Design and potential clinical impact of a noninvasive thermal diffusion sensor to monitor human peripheral microvascular perfusion in real-time

by

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1 Introduction

1.1 Motivation and Rationale

Perfusion in peripheral tissues is fundamental to the characterization of both local and global cardiovascular health. However, despite the inherent accessibility of tissues such as skin to microvascular measurements, there is still a need for routine, noninvasive methods for obtaining relevant assessments of the human microcirculation. Human microvasculature includes the arterioles, capillaries, and venules that supply tissues with nutrients, circulate humoral products, and remove waste. The process of circulatory distribution is an efficient and highly structured process that distributes output through a regulation of local and global mechanisms that match blood flow to tissue need. These mechanisms are still not fully understood, but they do indicate a practical and potentially valuable connection between coronary and peripheral vascular function. Current methods of measuring perfusion in a reliable way are invasive, involve complicated procedures, do not permit continuous data collection, and are relatively expensive. The design of a noninvasive perfusion monitoring system that can routinely and continuously monitor perfusion in accessible tissues such as skin would have significant potential in applications requiring an understanding of the microvasculature as well as its diagnostic potential in a variety of circulatory disease states including (but not limited to) atherosclerosis, ischemic events, and wound healing ability.

1.2 Context of Design and Implementation

An ideal monitor for measuring blood perfusion would be able to deliver objective, sensitive, and continuous data, with a rapid response in real time to any microcirculatory change. The

monitoring system would also be simple, safe, inexpensive and portable, and it would have the adaptable potential for measuring absolute perfusion (units of volume of blood per mass of tissue per minute) within a variety of tissues. Finally, the probe itself would be noninvasive, not only to minimize patient discomfort and physical trauma, but also to mitigate the effect of introducing a foreign object into the body that may change the local physiological state of the tissue and vessel architecture.

A method of monitoring perfusion is thus proposed that measures that intrinsic physiological heat transfer properties of perfused tissue (Bowman, 1985). Such a technique has significant potential for obtaining precise, continuous, and real-time assessments of absolute tissue blood flow. The design of a sensitive and responsive device that will monitor perfusion in skin using inert thermal diffusivity would therefore be a significant realization of the need for safe and widely applicable tissue blood flow quantification. To quantify the physiological phenomena of perfusion, an appropriate model must be used that accounts for the variation of thermal gradients in tissue over time, thereby relating heat transfer processes to tissue perfusion (Walsh, 1984). The dominant modes of heat transfer in a living tissue can be characterized in steady state by the tissue's thermal conductivity and in the nonsteady state by its thermal diffusivity. These embody the complex interactions between tissue conduction, perfusion, and metabolism in a largely anisotropic and heterogeneous architecture. Combined with an understanding of the mechanisms of how the body modulates vasomotor tone, empirical measurement of the thermal properties of the tissue can hold meaningful significance in quantifying and comprehending physiological phenomena.

Given the fundamental role of heat transfer in microvascular blood perfusion, it has previously been demonstrated that a simple and effective technique relating tissue heat transfer

ability and blood flow can accurately measure perfusion in a minimally invasive manner. The Thermal Diffusion Probe (TDP) developed by Bowman, et al. has been successfully used for quantification of microcirculation after human liver transplantation for up to 7 days. In this study, distinct thresholds of perfusion recovery correlated with post-operative graft function (Klar et al., 1996). Other areas in which this probe has proven useful are in the monitoring of cerebral blood flow in patients with traumatic brain injury (Vajkoczy et al., 2000) and in determining viability of prefabricated skin flaps used for reconstructive surgery (Maitz et al., 2004).

For my thesis, I propose to design a noninvasive sensor based upon the thermal heat flux character of cutaneous microvascular blood flow that will provide a real time measure of peripheral perfusion. As the largest organ in the human body, the skin is the most accessible and noninvasive interface for assessing microcirculatory flow. Thus, the device will employ the method of measuring thermal diffusion processes due to blood flow in a similar fashion to that of the TDP, with inherent adaptations to the noninvasive constraints of cutaneous microcirculation. Considerations will be made regarding selection of appropriate hardware, analysis of heat transfer properties of the sensor and human cutaneous microvasculature, and appropriate specifications to implement this within a clinical setting. The ability to make the measurements of peripheral blood flow described here will ultimately contribute to a new method of disease diagnosis and a deeper understanding of the mechanisms of endothelial dysfunction and regulation of vasomotor activity.

In the document which follows, I will perform an extensive review of the wide field of perfusion research, focusing on noninvasive modalities and their clinical applicability. Not only will this reveal the breadth of previously documented findings for studies within the peripheral circulation, but it will also aid in the selection of appropriate design parameters such as device

operation, cost, complexity, clinical applicability, functional constraints of user and patient interaction, and a variety of other future design considerations that must be considered for a clinical tool intended for extensive use. Furthermore, knowledge of both the epidemiological field in which such a tool could be applied and also the functional and societal limitations of currently available technologies strongly influences how such a design problem should be approached. Therefore, the present goal will be to illuminate current noninvasive perfusion technologies and their functional constraints in the context of clinical applications. The significance of the intended design (noninvasive cutaneous measure of perfusion) will also be considered with respect to utility in the diagnosis and screening of endothelial dysfunction in subclinical atherosclerosis and as a simple, continuous monitor of vital, nutritive perfusion in peripheral tissues.

1.3 Thermal Diffusion Probe (TDP) technology

The TDP previously mentioned contains two self-heating thermistors, an active one at the tip that is heated to a constant 2°C above the tissue baseline temperature and another passive one 8 to 10 mm proximal that is placed outside the heated field to measure the tissue baseline temperature fluctuations over time. The active thermistor dissipates a specific amount of power in the constant spherical temperature field and therefore provides a direct measure of the tissue's ability to carry heat by both thermal conduction within the tissue and by thermal convection due to local perfusion blood flow. Quantification of perfusion is made possible by modeling the heat transfer interactions between the probe and the tissue augmented by underlying blood flow.

The TDP heat transfer model employs a coupled probe-tissue thermal model based upon the bio-heat transfer model first proposed by Pennes in 1948, which describes heat transfer

between tissue and blood. Here it is assumed that the heated thermistor is a uniformly heated sphere that exists in perfect thermal contact with the tissue. It can thus be mathematically described by coupled partial differential equations where the solution assumes a constant temperature within the thermistor. In practice, the thermal transfer must consider intrinsic conductive properties of the tissue and the convective effects of perfusion. These effects were previously separated by calibrating in no-flow conditions but had obvious limitations in a clinical setting. The current method employs algorithms that are able to quantify the tissue conduction based upon the initial rate of propagation of the thermal field. In this way, the convection term from perfusion can be isolated and a physiologically meaningful measure of blood flow is obtained (Martin et al., 2000).

Adapting thermal diffusion methods for noninvasive applications will undoubtedly require reevaluation of the both the functional design of the probe apparatus as well as the heat transfer model to account for new tissue geometries and transfer properties. The advantages of using thermal diffusion to measure tissue blood flow stems from the use of effective thermal conductivity, which is an experimentally determined tissue property that accounts for heat transport due to tissue perfusion. Therefore, the minute details of vascular geometries, which are exceedingly complex and heterogenous in skin, do not limit the applicability of such a device. In addition, heat transfer in tissues perfused by small vessels has a distinct advantage of being in a reliable blood-tissue thermal equilibrium such that thermal diffusion provides a highly dependable measure of blood flow. Finally, it is important to note the undeniable benefits of using heat as an inert tracer in tests where safety and simplicity are a principal concern. These considerations will be further explored and elaborated upon in subsequent thesis research.

1.4 Preliminary TDP Data

Using the existing invasive thermal diffusion probe (Hemedex, Inc.) calibrated for skin, an experiment was performed to measure thermal clearance from blood flow in the thumb pad of the hand. The TDP was affixed to the skin of the thumb pad using insulting adhesive padding that completely covered the area. Glycerol was used as wetting agent to increase the thermal communication between the skin and the sensor, and the probe was connected to the perfusion monitor.

A blood pressure cuff was placed around the upper part of the same arm and inflated to a suprasystolic pressure to occlude circulation. This was maintained for 2 minutes during which time the thumb pad perfusion noticeably decreased. The cuff was then released and the perfusion monitor indicated an overshoot in blood flow (reactive hyperemia). The perfusion plot produced by the prototype sensor and the existing monitor is shown in Figure 1. The temperature of the skin during this experiment varied between 31.2°C and 31.7°C. Although the probe is not designed to measure perfusion of the skin, this experiment shows that the method of measuring thermal diffusion noninvasively on skin tissue is realistic and achievable with this technology. Design of an appropriate probe for skin would require adapting the probe and modeling the heat transfer properties of perfused skin to obtain an absolute measure of blood flow.

