16
Assessment of Human Microvascular Function

HARVEY N. MAYROVITZ

Introduction

Microvascular impairments impact on cardiovascular disease processes and complications and intensified efforts to deal with these at the clinical level are ongoing. Targets include diabetes, hypertension, peripheral vascular and cardiac disease, and complications including chronic skin ulcer development and healing potential. Goals include improved understanding of functional microvascular deficits and test development to aid in diagnosis, prognosis, and treatment. Central to the general issue of human microvascular assessment is the concept of the particular function one is attempting to determine. The set of blood vessels, cells, and perivascular environment that broadly represents the diffuse boundary of what is termed the microcirculation has multiple functions, many of which are likely unknown to us even now. Classically, however, the microcirculation has been viewed in a narrower sense: being associated with the movement of cells, fluids, and other materials to maintain an adequate functional environment for the many tissues it subserves. Thus, the notion of adequacy of microvascular function is tightly coupled to its ability to maintain dependent tissue functions in accordance with their needs. Changes or derangements in one or more of the native microvascular elements can induce or facilitate tissue dysfunction. Though molecular details of specific microvascular elements involved are difficult to evaluate in humans, old and new microvascular assessment methods provide much information, mostly based on skin microvasculature assessments. Targets of these may broadly be placed into three categories: mechanistic discovery, diagnostic aids, and treatment evaluations. In practice these areas often overlap, and a discussion of the topic of human microvascular assessments is probably best done on the basis of uses in some specific cardiovascular conditions. This approach is adopted in this chapter and will follow a description of pertinent background material. Space limitations prevent citation of all relevant work, but the references will aid in obtaining needed details.

Cutaneous Circulation

Since most microvascular assessments target skin, a summary of relevant features is useful. Skin epidermis is avascular stratified epithelium between 75 and 150 µm thick, except on palms and soles (400 to 600 µm thick). Dermal thickness varies widely; about 4 mm on the back, 2.5 mm on the thigh, and 1.25 mm on the palm. Dermal vasculature is complexly arranged with multiple interconnections and blood flow pathways running vertically toward the surface as well as horizontally in different layers of vessel plexuses. Penetrating small arteries arise from subcutaneous tissue, deeper muscle (musculocutaneous perforators), and from still deeper tissue (fasciocutaneous perforators). Penetrating vessels form deep dermal arteriolar arcades (reticular arteriolar plexus), which give rise to arterioles to supply skin appendages and also ascend in a candelabralike branching pattern to eventually form nutritional capillaries. Also emanating from the deep arteriolar plexus are arterioles that spread to form a subpapillary arteriolar plexus lying just below the epidermis, which also give rise to capillary loops to supply the epidermis (20). Capillary density estimates from superficial skin are low compared with most tissues, ranging from 10 to 70 capillaries per mm^2 with each capillary loop supplying an area from 0.04 to 0.27 mm^2. On the basis of one loop/papillae, a near maximum density of 60 to 70/mm^2 is expected. At most skin sites, the capillary loops when visualized with microscopy appear as dots or truncated vessel segments because only the tip or apex of the ascending loops can be well seen. However, at the base of the nail, the hairpinlike shape of the loops can be seen since the vessels lie in a longitudinal plane parallel to the dorsal surface. These have a narrow arteriolar portion, which progresses to a wider descending venular segment following a hairpinlike turn at the apex; the loops show a wide range in density, from 20 to 150 loops per mm^2. Lumen diameters (forearm skin) are 5 to 7.5 µm in the arteriolar portion and 6 to 10 µm in the descending venular portion and have lengths from 150 to 300 µm (19). Blood from the capillary loops and other areas drains into a series of dermal venous plexuses, which mostly lie parallel to the surface at various depths. The deepest plexus lies near the dermal-subcutaneous tissue margin.

Methodological Overview

Noninvasive assessments of human microcirculation may be conveniently categorized into two broad types: those relying on visualized vessels and those that do not. Early methods were visually based and were restricted to anatomical sites in which blood vessels or circulating cells could be microscopically seen. Such sites include the bulbar conjunctivae, lip, tongue, and the nail fold area of fingers and toes. With patience and undaunting

perseverance, early workers were able to amass a large body of qualitative and semiquantitative data characterizing many important features of human microcirculation. Sometimes this information was gathered using tools as simple as a stopwatch to measure transit times of red cells moving through nail fold capillaries or conjunctiva venules. The first use of the nail fold for microscopic study occurred 85 years ago (45). Such studies gave the earliest estimates of blood cell velocity in human microvessels and provided early indications of the dynamic, intermittent nature of capillary blood flow (18).

Extensions of visual methods followed development of apparatus to measure more precisely microcirculatory parameters. These still relied on visualized vessels or cells but used instrumentation that served as the observer's eye and replaced certain manual measurement procedures. Introduction of combined microscopy and closed circuit television allowed vessel images and cellular movements within them to be recorded for subsequent dynamic and image analyses (14,30). Recorded signals and images were analyzed with methods already in use for animal research. Refinements and automation were introduced to facilitate assessments of human microcirculation (13,41). Dynamic analyses of cellular movements, including red blood cell velocity and flux, were still done one vessel at a time and were restricted to tissue regions where vessel visualization with adequate resolution was possible. Introduction of laser-Doppler (L-D) and transcutaneous oxygen ($TcPo_2$) measurement techniques eliminated these requirements and provided important complementary methods for assessing human microcirculation. L-D permits assessment of local skin blood perfusion, whereas $TcPo_2$ measurements assess local oxygen status. In both methods, the measured quantities are dependent on the composite of many vessels and are nonlinearly related.

Several other methods for assessing features of human microcirculation with "minimally" invasive techniques are available. Microvessel blood pressure measurement, long used (44) and many years ago applied to human nail fold capillaries (78) were refined and applied for more precise capillary pressure measurements in humans (46,49,79). Nail fold capillary measurements show a pressure gradient along the capillary loop ranging from 37.7 ± 3.7 mmHg in the arteriolar limb, 19.4 ± 1.0 mmHg in the apex, and 14.6 ± 0.5 mmHg in the venous limb (95). Capillary temporal pressure variations suggest intervals of both filtration and absorption throughout the capillary. Reconstruction of the capillary pulse pressure shows a pattern similar to the radial pulse but of a much smaller amplitude 3.6 ± 3.4 mmHg. New innovations (33) now allow for simultaneous measurements of nail fold capillary blood velocity and pressure. Enhancement of blood and lymphatic vessel microscopic images and quantitative transcapillary fluid and cellular assessments have been developed using intravital fluorescence. Application of these "minimally" invasive techniques for assessing human microcirculation are well described in the literature (16).

