq$40 mmHg are positive signs favorable for wound healing and values between 20 mmHg and 40 mmHg are a "gray" area. Resting $TcPO_2$ does not discriminate well between levels of LEAD, and generally only severe disease produces significant reductions under resting conditions[7]; the amount of change in limb $TcPO_2$ with leg elevation improves this discrimination.[8] Recent reviews document further applications in the areas of peripheral vascular disorders.[9-13]

Laser Doppler

Physiological applications of laser Doppler began about 30 years ago.[14,15] Less than 20 years ago it was shown to have promise for the measurement of skin microcirculation.[16] Subsequently, the basic theory and conditions for its use in monitoring skin microcirculation were laid down and refined.[17-22]

Principle

When skin is illuminated with laser light, some of the incident energy is scattered by moving RBC's within the laser beam, and the frequency of the light is shifted in accordance with the Doppler effect. Because the RBC's participating in this process have a distribution of speeds and directions, a spectrum of Doppler shifts occurs, with the spectral bandwidth primarily dependent on the RBC speed distribution. The total power of the Doppler-shifted signal primarily depends on the number of RBC's from which the scattered signals are derived, providing an estimate of the number density of moving RBC's. On the basis of a theoretical model of the process[19] and under certain conditions found clinically, a measure of the time varying component of the scattered signal, $\langle w \rangle$, is related to both mean RBC speed U and the mean number of scattering Doppler scattering events m in a linear fashion by $\langle w \rangle = U \cdot m \cdot k$, where k is a dimensionless constant related to the detector. The value of m can be determined as the ratio of the detected Doppler-shifted power to the total detected power (Pd/Pt). The value of U is then calculable as the ratio $\langle w \rangle / (k \cdot Pd/Pt)$.

Clinical Instrumentation

When data are presented by the instrumentation, they may be expressed either directly in volts, "relative perfusion units," or the actual frequency shift in Hertz. Some instruments incorporate fixed conversion factors based on the levels of blood velocity and volume concentration measured in various tissues with outputs expressed as flow-units in ml/min/100 g. Some instruments provide separate outputs proportional to the velocity signal (U) and/or the m signal; conversion factors may or may not be used. The signal related to m will be referred to as V (for volume) because as already described, m is dependent on the volume concentration of moving RBC's within the sampled tissue volume. A common unit for V as converted would be % (volume of moving RBC/tissue volume)·100; U is usually expressed as mm/sec. In vitro measurements on human skin indicate red light transmission is exponential with an effective penetration of about 0.6 mm,[23,24] though the topic of penetration depth is an ongoing issue and is not yet adequately resolved.[25] However, it is clear that for currently used laser wavelengths and methods the recorded signal depends on events within the nutritional circulation and underlying non-nutritive vessels.

The literature reporting laser Doppler studies is increasing as more investigators begin using this noninvasive modality. However, as with any methodology there are limitations of the technique that can make interpretation of results misleading and otherwise less reliable than perceived. It is important that users of this technology be aware of the potential limitations. The laser Doppler flow parameter, $\langle w \rangle$, may be proportional to volumetric RBC flow, but even if this flow is fixed, different values of $\langle w \rangle$ will be obtained if differences in the RBC pathlength in the laser beam occur. This may be a limitation when laser Doppler methods are used to compare and contrast skin blood perfusion in patient populations in whom the metric and morphological features of the skin vasculature are significantly different one from the other. For example, suppose laser Doppler were being used to evaluate the skin microcirculatory effects of

a drug therapy in patients with intermittent claudication. Suppose further that the only effect of this treatment was on small vessel properties causing increased tortuosity of the microvessels within the sampled volume without affecting either the RBC speed or number of moving RBC's. Laser Doppler measurements before and after treatment would indicate an increase in ⟨w⟩, but it would be erroneous to interpret this as a flow increase. As a consequence of this discrepancy between true volumetric RBC flow and ⟨w⟩, the latter has been referred to as blood perfusion and flux. Although LDF perfusion and flow have been shown to have a high degree of correlation in many studies,[26-29] the potential hazard is evident if measurements are made on the same network at different times with length changes occurring with time, or if measurements are made on networks differing in vessel length.

Most laser Doppler manufacturers also provide a well-controlled heating module for thermal provocation use. The fiber optic cable that houses the transmitting and receiving fiber bundles is placed concentrically within the heating module and the combined probe-heater assembly is affixed to the skin with double-sided tape. In our laboratory we choose to obtain a resting Q with the heating module set to 35°C and then rapidly increase the temperature to 45°C. The preheating serves to provide a standardized local skin temperature for comparative purposes. Under most conditions it is best to evaluate both resting Q and the responses from the preheated value.