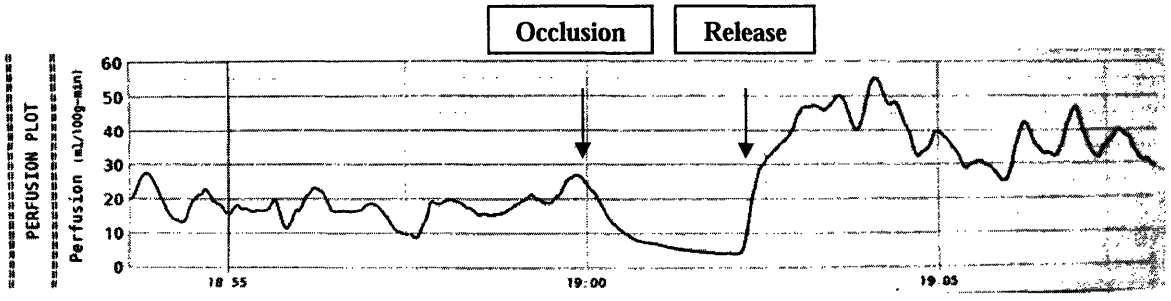

Figure 1 Perfusion plot from thermal diffusion probe on skin.

2 Perfusion and endothelial function assessment

2.1 Endothelial dysfunction

2.1.1 Pathophysiology

Endothelial dysfunction and other microvascular impairments are important and early events in the pathogenesis of major cardiovascular diseases, affecting vessels in both the coronary and peripheral circulation. This suggests a direct relation between cardiovascular disease processes, such as atherosclerosis, and state of microcirculation in the extremities. Serious health disorders such as diabetes, hypertension, peripheral vascular disease, atherosclerosis, complications of ulcerated extremities and non-healing wounds occur at the level of tissue blood flow in a process broadly defined as perfusion, or the rate at which a given quantity of blood is replenished to the capillary network. Perfusion is of primary importance in the local transport of heat, oxygen, drugs, nutrients, and waste products and is therefore a key parameter in normal and pathologic physiology. An understanding of such deficits would hold great value in clinical diagnosis, prognosis, and treatment as well as in research of the microcirculation. Furthermore, tissue oxygenation is frequently assessed clinically by using conventional global measurements such as blood pressure, oxygen derived variables, and blood lactate levels. However, the assessment of global hemodynamic parameters fails to reflect local increased blood lactate levels, the imbalance between oxygen demand and oxygen supply, or the status of the microcirculation. The usual methods for these purposes are either invasive and/or technically demanding. Endothelial function should instead be assessed in vivo and, as far as possible, noninvasively. Therefore, a noninvasive, continuous, real-time means of monitoring

and quantifying perfusion in a specific tissue volume holds great value in diagnosing and managing numerous medical problems.

Although the precise mechanisms of endothelial dysfunction are not fully understood, it appears to involve a positive feedback loop in which inflammatory factors promote monocyte and T-cell adhesion, foam cell formation, extracellular matrix digestion, and vascular smooth muscle migration and proliferation leading to atherosclerotic plaque formation. Dysfunction is characterized by abnormal vasoreactivity, which has traditionally been measured as a reduction of the bioavailability of vasodilators, in particular, nitric oxide (NO). This imbalance leads to an impairment of endothelium-dependent vasodilatation, which represents the functional characteristic of endothelial dysfunction. Hypertension, chronic heart failure, diabetes, hypercholesterolemia and generalized atherosclerosis, for example, are all associated with impaired endothelium-mediated vasodilatation. Therefore, the evaluation of endothelial function in humans is of great clinical relevance.

2.1.2 Methods of Noninvasive Assessment

The noninvasive assessment of endothelium-dependent vasodilatation has emerged as an accessible indicator of endothelial health. In particular, stimuli that increase production of endothelium-derived NO have proven useful in assessing endothelium-dependent vasodilatation in humans. Such stimuli include increased shear stress from increased blood flow, and receptor-dependent agonists, such as acetylcholine, bradykinin, or substance P. Basal NO release can be assessed using specific inhibitors of NO synthase, such as NG-monomethyl-L-arginine. Although this response has been shown to depend mainly on NO synthesis, it also reflects release of other endothelium-derived vasodilators and vasoconstrictors, a key observation that both complicates and illustrates mechanistic interactions of endothelial function.

The physiological microvasculature of the body in organs such as the skin, brain, heart, and extremities is an important regulatory network that provides information regarding global cardiovascular health. Systemic endothelial dysfunction is often found to be reflected in the peripheral circulation such that macrovascular and microvascular abnormalities share common functional characteristics (Anderson et al., 1995). The microcirculation is of interest because this is where fine control of blood supply takes place, and tissue ischemia is dependent on microvascular flow in many circumstances. In general during circulatory failure, blood flow is diverted from the less important tissues (skin, subcutaneous, muscle, gastrointestinal tract) to vital organs (heart, brain, kidneys). Thus monitoring perfusion in these less vital tissues such as skin could be an early clinical marker of vital tissue hypoperfusion. Indeed, the assessment of perfusion in peripheral tissues is more easily obtainable using noninvasive monitoring techniques, thus facilitating earlier recognition of abnormalities. The skin can provide a good platform for this, and in diabetes, for example, measurable changes in the skin have been found to pre-date the symptoms of microvascular disease in other organs by many years (Khan et al., 2000). In the dermal microcirculation, endothelial function may be assessed noninvasively using several methods, although no one technique is entirely satisfactory. Nevertheless, this vascular bed is highly suitable for assessment of the endothelium, as well as being particularly convenient.

By posing the dermal microcirculation as a way to interrogate overall vascular health, it is important to recognize that cutaneous blood flow is a dynamically fluctuating biological variable which possesses substantial spatial heterogeneity. Its magnitude and regulation is modulated through many external and internal parameters by virtue of its central role in thermoregulation and tissue perfusion. Microcirculatory flow adjusts as a result of variations in blood gas concentration, hormones, and physical factors like temperature and pressure. It is also carefully

controlled by the autonomous nervous system. Such signals increase or decrease the microvascular perfusion by stimulating the endothelium to release substances leading to vasoconstriction or vasodilatation of the upstream arterioles. Characterizing these systemic regulators of blood flow also impacts current understanding of perfusion. Several important clinical goals arise from these local and global relationships. Not only is the status of a patient's generalized microvasculature important, but direct associations must be determined to provide information as it relates to underlying pathophysiology.

2.1.3 Current Limitations and Practical Design Specifications

There is a need for a simple noninvasive diagnostic tool for endothelial dysfunction in both experimental and clinical settings to evaluate and monitor the microvascular sequelae of conditions known to affect the microcirculation, such as shock (hemorrhagic, septic), hypertension or diabetes. In addition to noninvasive applications, such a device could provide quantitative analysis of the microcirculation during surgery (transplant, cardiac, vascular, neurological, plastic, etc.), wound healing, tumor therapy and intensive care medicine. It could also become a screening tool to aid in detecting subclinical endothelial dysfunction related to systemic atherosclerosis. Such a test might identify presymptomatic subjects at high risk of atherosclerotic complications and therefore allow specific "targeting" of primary preventive strategies. Although endothelial function testing is available in the research setting, no technique yet exists that is simple, safe, reproducible and easily performed as a screening method.

Numerous techniques have been devised to measure tissue blood flow, but they have limited utility and are generally not routine or clinically applicable. Furthermore, they are sometimes surgically invasive or introduce foreign agents into the body. A few such techniques include

2.2 Current noninvasive modalities of perfusion measurement

2.2.1 Capillary Refill Time (nail blanch test)

Used as a quick test performed on nail beds to monitor tissue perfusion, pressure is applied to the nail bed until it turns white, indicating that the blood has been forced from the tissue (blanching). Once the tissue has blanched, pressure is removed and the time it takes for blood to return to the tissue, indicated by a pink color returning to the nail, is measured. This is based on the assumption that a delayed return of a normal color after emptying the capillary bed by compression is due to decreased peripheral perfusion. CRT has been validated as a measure of peripheral perfusion with significant variation in children and adults. A study on a normal population reported that CRT varied with age and sex (Schriger *et al.*, 1988). It was found that a CRT of <2 s was a normal value for most young children and young adults, but the lowest CRT was substantially higher in healthy women (2.9 s) and in the elderly (4.5 s). Furthermore, several clinical studies have reported a poor correlation between CRT, heart rate, blood pressure, and cardiac output (Bailey et al., 1990; Schriger et al., 1991; Tibby et al., 1999).