Clinical goals are to optimize the use of the various methods either singly or in combination to (1) provide indirect information on the generalized microvasculature status of a 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 diagnostic or treatment outcome assessments. Because most assessments are applied to skin, it is important to recognize that critical aspects of nutritional (capillary loop) blood perfusion depend on both the number of patent capillaries available for flow and the speed and number of red blood cells moving through these capillaries. The only direct measurement of this is quantitative capillaroscopy, which as has been noted is limited to nail fold capillaries. Assessments by L-D and $TcPo_2$ reflect events in nutritional capillaries and other microvessels within the measured sample volume. Absence of specificity with the latter methods is partially offset by the fact that significant information is contained in the composite and integrated microvascular responses reflected in parameters they measure. However, oppositely directed changes in capillary blood velocity and L-D perfusion may occur (45).

Capillary perfusion depends on nutrient capillary integrity and also on functional aspects of arteriolar vasodilation and conditions in proximal arterial vessels and distal venular network. Reduced nutritional perfusion with its associated impending pathological sequelae may occur with normal vasodilatory function but a reduced number of available capillaries and may be caused by an encumbrance of the movement of erythrocytes through a normal number of available capillaries or by deficits in oxygen movement within the dependent tissue. Contrastingly, a normal set of capillaries may be present, but because of a deficit in microvascular inflow capacity, diminished outflow, or deranged proximal microvascular control, a reduced perfusion may occur. Selective and appropriate use of laser-Doppler, $TcPo_2$, capillaroscopy, and related methods, usually in combination with traditional macrovascular tests, is the best strategy to provide the necessary data to help clarify microcirculatory function on a patient-by-patient basis.

Transcutaneous Oxygen Tension

Red blood cells (RBCs) moving through capillaries carry and subsequently release oxygen (O_2) to supply the O_2 needs of the tissue cells. Released O_2 diffuses through vascular walls 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 $TcPo_2$ measurements and is related to the O_2 concentration and solubility in the region of the measurement. Normally, skin oxygen consumption is low and O_2 delivery is physiologically regulated to match, but not significantly

exceed the O_2 demand. Within this volume, the net RBC blood flow, and hence the rate of O_2 delivery, depends on the product of the number of capillaries that are actively perfused with flowing RBCs and the speed with which the RBCs move through the capillaries. If heat is used to increase blood flow, then a mismatch between O_2 delivered and consumed results; a $TcPo_2$ measurement would record increased values proportional to the intravascular Po_2. However, if the same skin-heating maneuver were done in a patient with a compromised circulation, then lower $TcPo_2$ values would generally be recorded. The reduction depends on many factors. At one extreme is a patient with severe lower extremity arterial disease in whom arterioles are near maximally dilated under nonheated conditions. Heating causes less vasodilation and less change in the O_2 delivery. Lower $TcPo_2$ values thus reflect reduced microvascular reserve.

Most devices measure $TcPo_2$ by a polarographic technique with a platinum-sensing electrode and a silver/silver chloride reference electrode. Both electrodes are immersed in electrolyte and covered by a thin membrane. Oxygen diffuses through the membrane and, at the cathode, a chemical reaction occurs resulting in the reduction of the oxygen and a generation of electrical current, which forms the basis of the $TcPo_2$ measurement. At normal temperatures, the stratum corneum of the skin has a high diffusion resistance and thereby restricts the current passing across the epidermis to the $TcPo_2$-sensing electrode. Local skin heating to above 40°C causes a reversible structural change with a large increase in diffusivity. At a fixed temperature, measured $TcPo_2$ depends on arterial Po_2 and the nutritional skin blood flow in the region of the measurement. Further technical and mathematical details may be found in the literature (48). Various skin properties affect the measured $TcPo_2$ including skin composition, thickness, metabolism, capillary density, interstitial constituents, and edema. Basal $TcPo_2$ measurements combined with assessments of changes on breathing 100% oxygen or limb position changes have shown utility in assessing microcirculatory adequacy when prediction of healing of wounds and amputations are needed (5).

Laser Doppler

Laser-Doppler (L-D) showed promise for skin microcirculation measurement about 20 years ago (86) and has been progressively refined (18,71). If skin is illuminated with laser light, some incident energy is scattered by moving RBCs, and the light frequency is shifted in accordance with the Doppler effect. Because speeds and directions of RBCs involved in this process have a statistical distribution, a spectrum of Doppler shifts occurs with a spectral bandwidth mainly dependent on the RBC speed distribution. Total power of the Doppler shifted signal mainly depends on the number of RBCs from which scattered signals are derived and provides an

estimate of the number density of moving RBCs. Based on a theoretical model of the process (17), 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 Doppler scattering events, m, in a linear fashion by $\langle w \rangle = Umk$, with k a dimensionless detector constant. The ratio of detected Doppler shifted power to total detected power (Pd/Pt) determines m and the ratio

$$\frac{\langle w \rangle}{k * \frac{Pd}{Pt}}$$

determines U. Continuous data from small areas are made with a probe on the skin, but larger skin areas are measurable via noncontact laserDoppler imaging (51,92).

L-D data are expressed in volts or arbitrary perfusion units. Conversion factors from RBC velocity and volume concentrations in tissues are sometimes used to express perfusion in flow units such as mL/min per 100g. This can be misleading since flow calibrations with L-D are not available. Besides the composite perfusion signal (symbol Q in this chapter), some instruments provide separate signals proportional to the velocity signal (U) and/or the m signal; conversion factors may or may not be used. Signals related to m are here referred to as V (for volume signals) since m is dependent on the volume concentration of moving RBCs within a sampled tissue volume. A common unit for V as converted would be percent (volume of moving RBCs per tissue volume)*100. L-D signals depend on events within the nutritional circulation and underlying nonnutritive vessels in most skin areas. The L-D "flow" parameter, $\langle w \rangle$ may be proportional to volumetric RBC flow, but even if this flow is fixed, different values of $\langle w \rangle$ may be obtained if differences in RBC path length in the laser beam occur. This may be a limitation when L-D is used to compare skin blood perfusion in patient populations in whom the metric or morphological features of the skin vasculature are significantly different from one another. Because of this discrepancy between true volumetric RBC flow and $\langle w \rangle$, the latter has been referred to as blood perfusion. Though L-D perfusion and blood flow show a high degree of correlation in many studies, potential hazards of assuming a flow relationship are evident. Further details may be found in several monographs (10,11).