Variability in the magnitude of the measured signal is to be expected due to normal variations in the underlying vasculature and flow conditions that may be present at adjacent skin sites[30-33] and are present in different anatomical sites (e.g., toe pulp vs. foot dorsum). In the present author's experience variability at a given anatomical site is predominantly related to variations in V secondary to differing microvessel densities between nearby sites, whereas the RBC velocity is less variable. Changes due to external pressure[34] and room temperature can affect the underlying perfusion as can external stimuli of various types,[35] including changes in the patient's state of arousal. Testing is best done in a controlled environment.

A physiological temporal variability may also be present at a fixed measuring site, characterized by a semirhythmic waxing and waning of the measured perfusion. The primary source is likely spontaneous changes in arteriolar diameter; though the physiological function of this process has not been resolved. Some of the hemodynamic implications have been analyzed,[36,37] and differential features characteristic of various disease processes are at present being studied.[38,39]

Under certain circumstances the presence of a non-zero LDF signal has been noted when suprasystolic cuff pressures should have reduced flow to zero at the measured site. This non-zero value has been called the biological zero (BZ).[40,41] The source of the BZ is unclear but may in part be caused by cellular movements between microvascular regions; further work on this issue is necessary.

Clinical Applications of Laser Doppler Methods

The clinical utility of laser Doppler measurements has potential in conditions in which the status of the limb microvasculature is the crucial element. For example no skin ulcer will heal unless there is an adequate blood supply in the region of the ulcer to support that healing; this requires sufficient macrovascular flow to the general territory of the ulcer and also adequate flow to, and within, the periulcer microvasculature. The microvascular component can be assessed with laser Doppler. The practical advantages of LDF are that the method is noninvasive, it is quantitative and relatively free of operator bias or expertise, the equipment occupies relatively small space and is portable, most test procedures require relatively little time to perform and are not difficult to learn, and the procedure cost is relatively low.

Most clinical applications rely on some form of vascular provocation, which causes a change in blood perfusion at the measuring site. The response to this perturbation is then measured and the data used to make statements with regard to the status of the patient's blood circulation at the site of interest. Because skin perfusion is dependent on both environmental and local skin temperature,[42] gender,[43,44] age,[45,46] and anatomical site, due consideration of these factors should be included in any test result interpretation.

Response to Local Heating

One useful provocation is local skin heating.[47] The peak response to local heating has been used to discriminate between normal subjects and claudication patients with ankle–brachial index (ABI) ratios ≤ 0.5,[42] and clear differences in thermal responses have been reported between normals, claudicants, and rest pain patients.[48] Thermal provocation has also been useful in the study of diabetic neuropathy[49] and amputation-level screening.[50,51] Figure 1 illustrates a typical response for U, V, and Q as measured on foot dorsum. In practice, recording should not start until the resting value has stabilized, which typically occurs in about 2–3 min. The LDF data are recorded at the stabilized preheated value (35°C in Figure 1) for 2 min; heating to the upper temperature value is then initiated and maintained for 5 min. Increases in each parameter should be observed as shown in Figure 1. We routinely record the following data for each of the response variables Q, V, and U.

X_{35} = average 35° value measured over 2 min
X_{max} = maximum 45° value
X_{45} = average 45° value measured ± 1 min around X_{max}
T20 = time (sec) to reach 20% of the maximum response

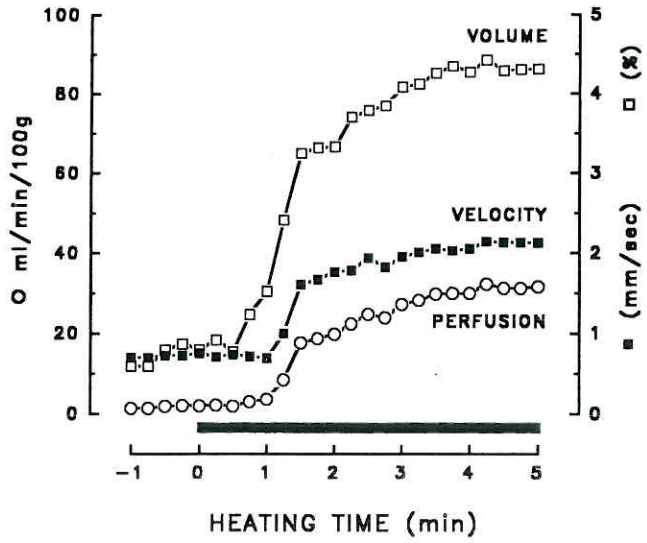

Figure 1

LDF response at foot dorsum of a normal subject. Data points are 15-sec averages.