2.2.2 Peripheral Perfusion Index (PPI) with Pulse Oximetry

The peripheral perfusion index (PFI) is derived from the photoeletric plesthysmographic signal of pulse oximetry and has been used as a noninvasive measure of peripheral perfusion. This monitoring technique is used in probably every trauma, critically ill and surgical patient. Pulse oximeters measure the arterial oxygen saturation of hemoglobin, which is a measure of the average amount of oxygen bound to each hemoglobin molecule. The principle of pulse oximetry is based on two light sources with different wavelengths (660 nm and 940 nm) emitted through

arterial catheterization, capillaroscopy, orthogonal polarization spectral imaging, transcutaneous oxygen measurement, hydrogen clearance, Xenon CT, or magnetic resonance imaging (MRI). Most methods suffer from low reproducibility and are restricted to a metric corresponding to the particular aspects of microcirculation being measured (tracers or objects in the flow rather than volume of blood). In addition, they often either assess only a fraction of the microvessels involved in underlying blood perfusion or fail to distinguish blood flow contributions from multiple tissues. Imaging techniques such as CT or MRI provide only a snapshot in a single moment rather than a continuous measurement.

These restrictions on current technology thus limit the use of routine perfusion monitoring in a noninvasive and quantifiably significant way, especially in instances of diagnostic potential. As will be shown herein, while some methods do provide a noninvasive assessment of blood flow, the same methods fail to provide a truly quantitative measure of perfusion essential for further application in clinical settings. This problem will form the basis for the current discussion and future design. More specifically, a perfusion monitor modeled upon the physiological tissue heat transfer properties holds promise as a way to obtain precise, continuous, and real-time assessments of tissue blood flow in absolute units. The design of a sensitive and responsive technique that will monitor perfusion in skin using inert thermal diffusivity would therefore be a significant realization of the need for safe and widely applicable tissue blood flow quantification. Before designing such a device, the current field of noninvasive perfusion measurement will be assessed and clinical implications of this technology will be explored. Particular attention will be paid to the area of screening for subclinical atherosclerosis and similar diseases that manifest at the level of dysfunctional microvascular perfusion.

the cutaneous vascular bed of a finger or earlobe. The Hb absorbs more light at 660 nm and HbO_2 absorbs more light at 940 nm. A detector at the far side measures the intensity of the transmitted light at each wavelength, and the oxygen saturation is derived by the ratio between the red light (660 nm) and the infrared light (940 nm) absorbed. Since other tissues also absorb light, such as connective tissue, bone, and venous blood, the pulse oximetry distinguishes the pulsatile component of arterial blood from the nonpulsatile component of other tissues. Using a two-wavelength system the nonpulsatile component is then discarded, and the pulsatile component is used to calculate the arterial oxygen saturation. The overall hemoglobin concentration can be determined by a third wavelength at 800 nm, with a spectrum that resembles that of both Hb and HbO_2. The resulting variation in intensity of this light can be used to determine the variation in arterial blood volume (pulsatile component). The PFI is calculated as the ratio between the pulsatile component (arterial compartment) and the nonpulsatile component (other tissues) of the light reaching the detector of the pulse oximetry, and it is calculated independently of the patient's oxygen saturation. A peripheral perfusion alteration is accompanied by variation in the pulsatile component, and because the nonpulsatile component does not change, the ratio changes. As a result the value displayed on the monitor reflects changes in peripheral perfusion (A. P. Lima et al., 2002).

The function of a pulse oximeter is affected by many variables, including: ambient light; shivering; abnormal hemoglobins; pulse rate and rhythm; vasoconstriction and cardiac function. A pulse oximeter gives no indication of a patient's ventilation, only of their oxygenation, and thus can give a false sense of security if supplemental oxygen is being given. In addition, there may be a delay between the occurrence of a potentially hypoxic event such as respiratory obstruction and a pulse oximeter detecting low oxygen saturation.

2.2.3 Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) offers a technique for continuous, noninvasive, bedside monitoring of tissue oxygenation. As with pulse oximetry, NIRS uses the principles of light transmission and absorption to measure the concentrations of oxygenated and deoxygenated hemoglobin, oxygen saturation (StO_2), and the redox state of cytochrome aa_3 noninvasively in tissues as an average value of arterial, venous and capillary blood according to the law of Lambert-Beer. NIRS has a greater tissue penetration than pulse oximetry and provides a global assessment of oxygenation in all vascular compartments (arterial, venous, and capillary). Tissue penetration is directly related to the spacing between illumination and detection fibers. At 25 mm spacing approx. 95% of the detected optical signal is from a depth of 0 to 23 mm.

NIRS has been used to assess forearm skeletal muscle oxygenation during induced reactive hyperemia in healthy adults and produces reproducible measurements of tissue oxygenation during both arterial and venous occlusive events. Using the venous and arterial occlusion methods NIRS can be applied to measure regional blood flow and oxygen consumption by following the rate of HbO_2 and Hb changes. In the venous occlusion method a pneumatic cuff is inflated to a pressure of approx. 50 mmHg. Such a pressure blocks venous occlusion but does not impede arterial inflow. As a result venous blood volume and pressure increase. NIRS can reflect this change by an increase in HbO_2, Hb, and total hemoglobin. In arterial occlusion method, the pneumatic cuff is inflated to a pressure of approx. 30 mmHg greater than systolic pressure. Such a pressure blocks both venous outflow and arterial inflow. Depletion of local available O_2 is monitored by NIRS as a decrease in HbO_2 and a simultaneous increase in Hb, whereas total Hb remains constant. After release of the occluding cuff a

hyperemic response is observed. Blood volume increases rapidly, resulting in an increase in HbO_2 and a quick washout of Hb. In addition to blood flow and evaluation of HbO_2 and Hb changes, NIRS can assess $cytaa_3$ redox state. Cytochrome aa3, the terminal cytochrome of the respiratory chain, is responsible for approximately 90% of cellular oxygen consumption through oxidative phosphorylation. Since the redox state of cytochrome aa3 is primarily determined by available oxygen, a decrease in cellular oxygen delivery results in a reduction of oxidative phosphorylation and a decreased oxidation level of cytochrome aa3. Monitoring the redox state of cytochrome aa3 might therefore be a key indicator of an impaired cellular oxidative metabolism and tissue dysoxia.

Tissue oxygen tension measures the difference between oxygen delivery and oxygen consumption, thus providing a reliable indicator of impaired circulation. NIRS offers the technology to monitor tissue oxygenation noninvasively and has become a tool in exercise physiology to measure muscle oxygenation. Despite the potential clinical applications of NIRS, some limitations still exist. The contribution of the $cytaa_3$ signal is small, and its interpretation remains controversial, requiring more rigorous development. There is no a gold standard to which NIRS data can be directly compared, and one of the reasons is that a variety of NIRS equipment is commercially available with different working systems. In both small- and large-animal models of hemorrhagic shock and resuscitation NIRS has demonstrated sensitivity in detecting skeletal muscle and visceral ischemia. As a noninvasive measure of peripheral perfusion NIRS has been applied in superficial muscles (brachioradialis muscle, deltoid muscle, tibialis anterior) of trauma ICU patients to monitor the adequacy of tissue oxygenation and detect a compartment syndrome. Although NIRS may be applied to almost any organ, it has mainly been used in studies investigating cerebral or muscle oxygenation after different types of hypoxic

injuries. The main limitation of NIRS in the clinical setting is the inability to make quantitative measurements because of the contamination of light by scatter and absorption.

2.2.4 Orthogonal Polarization Spectral imaging

Orthogonal polarization spectral (OPS) imaging is a noninvasive technique that uses reflected light to produce real-time images of the microcirculation. Light from a source passes through the first polarizer, and it is directed towards the tissue by a set of lens. As the light reaches the tissue, the depolarized light is reflected back through the lenses to a second polarizer or analyzer and forms an image of the microcirculation on the charge-coupled device, which can be captured through a single videotape. If a wavelength within the hemoglobin absorption spectrum (e.g. 548 nm) is chosen, red blood cells will appear dark and white blood cells may be visible as refringent bodies. The vessel walls themselves are not visualized directly and their imaging depends, therefore, on the presence of red blood cells. The technology has been incorporated into a small hand-held video-microscope which can be used in both research and clinical settings (Groner et al., 1999). OPS can assess tissue perfusion using the functional capillary density (FCD), defined as the length of red blood cell (RBC)-perfused capillaries per observation area (measured as cm/cm^2).