Variability arising from normal variations in underlying vasculature and flow at adjacent skin sites (52,88) and between different anatomical sites (e.g., toe pulp vs. foot dorsum vs. forearm vs. finger) needs to be taken into account. Site variations are often caused by variations in the L-D volume component because of differing microvessel densities; the L-D velocity component is less variable. Changes caused by external pressure affect perfusion (53) as do external stimuli of various types (54). A nonzero L-D perfusion signal may be present even when suprasystolic cuff pressures are applied; the source of this "biological zero" (BZ) (1) is unclear but it may be caused by cellular movements between microvascular regions. If

possible, the BZ should be taken into account. Skin perfusion is dependent on environmental and local skin temperature, gender (26,55), age and anatomical site, and due consideration of these factors should be included in test interpretations.

Flowmotion (Figure 16.1) is a physiological rhythmic perfusion change sometimes present. The primary source is likely spontaneous changes in arteriolar diameter, but its function has not been resolved. Some of the hemodynamic implications have been analyzed (4,56), and differential features characteristic of various disease processes are being studied (35,81). Early workers described rhythmic vascular phenomena in digits, only some of which were synchronous with heart and respiratory rhythms. These changes, originally detected as digit volume changes, appeared to be enhanced during sleep, suggesting they were not related to external stimuli, but were indeed spontaneous. Intense vasoconstriction or maximal vasodilation produced by heating eliminates this phenomena. A role of the venous system has been inferred since elevation of venous pressure to 60 mmHg eliminates the rhythmic volume changes. Recent research (12) provides spectral analysis evidence of a strong neural control aspect for some dynamics. Quantitative analyses of associated rhythmic diameter changes in both arterioles and venules showed a direct effect on capillary perfusion and transcapillary exchange leading to intervals of either total filtration or absorption (56). Subsequent assessment and analysis of L-D measured rhythmic perfusion changes in humans showed pattern variations and phase differences among the L-D components (57). Analytical models were used to explain these variations in terms of proximal and distal microvascular conditions. Other studies have focused on possibilities to use these dynamics in clinical assessments (15,35,81,83).

Microvascular Provocations

Most microvascular assessments rely on provocations that change blood perfusion or cell velocity. Responses are recorded, and analysis of parameters from sites of interest are used to make statements about the patient's microcirculatory function. Provocations are chosen to elicit either vasodilatory or vasoconstrictive responses, which may be reflexly or directly induced. Vasoconstrictive provocations used include mental stress, contralateral limb cooling, isometric hand grip, inspiratory gasp, postural shifts,

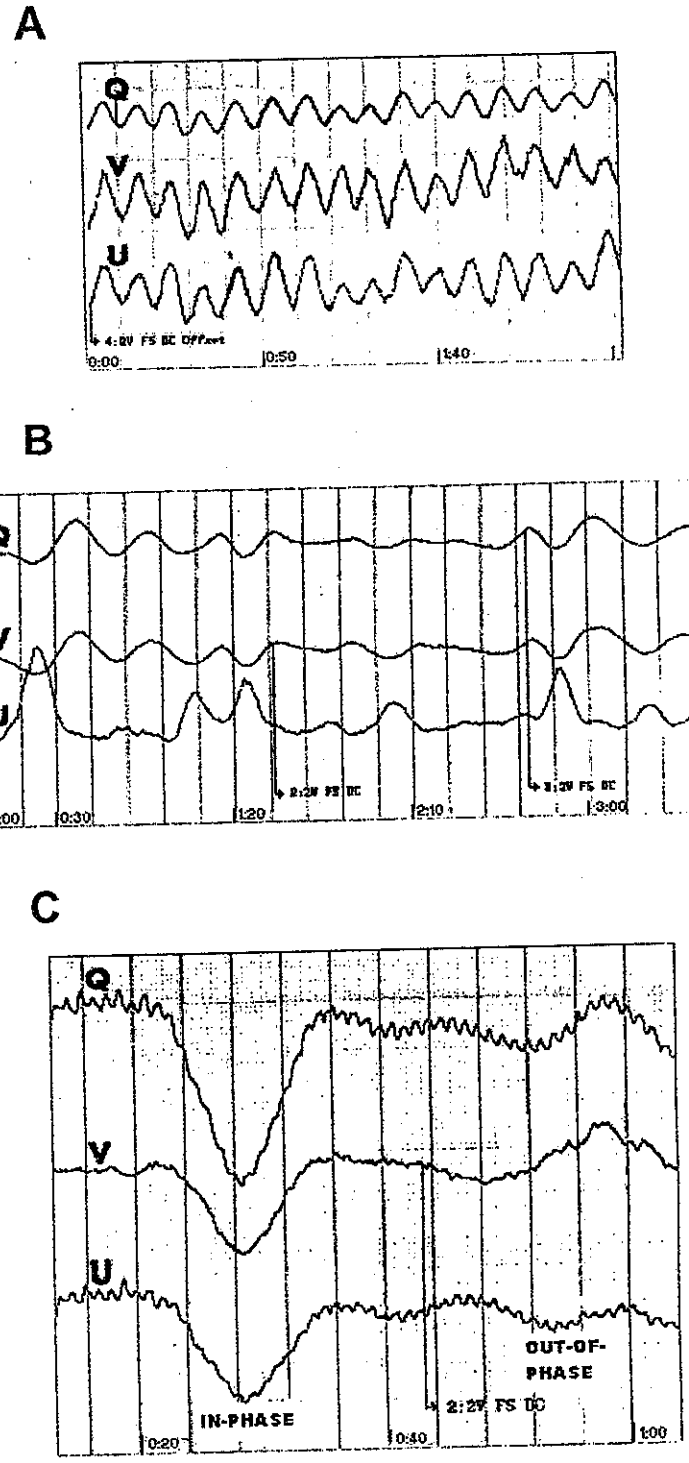

FIGURE 16.1. Flowmotion dynamics recorded by laser-doppler. *Q*, *V*, and *U* are laser-Doppler perfusion, volume, and velocity signals respectively. In (*A*), all components are in phase. In (*B*), *Q* and *V* signal patterns are nearly identical, but *U* is fully out-of-phase with both. In (*C*), a large decrease in all components is followed by a smaller wave-set with an Mixed-phase change in velocity. The high-frequency wave is synchronous with the heartbeat with an amplitude that decreases during the vasoconstrictive phase of flowmotion. Large vertical time lines are 10 s apart in *A* and *B* and 4 s apart in *C*.

and vasoconstrictive topicals. Vasodilatory provocations include local heating and postocclusion hyperemia.