T80 = time (sec) to reach 80% of the maximum response

X_{rate} = rate of response (X units/min) between 20% and 80% limits

X_{resv} = microvascular reserve (%) calculated as $(1 - [X35/X45]) \cdot 100$

$X_{ratio} = X45/X35$

Table I lists some of the values we have obtained from the foot dorsum in 104 consecutive evaluations (206 legs). The data were obtained after at least 10 min of supine rest. In this table the values are stratified with respect to an assessment of the limb vasculature being normal (NORM) or having evidence of LEAD by noninvasive testing. Figure 2 graphically illustrates the response differences in a patient with unilateral LEAD.

For a number of reasons use of resting perfusion as a discriminating variable has not been widely reported.[52-55] As pointed out previously the LDF measurement includes contributions from both the nutritional and subpapillary vessels. In an attempt to obtain more selective data on the nutritional perfusion, we have used a modified technique in which a spacer is placed between the laser probe and skin.[56] The spacer is made of material that has optical properties similar to skin. Preliminary data obtained from 37 patients (58 legs) are summarized in Table II. It may be noted that for these measurements made without heating or other provocations, significant differences are demonstrated for nutritional components but not for values obtained using the standard method (probe directly on skin). Use of the modified method may enhance the use of resting perfusion levels in several areas of clinical application, but this remains to be tested.

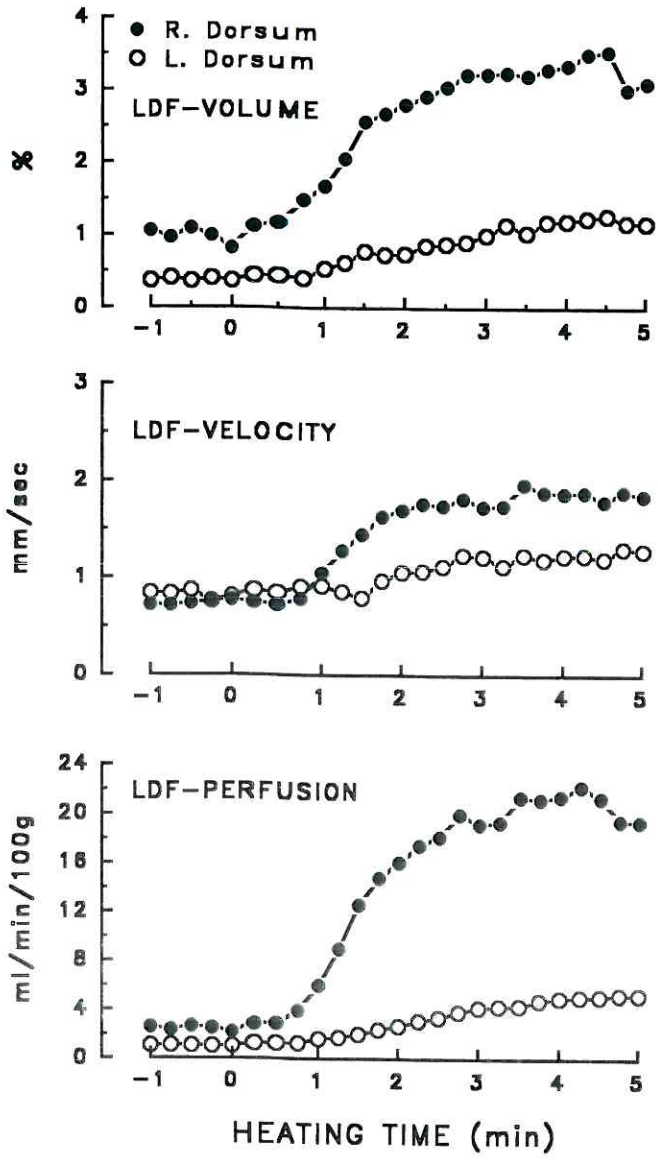

Figure 2

LDF response of a patient with intermittent claudication in the left leg only.

Skin Postocclusive Hyperemic Response (SPOHR)

This procedure is designed to ascertain the ability of the skin microvasculature to compensate for and respond to a standard interval of nutritive flow deprivation induced by an interval of suprasystolic limb compression with an appropriate cuff. The site can be the thigh, calf, or ankle and the duration from 2 to 5 min. Both the magnitude of the peak postocclusive response and the temporal pattern of the response have been used as measures of the prevailing microvascular status and may serve as useful indicators of wound healing likelihood in patients with skin ulcers[57] and characterize other limb vascular features.[58] A supramalleolar, suprasystolic occlusion of only 2 min with skin at 36°C is reported to have permitted correct retrospective classification of LEAD disease stage in 73% of patients based on the time