FCD is a direct and very sensitive parameter for determining the status of nutritive perfusion to the tissue and is an indirect measure of oxygen delivery. One of the most easily accessible sites in humans for peripheral perfusion monitoring is the mouth. OPS produces excellent images of the sublingual microcirculation by placing the probe under the tongue. The use of sublingual tissues with OPS provides information about the dynamics of microcirculatory blood flow, and therefore it can monitor the perfusion during clinical treatment of circulatory

shock. However, alterations in the sublingual microcirculation may not be representative of blood flow in other microvascular beds. Moreover, to be a useful monitor of perfusion, the obtained images must be interpreted uniformly and quantification of microcirculatory flow must be reproducible. Movement artifacts, semiquantitative measure of perfusion, the presence of various secretions such as saliva and blood, observer-related bias, and inadequacy of sedation to prevent patients from damaging the device are some of the limitations of the technique.

2.2.5 Laser Doppler flowmetry

Laser Doppler flowmetry (LDF) is a noninvasive, continuous measure of microcirculatory blood flow, and it has been used to measure microcirculatory blood flow in many tissues including neural, muscle, skin, bone, and intestine. The principle of this method is to measure the Doppler shift—the frequency change that light undergoes when reflected by moving objects, such as red blood cells. LDF works by illuminating the tissue under observation with a monochromatic laser from a probe. When the tissue is illuminated, only 3–7% is reflected. The remaining 93–97% of the light is either absorbed by various structures or undergoes scattering. Another optical fiber collects the backscattered light from the tissue and returns it to the monitor. As a result LDF produces an output signal that is proportional to the microvascular perfusion. Depending on the device and the degree of invasiveness it can be used to assess blood flow in muscle, gastric, rectal, and vaginal mucosae. As a noninvasive measure of peripheral blood flow, however, its use is limited to the skin.

LDF has been applied to obtain information on the functional state of the skin microcirculation during either reactive hyperemia or the noninvasive local application of acetylcholine or sodium nitroprusside in several conditions, including diabetes mellitus, essential

hypertension, atherosclerosis, and sepsis. This characteristic of LDF was used in critically ill patients to evaluate endothelial dysfunction in sepsis. The ability of LDF to assess abnormalities of skin blood flow control in sepsis could be of clinical use for early detection of microcirculatory derangements in high risk patients.

A major limitation of this technique is that it does not take into account the heterogeneity of blood flow as the velocity measurements represent the average of velocities in all vessels of the window studied. Moreover, the device only examines a superficial layer of dermis in a small yet undefined volume of tissue and requires several measurement sites for an accurate reading. However, the heterogeneity of skin lowers the reproducibility of the desired perfusion index since skin blood flow signal varies markedly depending on probe position. Finally, no current laser Doppler instrument can present absolute perfusion values (e.g., ml/min per 100 g tissue) and measurements are expressed as perfusion units, which are arbitrary. Thus, LDF measurements are only valid on a relative scale within the specific patient and tissue of interest.

2.2.6 PO_2 and PCO_2 transcutaneous measurements

Transcutaneous oxygen tension (PO_2) has been an integral part of noninvasive microcirculatory blood flow since the 1970s. Continuous noninvasive measurement of oxygen and carbon dioxide tensions is possible because both gases can diffuse through the skin, and thus their partial pressures can be measured in transcutaneous tissue. Normally the skin is not very permeable to gases, but at higher temperatures the ability of the skin to transport gases is improved. This method allows quantification of oxygen molecules transferred to the skin microcirculation after heating. This device consists of a polarographic oxygen electrode which is maintained in contact with the skin through a thin membrane. The skin immediately below the

electrode is heated to produce vasodilatation and the increase in local blood flow thus produced makes the amount of oxygen consumed by the skin small in comparison with the amount available through hyperfusion. Oxygen diffuses from the top most dilated capillaries and is detected by the electrode. The success of the method critically depends upon the skin being heated sufficiently to allow diffusion of the oxygen but not so much as to cause skin damage. The sensor heats the skin to 43– 45°C. These transcutaneous sensors make it possible to directly to estimate arterial oxygen pressure (PaO_2) and arterial carbon dioxide pressure ($PaCO_2$), and it has been successfully used for monitoring PaO_2 and $PaCO_2$ in both neonates and in adults. Although this technique is widely available, it is time consuming and does not assess all ischemic regions unless specifically placed on the tissue in question.

2.2.7 Temperature Gradients

In the presence of a constant environmental temperature a change in the skin temperature is the result of a change in skin blood flow. The temperature gradients peripheral-to-ambient (dTp-a) and central-to-peripheral (dTc-p) can better reflect cutaneous blood flow than the skin temperature itself. Considering a constant environment condition, dTp-a decreases and dTc-p increases during vasoconstriction (A. Lima et al., 2005).The peripheral skin temperature is measured using a regular temperature probe attached to the ventral face of the great toe. This site is more convenient for peripheral temperature measurement because of the negligible local heat production and the distal location from other monitoring devices. The concept of the dTc-p is based on the transfer of heat from the body core to the skin. The heat conduction to the skin by the blood is also controlled by the degree of vasoconstriction of the arterioles and arteriovenous anastomoses. High blood flow causes heat to be conducted from the core to the skin, whereas

reduction in blood flow decreases the heat conduction from the core. During vasoconstriction the temperature of the skin falls and the heat conduction from the core decreases, and therefore the central temperature rises and the dTc-p increases. A gradient of 3–7°C occurs in patients with stable hemodynamics. Hypothermia, cold ambient temperature (<20°C), and vasodilatory shock limits the use of dTc-p as an estimate of peripheral perfusion.

Forearm-to-fingertip skin-temperature gradient (Tskin-diff) has also been used as an index of peripheral circulation to identify the initiation of thermoregulatory vasoconstriction in patients following surgery (Rubinstein et al., 1990). Fingertip temperature is measured with the temperature probe attached to the ventral face of the finger. The use of Tskin-diff is based on assumption that the reference temperature is a skin site exposed to the same ambient temperature as the fingertip. It has been applied in conditions where an ambient temperature is not stable, such as in patients undergoing surgery. A change in ambient temperature therefore affects similarly forearm and fingertip temperature, producing little influence in the gradient. Basically, when vasoconstriction decreases fingertip blood flow, finger skin temperature decreases, and Tskin-diff increases. Experimental studies have suggested a Tskin-diff threshold of 0°C for the initiation of vasoconstriction, and a threshold of 4°C for severe vasoconstriction in anesthetized patients.

The body temperature gradient was first applied to assess patients with circulatory shock and to differentiate central heat retention caused by fever from peripheral vasoconstriction. A number of studies have examined the correlation between body temperature gradient and global hemodynamic variables in hypovolemic, septic and cardiogenic shock, but these have produced conflicting results. One reason for the inaccurate relationship between body temperature gradient and global hemodynamic parameters could be related to an unstable environment, as skin

temperature depends also on ambient temperature, and the thermoregulatory response is suppressed in anesthetized patients. In addition, global hemodynamic parameters may not be sensitive enough to reflect changes in peripheral blood flow in critically ill patients. Tskin-diff may be an alternative, but its use in these conditions has not yet been defined.

3 Microvascular Blood Flow

3.1 Network model of the microcirculation

The blood vessels are a closed system of conduits that carry blood from the heart to the tissues and back to the heart. The resistance to flow depends to a minor degree on the viscosity of the blood but mostly on the diameter of the vessels, principally the arterioles. The blood flow to each tissue is regulated by local chemical and general neural and humoral mechanisms that dilate or constrict the vessels of the tissue. A parallel arrangement permits wide variations in regional blood flow without changing total systemic flow. In humans and other mammals, multiple cardiovascular regulatory mechanisms have evolved. These mechanisms increase the blood supply to active tissues and increase or decrease heat loss from the body by redistributing the blood. In the face of challenges such as hemorrhage, they maintain the blood flow to the heart and brain. When the challenge faced is severe, flow to these vital organs is maintained at the expense of the circulation to the rest of the body.

3.1.1 Arteries & Arterioles

The walls of all arteries are made up of an outer layer of connective tissue, the adventitia; a middle layer of smooth muscle, the media; and an inner layer, the intima, made up of the endothelium and underlying connective tissue. The walls of the aorta and other arteries of large diameter contain a relatively large amount of elastic tissue, primarily located in the inner and external elastic laminas. They are stretched during systole and recoil on the blood during diastole.

The walls of the arterioles contain less elastic tissue but much more smooth muscle. The muscle is innervated by noradrenergic nerve fibers, which function as constrictors, and in some instances by cholinergic fibers, which dilate the vessels. The arterioles are the major site of the resistance to blood flow, and small changes in their caliber cause large changes in the total peripheral resistance. Pulsatile flow appears, in some poorly understood way, to maintain optimal function of the tissues. If an organ is perfused with a pump that delivers a nonpulsatile flow, there is a gradual rise in vascular resistance, and tissue perfusion fails.