Thermal Provocations (Figure 16.2)

Skin blood flow is sensitive to ambient, internal, and local skin temperature. Heating increases and cooling decreases flow by neural and local vasoactivation. This is exploited by assessments using local temperature changes to test microcirculatory responses. Most L-D systems have a heating module. Typically, a fiber optic cable that houses the transmitting and receiving fibers is placed concentrically within the heating module and the combined probe-heater assembly is affixed to skin with tape. A resting baseline blood perfusion, here denoted as Q, is obtained and responses to rapid or stepped heating determined. Preheating provides a standardized local skin temperature for comparative purposes, but its value alone is

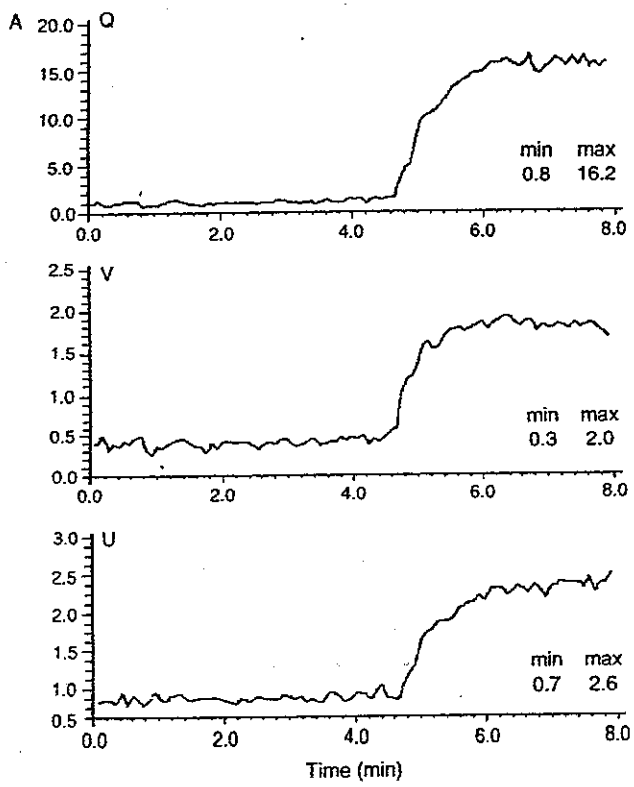

FIGURE 16.2. Thermal provocation responses. Responses to rapid heating to 42°C after a 4-min baseline recording. (*A*) Normal large and rapid response of all laser-Doppler components. (*B*) Impaired response with significantly reduced response peak, rate, and MVR. Note the pronounced flowmotion in the baseline velocity (*U*) signal and its decrease during the 4-min heating interval. L-D values in arbitrary units.

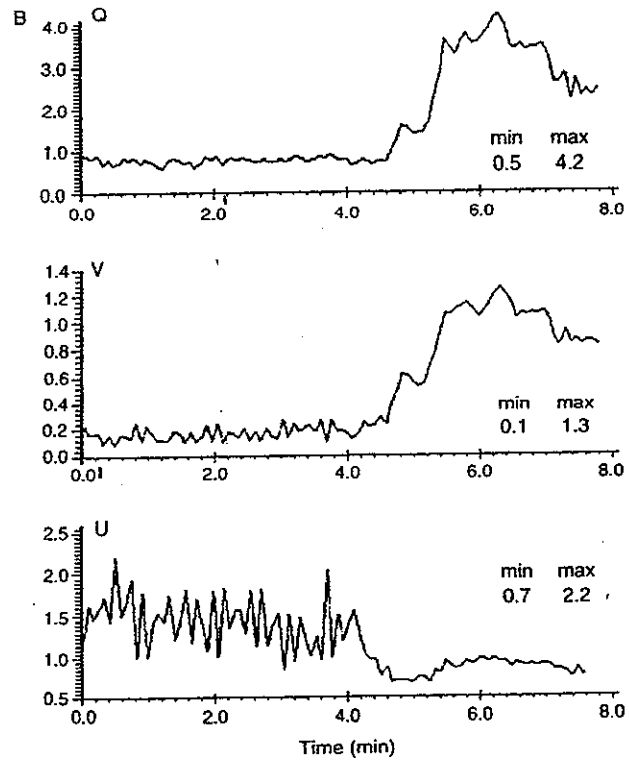

FIGURE 16.2. *Continued.*

limited. It is best to also evaluate some feature of the responses (denoted as QQ). Recording starts after stabilization from probe placement, which takes 1 to 2 min. Data are recorded at a stable baseline, or preheated value for 2 to 4 min; heating to the upper temperature is initiated and maintained for 4 to 6 min. Increases as seen in the figure are typically observed. Several quantitative parameters characterizing thermal (and other) provocation responses are used. These include peak responses, average response centered around the peak response (QQ), rates of increase, ratios (QQ/Q) and various time delays between provocation initiation and response. A useful parameter called microvascular reserve (MVR) is calculated as $[1 - (Q/QQ)] \times 100$.