Table I

*Foot Dorsum LDF Parameters**

	U		V		Q	
	NORM	LEAD	NORM	LEAD	NORM	LEAD
X_{35}	0.80 (0.21)	0.89[b] (0.30)	1.36 (0.62)	1.56 (0.82)	3.8 (2.3)	4.6[a] (3.0)
X_{45}	1.83 (0.60)	1.66 (0.87)	4.33 (1.12)	3.91[a] (1.29)	27.9 (13.9)	20.8[c] (11.8)
X_{rate}	0.76 (0.61)	0.51[b] (0.61)	1.55 (1.25)	1.63 (3.80)	10.9 (6.8)	7.0[c] (4.4)
X_{resv}	53.3 (16.0)	40.8[c] (21.7)	67.4 (14.1)	59.9[c] (16.6)	84.7 (10.2)	75.1[c] (16.8)
X_{ratio}	2.4 (0.9)	2.0[b] (1.3)	3.74 (1.76)	2.92[c] (1.25)	9.2 (6.0)	5.7[c] (4.2)

* Values are mean and (SD). NORM: $n = 123$ legs; LEAD: $n = 83$ legs.

[a] $p < 0.05$ vs. NORM, [b] $p < 0.01$ vs. NORM, [c] $p < 0.001$ vs. NORM. Subscripts 35 and 45 refer to skin temperature at which measurements were made.

required for the reperfusion to reach preocclusion values.[59] Total leg occlusion at the thigh level reveals similar findings,[60] but this procedure would be contraindicated in many patients, such as those with an infrainguinal bypass graft.

Veno-Arteriolar Response (VAR)

This procedure is designed to ascertain the ability of the arteriolar microvasculature to compensate for and respond to a standardized postural-induced increase in lower extremity hydrostatic pressure. Movement from a supine to a standing position provokes this response as does placing the foot in a dependent position.[61] A normal response is characterized by a rapid and sustained arteriolar vasoconstriction related mainly to local neurogenic reflexes and small central effect. This serves to buffer the capillary network from the increased pressure load. Normally the total skin perfusion response is characterized by a parallel reduction in LDF perfusion. Abnormalities in this response have been noted in patients with diabetes in whom the vasoconstrictive component has been shown to be reduced or absent.[62] The amount of this deficit has been linked to skin ulcer prevalence and healing likelihood. In patients with diabetes, the VAR is significantly depressed and even worse in patients with superimposed peripheral neuropathy.[62] Other aspects of reflex vasoconstrictive responses in neuropathic patients have been demonstrated.[63]

Diabetes

As discussed above a significant application concerns the microcirculation in patients with diabetes, with or without complications of peripheral neuropathy and/or skin ulcerations. Because of the complexity of the skin nutritional and thermoregulatory

Table II

*Foot Dorsum LDF Values with and without Spacer**

	U (mm/sec)		V (%)		Q(ml/min/100 g)	
	NORM	LEAD	NORM	LEAD	NORM	LEAD
Spacer	1.06 (0.29)	1.02 (0.34)	0.08 (0.04)	0.05[b] (0.04)	0.32 (0.22)	0.20[a] (0.15)
Standard	0.92 (0.25)	0.91 (0.27)	0.66 (0.37)	0.56 (0.33)	2.13 (1.55)	1.62 (0.88)

* Values are mean and (SD). NORM: $n = 27$ legs; LEAD: $n = 31$ legs.

[a] $p < 0.05$ vs. NORM, [b] $p < 0.01$ vs. NORM. All measurements on unheated skin.

circulation, changes in any of several components that occur in diabetes may impact on measurable microcirculatory function. These include the composite structural and functional integrity of (1) the skin microvessels, (2) the vessels that supply and drain the skin microvasculature, (3) the neural, hormonal, and metabolic control systems regulating the microvessels and the blood flow through them, and (4) the rheological properties of the blood flowing in the microvessels. Data obtained using LDF have revealed certain quantitative perfusion differences between diabetic patients and control subjects.[64] Resting foot perfusion levels appear to correlate inversely with blood viscosity,[65] and controlled localized skin heating suggests a deficit in the vasodilatory response.[64,66]

Conclusion

Critical nutritional blood perfusion at the microvascular level depends on the number of patent capillaries available for flow and the speed with which the RBC's move through these capillaries. Given a set of potentially available capillaries, the number that are actively perfused with blood depends on the integrity of the nutrient capillaries, the extent of arteriolar vasodilation, and on conditions in more proximal arterial vessels and distal venular network. A reduction in nutritional perfusion with its associated impending pathological sequelae may occur with normal vasodilatory function, but a reduced number of available capillaries may be due to an encumbrance of the movement of erythrocytes through a normal number of available capillaries or may be caused by deficits in oxygen movement within the dependent tissue. Contrastingly, a normal set of capillaries may be present but because of a deficit in macrovascular inflow capacity or diminished outflow, a reduced perfusion may occur. The selective and appropriate use of laser Doppler and $TcPo_2$ methods, usually in combination with traditional macrovascular assessment testing, can provide the necessary data to help clarify these issues on a patient-by-patient basis.

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