3.1.2 Capillaries

The arterioles divide into smaller muscle-walled vessels, sometimes called metarterioles, and these in turn feed into capillaries. In some of the vascular beds that have been studied in detail, a metarteriole is connected directly with a venule by a capillary thoroughfare vessel, and the true capillaries are an anastomosing network of side branches of this thoroughfare vessel. The openings of the true capillaries are surrounded on the upstream side by minute smooth muscle precapillary sphincters. It is unsettled whether the metarterioles are innervated, and it appears that the precapillary sphincters are not. However, they can of course respond to local or circulating vasoconstrictor substances. The true capillaries are about 5 um in diameter at the arterial end and 9 um in diameter at the venous end. When the sphincters are dilated, the diameter of the capillaries is just sufficient to permit red blood cells to squeeze through in "single file." As they pass through the capillaries, the red cells become thimble- or parachute-shaped, with the flow pushing the center ahead of the edges. This configuration appears to be due simply to the pressure in the center of the vessel whether or not the edges of the red blood cell are in contact with the capillary walls.

The total area of all the capillary walls in the body exceeds 6300 m^2 in the adult. The walls, which are about 1 um thick, are made up of a single layer of endothelial cells. Located between the circulating blood and the media and adventitia of the blood vessels, the endothelial cells constitute a large and important organ. They respond to flow changes, stretch, a variety of circulating substances, and inflammatory mediators. They secrete growth regulators and vasoactive substances.

Capillaries and postcapillary venules have pericytes outside the endothelial cells. These cells have long processes that wrap around the vessels. They are contractile and release a wide variety of vasoactive agents. They also synthesize and release constituents of the basement membrane and extracellular matrix. One of their physiologic functions appears to be regulation of flow through the junctions between endothelial cells, particularly in the presence of inflammation.

3.1.3 Venules & Veins

The walls of the venules are only slightly thicker than those of the capillaries. The walls of the veins are also thin and easily distended. They contain relatively little smooth muscle, but considerable venoconstriction is produced by activity in the noradrenergic nerves to the veins and by circulating vasoconstrictors such as endothelins. Anyone who has had trouble making venipunctures has observed the marked local venospasm produced in superficial forearm veins by injury. Variations in venous tone are important in circulatory adjustments.

3.2. Vasomotor Regulation of Cutaneous Microcirculation

At rest, at least 50% of the circulating blood volume is in the systemic veins. Twelve percent is in the heart cavities, and 18% is in the low-pressure pulmonary circulation. Only 2% is in the aorta, 8% in the arteries, 1% in the arterioles, and 5% in the capillaries. Although only 5% of the circulating blood is in the capillaries, this 5% is in a sense the most important part of the blood volume because it is across the systemic capillary walls that O_2 and nutrients enter the interstitial fluid and CO_2 and waste products enter the bloodstream. The exchange across the capillary walls is essential to the survival of the tissues.

In resting tissues, most of the capillaries are collapsed, and blood flows for the most part through the thoroughfare vessels from the arterioles to the venules. In active tissues, the metarterioles and the precapillary sphincters dilate. The intracapillary pressure rises, overcoming the critical closing pressure of the vessels, and blood flows through all the capillaries. Relaxation of the smooth muscle of the metarterioles and precapillary sphincters is due to the action of vasodilator metabolites formed in active tissue and possibly also to a decrease in the activity of the sympathetic vasoconstrictor nerves that innervate the smooth muscle.

The vascular endothelium modulates smooth muscle tone by releasing several vasoactive substances. Nitric oxide (NO) is involved in regulation of vascular tone and plays an important role in regulation of blood pressure and blood flow distribution. It is released during basal conditions in response to chemical stimuli, such as acetylcholine (ACh), and in response to mechanical stimuli, such as shear stress. The terminal guanidino nitrogen of the amino acid L-arginine is the precursor of NO. L-arginine analogues, such as N^G-monomethyl-L-arginine (L-NMMA), inhibit the synthesis of NO.

Skin blood flow is controlled by two branches of the sympathetic nervous system, a noradrenergic vasoconstrictor system and a cholinergic active vasodilator system. Sympathetic vasoconstrictor and vasodilator nerves innervate all areas of nonglabrous skin, whereas areas of glabrous skin (palms, soles, lips) are innervated only by sympathetic vasoconstrictor nerves. Another important distinction between glabrous and nonglabrous skin is the existence of arteriovenous anastomoses (AVA), which are thick-walled, low-resistance conduits that allow high flow rates directly from arterioles to venules. In glabrous skin, AVA are numerous and richly innervated by sympathetic vasoconstrictor nerves. Therefore, in these areas, opening or closing of these AVA can cause substantial changes in skin blood flow. In contrast, nonglabrous skin has few if any AVA and is innervated by both sympathetic vasoconstrictor and vasodilator nerves.

As core body temperature begins to rise, the initial increase in skin blood flow is mediated by a release of vasoconstrictor tone; upon reaching a specific threshold, sweating and reflex active vasodilatation are initiated, stimulating the co-release of acetylcholine (ACh) and an associated vasodilator from sympathetic cholinergic nerves. ACh-induced vasodilatation depends on intact vascular endothelium, whereas sodium nitroprusside (SNP) acts directly on vascular smooth muscle cells. Endothelial dysfunction can therefore be evaluated by comparing ACh- and SNP-induced vasodilatation. ACh and SNP may be introduced transdermally by iontophoresis, and the increases in perfusion may be quantified by the laser Doppler flowmetry (LDF) technique. ACh-induced vasodilatation may be mediated by generation of NO, PGs, or endothelial-derived hyperpolarizing factor. Administration of exogenous acetylcholine to endothelial cells does indeed affect vasodilatation in skin, but it is not clear whether this is due to NO, prostaglandins (PGs), or other mechanisms. There are findings that support the role of PGs

(and not NO) for affecting the cutaneous vasodilatation caused by exogenous ACh as well as conflicting results which conclude that PGs are not involved in the vasodilator response of skin blood vessels to ACh. More recent evidence shows that exogenous ACh effects vasodilatation in human skin by mechanisms that involve both NO and PG production although it is not clear in what proportions.

The oscillations recorded in the blood flow reflect both vasomotion and flow motion. The vasomotion is usually defined as rhythmic changes in the diameter of the small blood vessels, produced by contraction and relaxation of the muscular components in their walls. The flow motion results from the motion of the blood cells and their interaction with the vessel walls. The cardiac frequency (~1 Hz in a resting, healthy subject) and the respiratory frequency (~0.3 Hz) have been reported in the peripheral blood flow signal, measured by laser-Doppler flowmetry. They were also demonstrated in simultaneous measurements of ECG, respiration, and peripheral blood flow recorded at different sites of human skin. Endothelial activity may also be evaluated by the dynamics of the blood perfusion signal. The application of endothelium-dependent (ACh) and an endothelium-independent (sodium nitroprusside) vasodilator demonstrated that endothelial involvement in blood flow oscillations is manifested in the frequency interval from 0.0095 to 0.021 Hz (Kvernmo et al., 1999; Stefanovska et al., 1999), representing the intrinsic myogenic activity of vascular smooth muscle. It was also found that the main difference between free flap and intact skin occurred in the frequency interval between 0.021 and 0.052 Hz, which is proposed to be compelling evidence for repetitive sympathetic control of blood flow oscillations (between ~20 and 50 s). There is further evidence of an oscillation with a period of around 1 min (0.01 Hz), which is modulated by the endothelium.

4 Clinical Significance of the Perfusion Record

4.1 Perfusion monitoring in post-operative and intensive care settings

Digit replantation and free tissue transfer are areas of clinical need in which postoperative monitoring devices that aid in recognizing vascular disturbances could lead to earlier detection of vascular occlusion and improved salvage rates. Several methods have been used to satisfy this need including pulse oximetry, temperature monitoring, Laser Doppler flowmetry and serial quantitative skin surface fluorescence, but no currently available device has become widely accepted for routine monitoring due to complexity, poor sensitivity and specificity, or in the latter case, adverse side effects (Colwell et al., 2006).