Hyperemic Provocations (Figure 16.3C)

These are used to ascertain the ability of skin microvasculature to compensate for and respond to standard intervals of flow deprivation induced by suprasystolic limb compression. Compression sites include thigh, calf, ankle, arm, hand, or finger with durations from 0.5 to 5 min. Peak postocclusive responses and temporal patterns of the response are used as measures of microvascular status, vasodilatory reserve capacity, and as

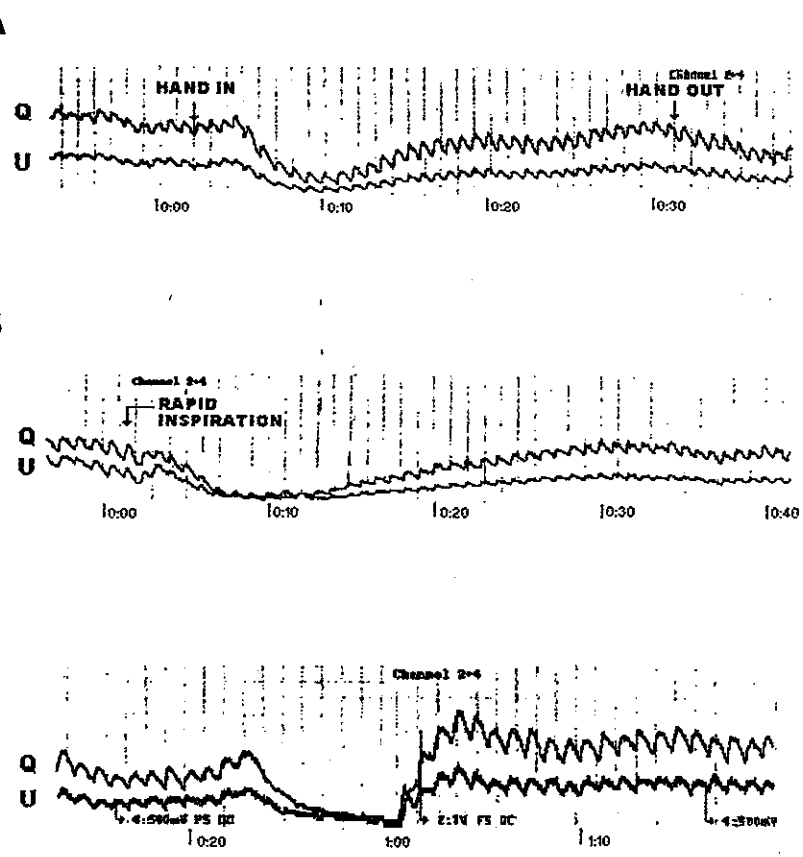

FIGURE 16.3. Other provocation responses. Laser-Doppler finger perfusion (Q) and velocity (U) decrease in response to contralateral hand cooling (ice water) as shown in (A) and because of a rapid and sustained inspiration as shown in (B). In both cases, the provocation was for 30s, but the response is seen to be transient and to normalize while the stimulus is still applied. In (C), the foot dorsum response to a 40-s suprasystolic ankle compression is illustrated. Large vertical time lines are 1 s apart. Small pulsations are synchronous with the heartbeat.

indicators of wound-healing likelihood in patients with skin ulcers and to characterize limb vascular features. A supramalleolar occlusion of 2 min is reported to permit correct retrospective classification of vascular disease stage in 73% of patients (75).

Postural Provocations (Figure 16.4)

Assessments of this type use gravity as a noninvasive way of inducing changes in intravascular blood volume, flow, and pressure distribution to

invoke nervous system responses linked to vasomotor control. These are induced by whole body postural shifts (e.g., supine to standing) or by limb positional changes (e.g., passive elevation of the arm or leg). Shifting the lower extremities to a dependent position stresses, and thereby tests, the ability of the microvasculature to compensate for increased hydrostatic pressure. Movement from supine to standing provokes this response as does placing a body part in a dependent position (47). Normal responses are characterized by rapid and sustained arteriolar vasoconstriction caused mainly by local myogenic and neurogenic reflexes that act to buffer the capillary network from increased pressure load. Responses monitored with L-D show large decreases in perfusion in vascularly normal individuals, and the physiological response is sustained and associated with a measurable limb volume change (58). Abnormalities in several conditions have been shown (e.g., diabetes, venous disease), prompting the use of L-D as a microcirculatory assessment tool to detect early events and to assess disease progression. In some individuals, inadequate perfusion reduction has also been linked to increased skin ulcer prevalence and suppressed healing

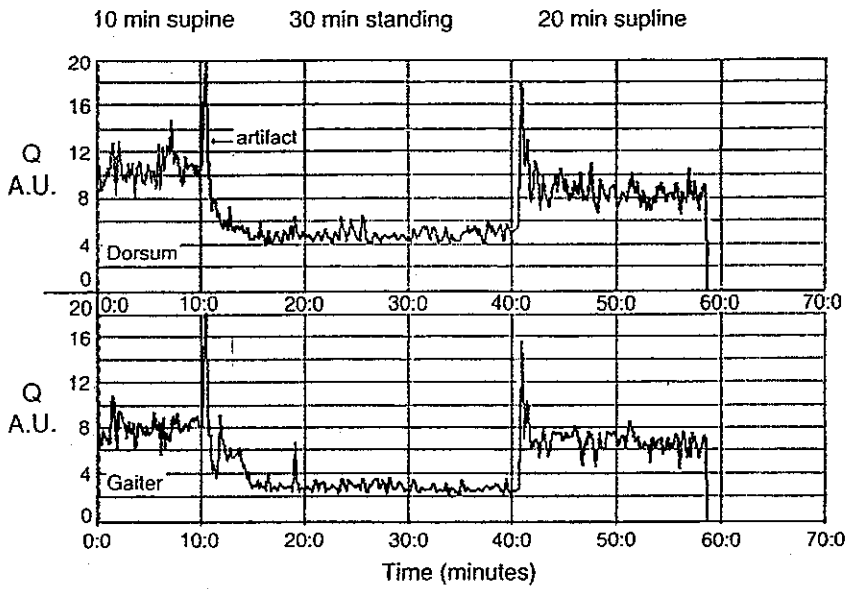

FIGURE 16.4. Postural provocation vasoconstrictive response. Simultaneous laser-Doppler perfusion recordings (Q) on foot dorsum and gaiter area. Patient is supine for 10 min, stands for 30 min, and returns to supine for 20 min. Dramatic decreases in perfusion on standing in this normal individual are noted. This response is not transient as it is maintained over the full 30-min provocation interval. Large transient increases during position change are movement artifacts. Perfusion values in arbitrary units.

likelihood. In diabetic patients, the response is depressed and worse in patients with superimposed peripheral neuropathy. The response magnitude has been calculated in different ways. One is the "venoarteriolar response" (VAR) (6), calculated as $100\times(Q-QQ)/Q$, where Q and QQ correspond to the baseline (supine) and posturally stressed (standing) perfusion values. Expressed in this way larger values of VAR represent normal. Alternately, the ratio QQ to Q expressed as a percentage is used, and smaller values represent normal.

Other Provocations

Assessment of microcirculatory responses to topically applied agents is old but useful for separating functional components of microcirculatory responses. Rubefacients such as nicotinic esters and mustard oil produce erythema, hyperemia, and usually local hyperthermia. Refinements in transdermal delivery of vasoactive substances using iontophoresis show promise. Iontophoresis of endothelial cell dependent and independent vasodilatory and vasoconstrictive substances allows for a more selective study and assessment of microcirculatory function (69). Respiratory provocations (Figure 16.3B) such as rapid deep inspiration (inspiratory gasp) are associated with a rapid but transient decrease in peripheral blood perfusion triggered by a neurogenic stretch reflex. Many other provocation stimuli have been studied, including mental stress, contralateral limb cooling (Figure 16.3A), noise, and skin trauma (43).

Diabetes

Microvascular Functional Involvements

Many assessments focus on patients with diabetes, with or without complications of peripheral arterial disease, peripheral neuropathy, and/or skin ulcerations. Because of the complexity of the skin nutritional and thermoregulatory circulation, changes in any of several components that occur in diabetes may impact on microcirculatory function. These include the composite structural and functional integrity of skin microvessels; vessels that supply and drain the microvasculature; neural, hormonal, and metabolic control systems regulating the microvessels and the blood flow through them; and the rheological properties of the blood. Assessments reveal quantitative perfusion differences between diabetic and control subjects (76), and provocation responses suggest deficits in vasodilatory function. Thermal provocations show reduced MVR (59), and postural provocations show reduced vasoconstrictor responses (6).

Early Detection of Functional Changes

Nail fold capillary changes including increased microaneurysms are seen in diabetic children (89). Peak responses to foot thermal provocations in diabetic children, free of detectible complications, are already functionally impaired (84), and postural vasoconstriction deficits precede overt diabetic complications in postpubertal children with insulin-dependent diabetes (85).

Assessment of Disease Progression

Responses to postural provocations in diabetic patients with and without peripheral neuropathy show progression of deficits that correlate with capillary filtration (7). This supports the concept that functional deficits are involved in capillary structural changes in diabetic lower extremities. Peripheral arterial disease (PAD) is a frequent complication of diabetes, and tests in diabetic patients with PAD using L-D, $TcPo_2$, and nail fold capillaroscopy show the macrocirculatory deficit may outweigh the microcirculatory component when significant ischemic disease is present (90). However, reduced $TcPo_2$ is of greater import in diabetes (60), and if the separate L-D components are examined, deficits in diabetics are better discerned (60). Early capillary pressure increases (79) are partially normalized by improved glycemic control, suggesting that control may reduce complications via this mechanism.

Complications and Mechanistic Assessments

Combined L-D and toe nail fold capillaroscopy in patients with and without peripheral neuropathy indicate neuropathy is associated with increases in capillary blood velocity and number density as well as total perfusion (70). Sympathetic stimulation induced via inspiratory gasps transiently reduces perfusion in normals by about one half, but in neuropathic patients by less than one third. This may indicate that the hyperdynamic circulation and not nutritional shunting (21) is the main culprit of delayed healing of neuropathic foot ulcers. This concept is in accord with the functional roles of early hyperemia and capillary hypertension in the etiology of diabetic complications. Assessment of finger nail fold capillary density before and after local venous compression suggest that impaired microvascular reserves in type II diabetes is not dependent on reduced capillarity (37). L-D imaging after iontophoresis of sodium nitroprusside and acetylcholine on forearm indicates the reduced microvascular reserve is caused by both vascular smooth muscle and endothelial deficits. Combed L-D and toe capillaroscopy gives compelling evidence of a nutritional deficit even in patients apparently free of arterial disease (39).

Hypertension

Hypertension is a well-known risk factor for cardiovascular disease with multiple involvements at the microcirculatory level. Decreases in microvessel density and many structural and hemodynamic alterations within the microcirculation have been shown in animals and in humans (73). Earlier workers used nail fold capillaroscopy and showed significant hypertension-related increases in the rates of spontaneous flowmotion. Absence of such increases in patients with overt atherosclerotic disease suggested an increased arteriolar vasoactivity. Biomicroscopy of conjunctival arterioles after low-dose topical adrenaline support this view since three fourths of hypertensive patients and two thirds of their first-degree relatives showed decreased vasoconstrictive thresholds. Recent work (96) also shows decreased capillary density and blood velocity in human retinae, a tissue that likely mirrors conditions in brain microcirculation. This suggests that data obtained from more accessible areas, such as skin, provide useful windows for microcirculatory assessments in hypertension. Based on capillaroscopy and $TcPo_2$, primary microcirculatory disorders were detected in about 50% of hypertensive patients without evidence of macrovascular disease (40). Foot dorsum resting L-D perfusion and vasoconstriction responses caused by leg dependency were both lower in hypertensives than in controls prior to treatment. After four weeks of calcium-channel blocker treatment, which reduced blood pressure, replication of measurements showed increases in both microcirculatory parameters. However, other studies show no or reversed effects of postural vasoconstriction (24). This may be explained by nonimpaired pretreatment levels. Long-term L-D studies in animals suggest that hypertension progression is associated with decreased basal skin perfusion but mainly at skin sites rich in arteriovenous vessels (paw pad) (36). This experimental finding may relate to observations, using L-D in humans, that nitric oxide appears to exert its main vascular effect on skin also rich in such vessels (thumb pulp) (77). Forearm blood flow changes accompanying intrabrachial infusions of acetylcholine in the presence of L-argenine indicate that age-related impairments in endothelial function are indeed accelerated by hypertension (72). Regulation of nail fold capillary RBC velocity has been shown (87) to be well controlled (0.7 to 0.8 mm/s) over an ambulatory blood pressure range of 80 to 115 mmHg but significantly reduced at pressures above and below. But, in patients with mild-to-moderate hypertension, RBC velocity is lower than for matched controls and inversely related to mean 24-hour ambulatory pressures (27). Nail fold capillary blood cell dynamics after cooling differed in hypertensives, being associated with a greater frequency of "stopped-flow" intervals (28). This may indicate a microcirculatory manifestation of augmented vasoactivity or may be caused by the diminished capillary density. Initial low levels of resting L-D perfusion and $TcPo_2$ and responses to postural provocations all were reported improved (increased) in hypertensive patients following calcium blocker treatment. These favorable peripheral responses to blood-pressure–lowering treatment were thus detectible via microcirculatory functional assessments (32). Angiotensin-converting enzyme inhibitors are effective agents for treating hypertension and congestive heart failure, but aspects of their action are unknown. L-D responses to local injections (93) showed perfusion increases that were blocked by coinjections of nitric oxide synthase inhibitor and bradykinin antagonists. It was concluded that microcirculatory responses were mediated via a bradykinin-dependent mechanism.