Studies in which pulse oximetry was used post-operatively in digit replantation surgery showed that values greater that 95% were associated with a viable digit whereas decreasing values were suggestive of arterial or venous occlusion (Graham et al., 1986). However, the pulsatile flow required for accurate readings is not always detectable in the immediate post-operative setting for which it is most important. While surface thermometry is easy and inexpensive, measurements are too often influenced by other factors and thus are not very reliable. Compared with thermometry, laser Doppler is more sensitive and specific, but adds expense, complexity, and false reports of increase flow with probe or tissue motion. Serial quantitative fluoroscopy is a sensitive and minimally invasive diagnostic tool for detecting disturbances in tissue microcirculation but requires fluorescein mixing and injection, the labor intensiveness of 3 measurements per hour, and a small risk of side effects (Colwell et al., 2006).

Inadequate tissue perfusion and oxygenation are likely to contribute to the development of organ failures and increased mortality in critically ill patients (Pittard et al., 1994). For this reason, assessment of the adequacy of oxygen supply to organs and tissues is essential. Monitoring of tissue oxygenation and organ function in the clinical setting is largely based on measuring traditional variables of resuscitation, such as global hemodynamics, pulse oximetry, capillary refill, urine output, or indirect biochemical markers (Boldt, 2002). However, continuing regional tissue dysoxia can persist despite the presence of an apparently adequate systemic blood flow, pressure, and arterial oxygen content, highlighting the need for specific indices of perfusion at tissue level.

4.2 Systemic endothelial dysfunction and the microvasculature

There is growing evidence that microvascular disease plays a prominent role in the pathogenesis of macrovascular disease and mortality (Marcus et al., 1990) and there is increasing evidence that coronary microvascular dysfunction may be one underlying mechanism in patients with symptoms and signs of myocardial ischemia without angiographically detectable coronary artery disease, and even in asymptomatic patients with cardiovascular risk factors (Chilian, 1997; Kaufmann et al., 2000). Microvascular processes, primarily in the coronary microcirculation, have also been implicated in left ventricular dysfunction and subsequent heart failure, particularly in people with diabetes and hypertension, as well as in patients with dilated or hypertrophic cardiomyopathy. However, the majority of these studies have been cross-sectional and have focused on small samples of highly selected symptomatic patients. In addition, since the coronary circulation cannot be visualized in vivo and methods to assess the coronary

microcirculation are invasive and applicable only in experimental settings, what is known about microvascular mechanisms in the pathogenesis of congestive heart failure has been derived indirectly from studies of functional parameters such as myocardial blood flow and coronary flow reserve.

One method of evaluating vascular abnormalities via the microvasculature has been to measure retinal microvascular abnormalities through a computer-assisted method which measures retinal vessel diameters from digitized photographs (Hubbard et al., 1999). Retinal microvascular abnormalities, such as retinal arteriolar narrowing and retinopathy, have been associated with hypertension (Hubbard et al., 1999; Sharrett et al., 1999; Wong et al., 2004), diabetes (van Leiden et al., 2003; Wong et al., 2002), and with prevalent and incident CVD and mortality (B. E. Klein et al., 2004; Van Hecke et al., 2003; Wong et al., 2001), suggesting that retinal microvascular disorders may be a marker of atherosclerosis. Additionally, retinopathy is an independent predictor of congestive heart failure, even in persons without preexisting coronary heart disease, diabetes, or hypertension. (Wong et al., 2005). These results suggest that microvascular disease may play an important role in the development of asymptomatic heart disease in the general population. However, several studies have failed to show an evident relationship between retinal microvascular abnormalities and markers of atherosclerosis or macrovascular disease (R. Klein et al., 2000; van Hecke et al., 2006; Wong et al., 2003). This may be due to alternative pathways linking retinal microvascular disease and macrovascular endothelial dysfunction. Secondly, the retinal microcirculation may not sufficiently resemble the systemic microcirculation.

Several studies have suggested that microcirculation in skin resembles the microcirculation in other tissues and could be used to mirror the state of the microcirculation in

other vascular beds and overall cardiovascular health. The surface area of dermal capillaries provides for about 30 m^2 of endothelial coverage and cutaneous blood flow usually averages 10–20 $ml \cdot min^{-1} \cdot 100\ g^{-1}$. However, this may vary between 1 and 200 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, indicating a remarkable plasticity of regulatory control in this vascular bed (Clough et al., 2002). The skin, therefore, is highly vascular and an easily accessible model of the microcirculation. In individuals with hypertension, microvascular defects can be demonstrated in heart, skeletal muscle and skin. Similarly, although muscle is the main peripheral site of insulin-mediated glucose uptake, an association of diabetes and/or insulin resistance with microvascular function has been reported in heart, skeletal muscle and skin (RG et al., 2003). Moreover, metabolic and vascular effects of insulin could be demonstrated in skin (Serne et al., 2002; Tooke et al., 1985). Taken together these studies suggest that microvascular function in skin resembles microvascular function in other tissues in many ways.

The skin microcirculation offers an opportunity to noninvasively explore the relation of systemic microvascular dysfunction to cardiovascular disease and its risk factors (Antonios et al., 2001). Several studies have demonstrated that impaired microvascular responses in skin are associated with elevated blood pressure and insulin resistance (Antonios et al., 2001; Caballero et al., 1999; Irving et al., 2002; Serne, Gans, ter Maaten, Tangelder et al., 2001; Serne, Gans, ter Maaten, ter Wee et al., 2001). Impairment of postischemic forearm skin reactive hyperemia as measured by laser Doppler signal has also been associated with increased heart disease risk according to the Framingham risk score (Vuilleumier et al., 2002) as well as with manifest cardiovascular disease such that cutaneous FMD offered higher specificity for the diagnosis of coronary artery disease compared with brachial FMD (Shamim-Uzzaman et al., 2002). Lower endothelium-dependent vasodilatation and capillary recruitment in skin was found in men and

women with increased coronary heart disease (CHD) risk scores independent of body mass index (RG et al., 2003). Additional support comes from studies demonstrating that impaired responses of the skin microcirculation may be reversed after cholesterol-lowering therapy (Haak et al., 2001; Khan et al., 1997; Rauch et al., 2000).These findings suggest that microvascular function in skin may be a valid model for the study of the relationships between cardiovascular risk factors and microvascular function. That is, individuals at an increased risk for cardiovascular disease are characterized by an impaired microvascular function in skin. It is nevertheless important to note that the relationship between the commonly assessed brachial FMD does not clearly correspond to the magnitude of skin reactive hyperemia (Hansell et al., 2004; Shamim-Uzzaman et al., 2002). Thus, further research must investigate the possibility of different vasodilatory mechanisms or factors that would account for this lack of correlation between conduit vessels and the cutanous microcirculation.

4.2.1 Atherosclerosis

Atherosclerosis is a chronic inflammatory disease that affects essentially all arterial beds including the thoracic and abdominal aorta, coronary arteries, carotids, peripheral, renal and mesenteric arteries. It results from a complex interaction between genetic and environmental factors that causes the arterial wall to respond to inflammatory stimuli through the actions of endothelial cells, smooth muscle cells, inflammatory cells, and platelets. These produce a large variety of substances such as growth factors, cytokines, reactive oxygen species, enzymes, signaling factors that profoundly alter the arterial wall structure, and finally lead to the development of the atherosclerotic plaque. The plaque is composed of varying amounts of smooth muscle cells; monocyte-derived macrophages; T lymphocytes; cholesterol; cholesteryl

esters; phospholipids; and extracellular connective tissue. This ensemble forms the extracellular matrix including collagen, proteoglycans, and the pericellular matrix formed by fibronectin and elastic fibers. The plaque eventually progresses to the point of obstructing the arterial lumen, Atherosclerotic cardiovascular disease is the main cause of death in the western hemisphere. For the past few decades, the principal focus of treatment and investigation has been its complications, notably myocardial infarction, angina, sudden death, and heart failure. Very substantial economic and human resources, including imaging studies, catheterizations, coronary bypass surgery, catheter interventions, pacemakers, and medical treatments, have been used for the diagnosis and treatment of these complications. In spite of undeniable progress in all these areas with improved overall outcomes, treating complications of a disease is in fact a medical failure; we are acting after the events A better alternative would be to prevent the development of the basic disease, i.e., atherosclerosis, or at least to identify those patients who are at risk of an acute event and to intervene before damage occurs. Identifying causative factors of the primary disease is the fundamental principle of so-called *primary prevention.* The rational basis for the application of the principle lies in the ability to recognize individuals who are at risk for development of the disease, as well as those who are at risk for complications; the logical consequence is the customized use of effective therapeutic approaches.