Vascular Disease

Microcirculatory-Macrocirculatory Linkages

Sorting-out interactions between micro and macrocirculatory dysfunction are increasingly important aspects of functional assessments. Noninvasive assessment tools are used to detect large artery disease and cardiac dysfunction, but potential additional diagnostic and prognostic benefits of microcirculatory assessments are being discovered. Combined measurements of lower extremity blood flow and L-D perfusion (Figure 16.5) have provided new insights into several aspects of these interactions (61,62). Capillary microscopy and $TcPo_2$ assessments in patients with varying levels of PAD have also showed increased diagnostic efficacy (38). When critical ischemia could not be detected via pressure measurements, microcirculatory assessments were adequate in more than three fourths of patients so classified (90). Postexercise assessments of foot L-D in patients with intermittent claudication show a significant correlation ($r = 0.667$) with walking distance (80), further suggesting potential new diagnostic uses of microcirculatory assessment methods. An abnormal thermal response is shown in Figure 16.6. Interactions between cardiac and peripheral vascular function are now being examined (29,61). Nail fold capillaroscopy in chronic heart failure shows capillary enlargement and reduced resting blood velocity and postocclusion responses correlated with the severity of cardiac dysfunction (29).

Raynaud's Phenomena and Disease

Raynaud's phenomena is characterized by episodic digital skin triphasic changes: initial pallor (arteriolar vasospasm), followed by cyanosis (reduced blood flow and stasis) and ending with rubor (hyperemic vasodilation) as an attack subsides. During an attack, digits initially feel cold but during the hyperemic phase, paraesthesia and some edema may be seen. Involvement may be a single finger and may be associated with mild pain during an attack or significant ischemic pain. When these phenomena

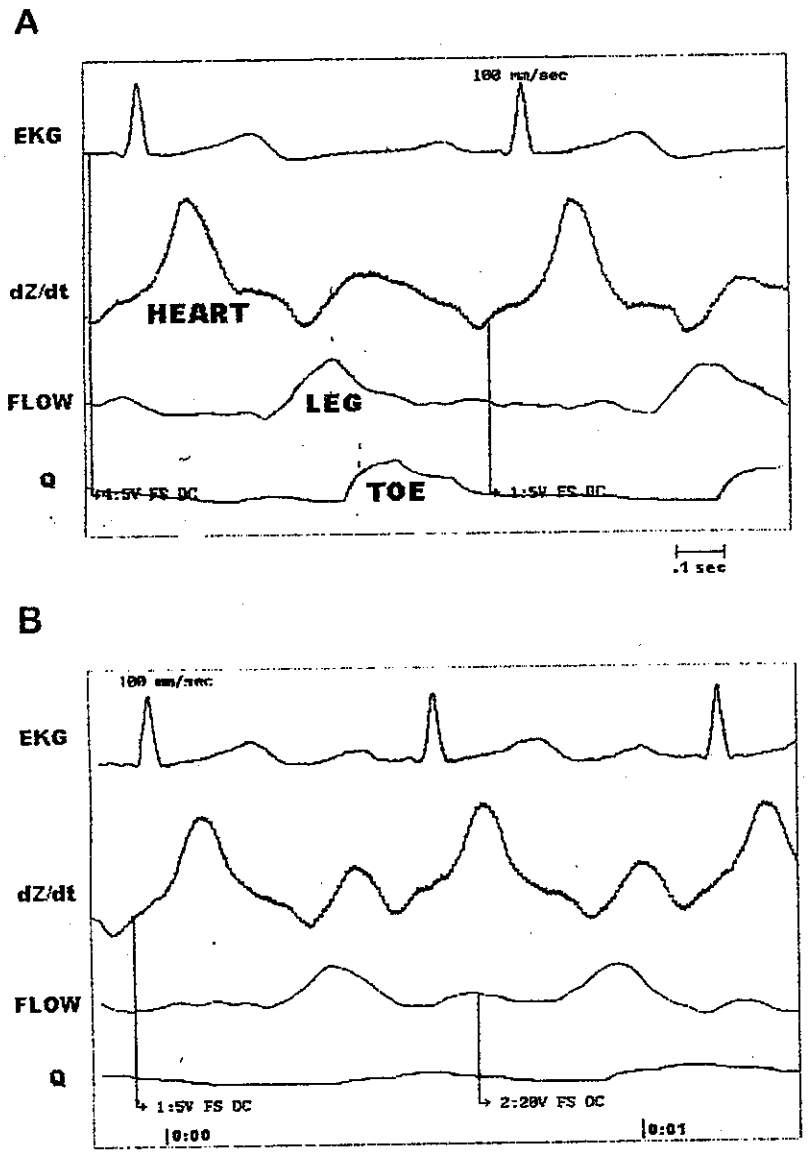

FIGURE 16.5. Macrovascular-microvascular interactions. (*A*) Resting supine waveforms showing electrocardiogram (EKG), rate of change of thoracic impedance (*dZ/dt*) that is coincident with cardiac ejection, leg blood flow pulse at midcalf measured by nuclear magnetic resonance, and toe perfusion pulse measured by laser-Doppler (before handgrip); (*B*) After 1 min of isometric handgrip at 40% maximum effort. Note dramatic decrease in toe-pulse perfusion. Large vertical time lines are 0.1 s apart.