Atherosclerosis begins very early in life and appears in asymptomatic infants, adolescents, and young adults, both male and female, as fatty streaks in the intima of systemic arteries (Stary, 1989). Data from the Bogalusa Heart Study (Berenson et al., 1992) and the multi-center Pathobiological Determinants of Atherosclerosis in Youth study (McGill et al., 1997), have demonstrated a strong association of specific antemortem risk factors with vascular lesions in children and young adults, showing that the development of atherosclerosis is an early

phenomenon in humans although its clinical manifestation may only occur decades later. Moreover, the extent of asymptomatic atherosclerotic lesions increased markedly in young people with multiple risk factors suggesting that early interventions focusing on modifiable risk factors early in life are of great importance (Berenson et al., 1998). The endothelial dysfunction present in childhood and teenage years relates to the presence of traditional risk factors, including hypercholesterolemia, a family history of premature coronary artery disease, hypertension and cigarette smoking.

It is also important to note that the development of atherosclerotic plaques progresses slowly and without significant narrowing of the arterial lumen due to compensatory enlargement and vascular remodeling (Glagov et al., 1987). Consequently blood flow may be normal even under conditions of increased oxygen demand, such as in intense exercise. The severity of lumen narrowing of atherosclerotic arteries has long been believed to depend on the extent of accumulation of plaque along the arterial wall while changes in overall dimensions of the arterial wall were not appreciated. Therefore, it was thought that there was a direct relation between the severity of lumen stenosis and plaque area size, but it is now well-documented that concomitant changes in the total cross-sectional area of the arteries occur that are of the utmost importance in determining the final size of the lumen and hence the degree of blood flow impairment and severity of ischemia (Birnbaum et al., 1997). In the initial phases of plaque formation there is a thickening of the arterial wall and external expansion that delays the development of lumen narrowing. In this phase, conventional coronary arteriography shows no luminal abnormalities or only arterial irregularities. However, in more advanced stages of atherosclerosis when the plaque occupies >30% to 40% of the vessel area, and especially after deep vascular wall injury (as seen after percutaneous interventions), this protective mechanism fails, and inflammatory changes and

fibrosis may lead to constriction of the arterial wall. Arterial remodeling explains the long asymptomatic period so often seen in patients with coronary artery disease. This is especially important when considering that lesions that are not hemodynamically significant or flow-limiting, e.g., those causing < 70% stenosis, may suddenly become unstable, suffer rupture, and cause partial or total occlusions precipitating an acute coronary crisis. Many factors contribute to this phenomenon, including structural characteristics of the plaque, endothelial erosions, inflammation, and thrombosis.

Because atherosclerosis is a generalized macrovascular disease, lesions in one vascular territory predict disease in other arterial regions. This systemic nature was documented by the Framingham study which showed that patients first presenting myocardial infarction, cerebral vascular accident, heart failure, or peripheral vascular insufficiency would, in the following 10 years, manifest disease in another vascular territory in proportions varying from 16% to 50%, both for men and women (Kannel et al., 1979). Similar risk factors are present among patients with coronary, peripheral, and carotid atherosclerosis. Of particular importance is evidence that disease in noncoronary arteries is a powerful predictor of CHD mortality. Aortic, peripheral, and carotid artery diseases have been termed "Coronary Heart Disease Equivalents" because the level of CHD risk and CHD event rates associated with these conditions is approximately equivalent to the level of risk seen in stable CHD. The rate of CHD events in persons with atherosclerotic vascular disease in other territories is similar to event rates in patients with known CHD. Thus, screening for atherosclerosis in other vascular regions has been considered for CHD risk evaluation.

Atherosclerosis manifests as a broad spectrum of clinical pathology involving both coronary and noncoronary circulation. Because the incidence of CAD is a direct function of the

development and progression of atherosclerotic plaque formation, the use of noninvasive surrogate markers of atherosclerosis can aid in the diagnosis of cardiovascular disease through the identification of subclinical disease. Cardiac catheterization and coronary angiography currently provide an accepted standard for visualizing the luminal surfaces of the epicardial coronary arteries and for detecting coronary artery stenosis. However, the invasive nature and procedure-related rate of morbidity and mortality remain a significant concern for the use of these modalities.

4.2.2 Diabetes

Macro- and microvascular disease are currently the principal causes of morbidity and mortality in patients with type I and type II diabetes mellitus. Microvascular disease is the hallmark of retinopathy, neuropathy, and nephropathy, whereas macroangiopathy in diabetes is manifested by accelerated atherosclerosis, which affects vital organs (heart and brain). Diabetes by itself is a powerful and independent risk factor for coronary artery disease (CAD), stroke, cardiomyopathy, congestive heart failure, and peripheral arterial disease. Cardiovascular disease accounts for up to 80% of premature excess mortality in diabetic patients. In fact, diabetes has been designated by the American College of Cardiology and the American Heart Association as a CAD risk equivalent. However, CAD is often silent and more advanced at the time of diagnosis, and is associated with a less favorable prognosis than in the general population (Heller, 2005). As a result, prevention of cardiovascular complications for diabetic patients is a pressing issue.

Vascular pathologies of both type I (insulin-dependent) and type II (non-insulin-dependent) diabetic patients, have mostly been described under enhanced oxidative stress. The abnormal metabolic state that accompanies diabetes causes arterial dysfunction. Relevant

abnormalities include chronic hyperglycemia, dyslipidemia, and insulin resistance. These factors contribute to microvascular endothelial dysfunction and render arteries susceptible to atherosclerosis. In particular, the hyperglycemia of diabetes leads to glucotoxicity, which causes insulin resistance. Similarly, elevated free fatty acid (FFA) levels in diabetes, obesity, and dyslipidemias lead to lipotoxicity, which underlies other shared mechanisms of insulin resistance and endothelial dysfunction. (Kim et al., 2006). Impaired endothelium-dependent (nitric oxide–mediated) vasodilatation has been demonstrated in various vascular beds of different animal models of diabetes and in humans with type I and II diabetes. Inflammation also plays a role such that the loss of endothelium-derived NO permits increased activity of the proinflammatory transcription factor nuclear factor kappa B (NF-κB), resulting in expression of leukocyte adhesion molecules and production of chemokines and cytokines (Creager et al., 2003). These actions promote monocyte and vascular smooth muscle cell migration into the intima and formation of macrophage foam cells, characterizing the initial morphological changes of atherosclerosis. Diabetes alters function of multiple cell types, including endothelium, smooth muscle cells, and platelets, indicating the extent of vascular disarray in this disease and underscoring the need for cardiovascular intervention in relevant diabetic populations.

Published data suggest that abnormal endothelial function precedes other evidence of vascular disease and that the progression of metabolic syndrome to type II diabetes parallels the progression of endothelial dysfunction to atherosclerosis. Both type I and type II diabetes, like metabolic syndrome and other cardiovascular risk factors determine an abnormal endothelium response thought to precede the development of atherosclerosis. Diabetes mellitus substantially increases the risk of developing coronary, cerebrovascular, and peripheral arterial disease and the pathophysiology of vascular disease in diabetes involves abnormalities in endothelial, vascular

smooth muscle cell, and platelet function. These abnormalities contribute to the cellular events that cause atherosclerosis and subsequently increase the risk of the adverse cardiovascular events that occur in patients with diabetes and atherosclerosis. Microvascular function in skin has been found to be impaired in young patients with diabetes using noninvasive laser Doppler methods (Golster et al., 2005), suggesting it is possible to detect subclinical abnormalities of the microcirculation. Furthermore, target-driven, intensive intervention directed at multiple risk factors (behavior modification and pharmacologic therapy) has been shown to reduce the risk of cardiovascular events, as well as microvascular events, by ~50% in patients with type II diabetes (Gaede et al., 2003). Thus, it is important to identify patients at risk for developing cardiovascular complications who may not demonstrate obvious symptoms. Appropriate screening and aggressive intervention can significantly benefit many patients with diabetes and identification of these patients early allows risk stratification while the disease progression is likely to be managed.

4.2.3 Peripheral Arterial Disease

Peripheral arterial disease is defined as any occlusive arterial disease beyond the coronary circulation and outside the cranium. However, it most commonly manifests as reduced blood flow in the lower extremities due to atherosclerosis and is diagnosed by the ankle/brachial blood pressure index (ABI) which detects vascular stenosis in the lower extremities. In population studies, people with low ABI have been found to have a considerably higher prevalence of cardiovascular disease, defined as a history of myocardial infarction, coronary artery bypass graft, stroke, or stroke surgery or other measures of clinical CVD such as angina or congestive heart failure. Compared with healthy individuals, patients with PAD have a 2- to 3-fold increased risk

of cardiovascular disease-associated mortality. This confirms that atherosclerosis is a diffuse disease that affects multiple vascular beds. Thus, an abnormal ABI is often indicative of clinically significant cardiovascular disease.