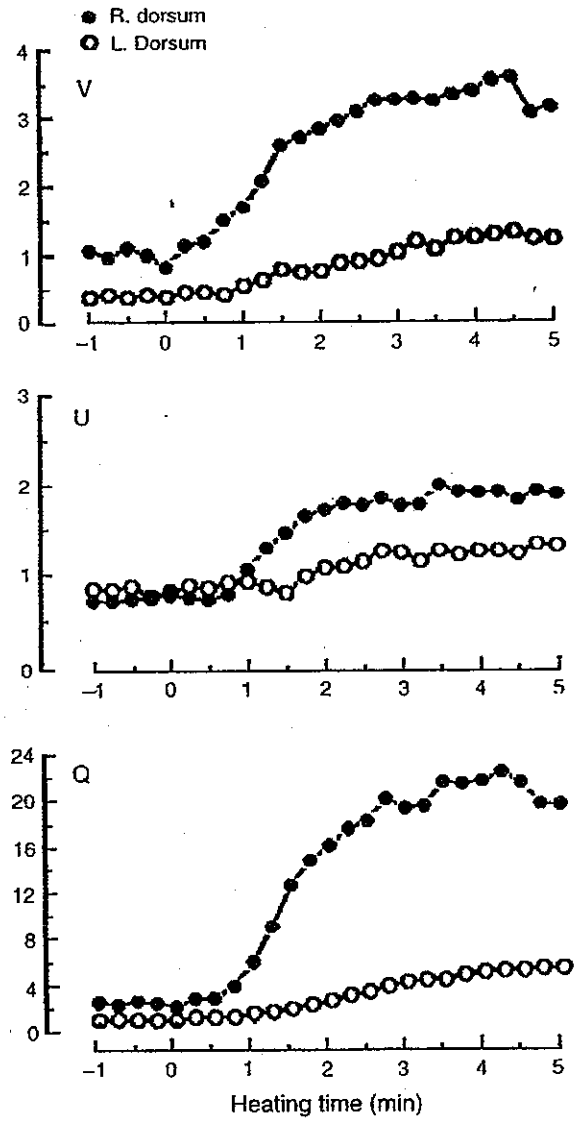

FIGURE 16.6. Impaired response in patient with unilateral peripheral arterial disease. Bilateral laser-Doppler thermal provocations show significantly impaired microvascular reserve in this patient with significant left leg arterial disease. Each data point is a 15-s average.

accompany an underlying systemic disease, a designation of secondary Raynaud's phenomena or Raynaud's disease is applied. Predisposing factors have been described, but mechanisms (local hypersensitivity or increased sympathetic activity) are unclear (97). Targets of microcirculatory assessments include diagnosis, severity estimation, differential diagnosis (primary vs. secondary), and therapeutic efficacy. At present, no microcirculatory assessment is fully adequate. Local cold exposure during nail fold capillaroscopy shows dramatic changes in erythrocyte velocity (50). Finger cooling L-D responses gave an 88% sensitivity and 87% specificity for diagnosis, and capillary microscopy showed a 67% sensitivity, 84% specificity, and 81% accuracy. Features of disturbed capillary morphology observed in Raynaud's disease yielded a 100% specificity and 74.4% accuracy (91). Heat and postocclusion hyperemic responses are used to help make differential diagnoses. Scleroderma, or "skin hardening," is a rare skin manifestation usually accompanying a systemic disorder (systemic sclerosis) and sometimes is an underlying feature of Raynaud's disease. This condition, which has a genetic predisposition, occurs three to five times more frequently in women and is associated with diffuse vascular damage, fibrosclerosis of cutaneous and visceral connective tissues, and immunological abnormalities. A review of microcirculatory assessments in this condition is available (34).

Venous Disease

Mechanistic Assessments

The event sequence whereby venous insufficiency leads to leg skin ulceration is not fully worked out, but changes in microvessel metrics, morphology, rheology, permeability, hemodynamics, and interstitium have been observed. Microcirculatory features of skin in high-risk preulcer areas or near existing ulcers have low $TcPo_2$, high $TcPco_2$ and increased L-D perfusion. Assessments with both L-D and capillaroscopy show that susceptible anatomical leg areas may have suppressed postural vasoconstrictive responses even in normal subjects (23). Periulcer skin L-D responses to heat and analyses of separate L-D components (63) indicate periulcer skin has fewer microvessels, but each carries more blood with increased RBC velocity. Blood flow data are consistent with this concept (64).

Diagnosis and Treatment

Treatment impacts on functional and clinical outcomes have been assessed (9), and microcirculatory correlates of compression bandaging, the mainstay of effective treatment, have been clarified (65–67). Intermittent limb compression therapy, which increases healing rate of some ulcers, produces a relative normalization of the increased L-D resting perfusion but with no effect on the abnormally low postural vasoconstrictive response (8). Foot microlymphatic pressures in primary lymphedema visualized with fluorescence microlymphography showed elevated values (12.8 ± 5.9 vs. 6.7 ± 3.8 mmHg) and characteristic low-frequency, high-amplitude (5.5 mmHg) pressure fluctuations (94).

Wound-Related Assessments

Assessments of adequacy of local microcirculation to minimize tissue loss, support wound healing of chronic skin ulcers, and predict suitable amputation levels when these are necessary are other clinical applications. L-D methods are used to estimate perfusion pressure at sites close to ulcers or likely amputation sites (3). Combined L-D perfusion with pressure yields an ulcer-healing prognosis with 100% sensitivity and 83% specificity when assessed by local heating (25). L-D assessments of VAR in nonulcerated skin near lower extremity ulcers help distinguish between venous and arterial disease (74). Diabetic neuropathic ulcers, often a precursor to amputation, are the focus of several microvascular assessments. Use of L-D and $TcPo_2$ show an inverse relationship between perfusion and oxygen responses. This suggests a blood flow "steal" phenomena perhaps related to neuropathic origin of an arteriolar-venous neural control deficit (22). Procedures to augment local microcirculation to aid the wound healing process have been evaluated using pre- and posttreatment L-D assessments (68). L-D and other methods to detect high-risk patients for pressure-ulcer development are being developed (42). Resting and hyperemic perfusion in the sacral area appears to show that patients with low values of each are at higher risk, which correlates with low systemic blood pressures (82). Assessment of microcirculatory changes at sites prone to skin breakdown while under pressure loading provides insight into the nature of the pressure-induced circulatory changes (2,31,59).

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