The major symptom of PAD is intermittent claudication (IC) which presents as pain, numbness, weakness, or fatigue in limbs cause by exercise-induced ischemia that subsides with rest. Data from the Framingham Study and other population studies have shown that reported IC, which manifests in later middle age groups, severely underestimates the true prevalence of flow-limiting PAD. Noninvasive testing for true PAD using ABI standards in populations indicates that the true prevalence is at least five times higher than would be expected from reported cases of IC. There are several reasons for this. Foremost, even after symptoms develop, many patients with IC consider their leg pain to be an inevitable consequence of aging and do not report their symptoms. Thus, PAD frequently remains undiagnosed until the condition progresses to a point at which the quality of life is significantly impaired. Another reason seems to be that IC does not occur in all people with PAD and may not be debilitating (Comerota, 2003). As a result, many physicians view PAD as being a relatively benign condition instead of as a marker of systemic atherosclerosis. Up to 60% of patients with PAD and 35% of those with concurrent IC remain undiagnosed (Hirsch et al., 2001).

The risk factors for PAD are similar to those for coronary artery disease (CAD) and cerebrovascular disease (CBVD), but diabetes and cigarette smoking have a particularly strong association with PAD. In fact, heavy risk factor burden appears to be the cause of PAD in people two to three decades earlier than conventional forms of the disease. If PAD is identified on the commonly used basis of an ABI of <0.90, its prevalence in diabetic patients may be as high as 29% (Marso et al., 2006). Smoking is the most powerful risk factor for PAD with a history of

smoking reaching a prevalence as high as 91% in some studies (Barretto et al., 2003). As with cardiovascular impairments that are diagnosed in diabetes, management of PAD already assumes widespread atherosclerosis and involves risk factor modifications that include multiple drug therapies and aggressive lifestyle changes. Surgical intervention is prescribed for the most severe cases, such as those who suffer from limb-threatening ischemia. However, in almost two-thirds of patients presenting for PAD surgery have clinically silent coronary disease, which is believed to cause a high rate of perioperative complications and mortality. ABI has been found in population-based cohorts to be highly specific but not sensitive for predicting cardiovascular disease. As a result, it is not appropriate for use as a generic screening test in asymptomatic populations (Doobay et al., 2005). It is, however, a useful addition global risk assessment. Furthermore, as with other forms of cardiovascular disease, it is important to institute screening and diagnosis of patients at risk for PAD or intermittent claudication when classic risk factors such as smoking, diabetes, dyslipidemia, hypertension, obesity, or increasing age are present.

5 Clinical Implications of Noninvasive Assessment

5.1 Significance of Atherosclerosis Screening

5.1.1 Risk Stratification

Despite many available risk assessment approaches, a substantial gap remains in the detection of asymptomatic individuals who ultimately develop coronary heart disease. The relationship between the demonstration of atherosclerosis has a variable, and often uncertain, relationship to the development of future CHD events. A valuable screening test should: a) identify high- and low-risk groups more accurately (e.g., low proportion of false negative and false positives); b) enhance the identification of high-risk individuals; c) result in a favorable impact on disease outcomes; d) be relatively free of risk; e) be cost-effective when compared to the current screening modalities; and f) educate the public concerning atherosclerosis and vascular disease risk (Wilson *et al.*, 2003). Measuring atherosclerosis also aids clinical and research-oriented applications beyond risk stratification and prediction, including evaluation of patient response to interventions and identification of novel genetic, cellular and molecular determinants of risk.

The Framingham risk score is a very popular and useful global risk score based on traditional risk factors such as age, sex, smoking, blood pressure, diabetes, arterial hypertension, and cholesterol values. According to the Framingham risk score, individuals are ranked into low-, intermediate-, and high-risk groups based on their chances of experiencing events within 10 years. The low-risk group has less than 6% risk over 10 years, the intermediate-risk group, 6% to 20% risk over 10 years, and the high-risk group, equal to or higher than 20% risk over 10 years (see Figure 2). Risk for the latter group is equivalent to those for patients with stable established

coronary artery disease. Greenland et al. (2001) analyzed the North American adult population and estimated that 35% are in the low-risk group, 40% in the intermediate-risk group, and 25% in the high-risk group. This scoring system is used to facilitate identification of patients at different risk levels with implications for counseling and treatment. Although of great practical value, this and other similar scoring systems are designed to predict risk and not the physical presence of the disease.

5.1.2. Primary Prevention

Vascular disease prevention is most cost-effective in high-risk patients, but the high-risk strategy of identifying patients with elevated levels of risk factors is problematic because traditional risk factors predict only half of vascular events. In an era of cost awareness and limited resources, this has also led to a debate about who should be treated. Individuals are often selected using surrogate markers of atherosclerosis, such as prior cardiovascular events, number of risk factors and lipids levels. This allows identification of the subjects at highest risk, but for the large population at moderate risk, better strategies are needed. With cardiovascular diseases as the leading cause of death in the Western world, prevention of atherosclerosis and its complications is a major goal of national and global healthcare. Recent advances in prevention research and the introduction of powerful lipid-lowering drugs has brought unprecedented mortality reductions in both secondary and primary prevention. Emerging coronary risk factors

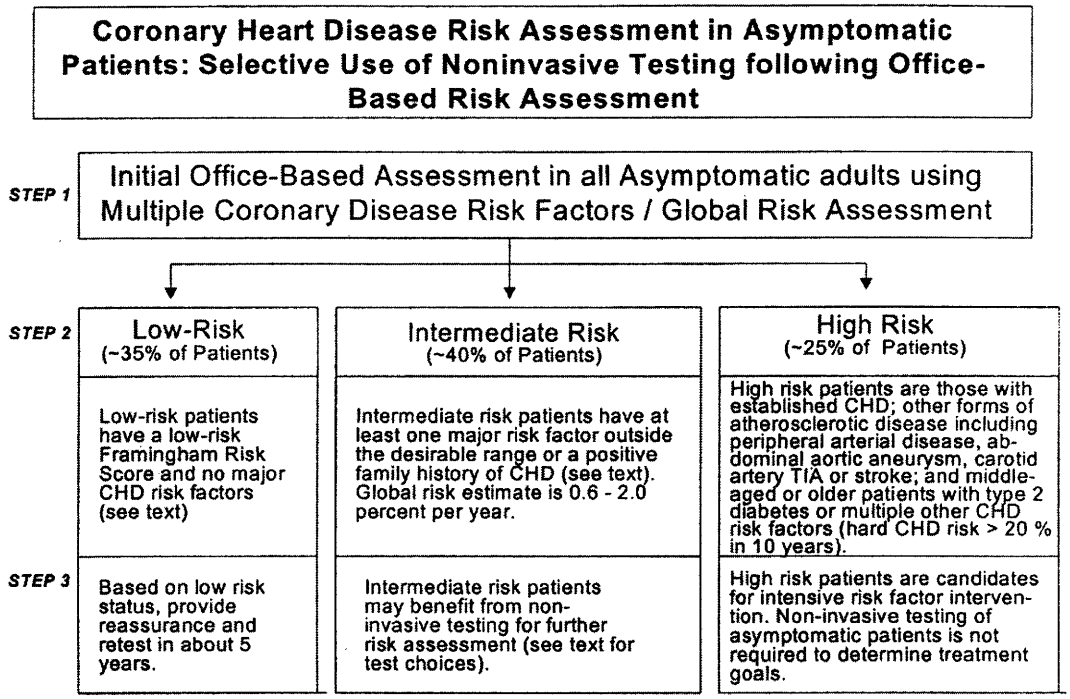

Figure 2 Flow chart of office-based risk assessment.(Greenland *et al.*, 1998)

have been described including inflammatory, infectious, and thrombotic markers, and there has been a steady flow of reports that focus attention on potential new ways of predicting coronary risk. In addition, noninvasive tests for subclinical atherosclerotic disease are available and becoming widely promoted for risk assessment in asymptomatic patients so that office-based measures can initiate the process of selecting patients for further intervention or additional testing.

Given the increasing atherosclerosis pandemic, advances in diagnostic modalities, and health economics issues, it is of increasing urgency to define the most effective prevention strategy for subjects at risk of atherosclerotic events. Patients at high risk (those with diabetes, other vascular disease, or multiple risk factors) clearly benefit from aggressive risk factor reduction including a combination of healthy eating and exercise with pharmacological therapy. Patients without traditional risk factors or at low risk over both the short and long term might

only require advice regarding healthy lifestyles. However, the majority of people fall into the intermediate group, which also represents the greatest challenge for treatment decisions. Patients in this intermediate risk group do not currently qualify for the most intensive risk factor interventions. The problem with this approach is that the treatment algorithms take into account short-term (10 years) rather than long-term risk (30 years), and there is a wide range of risk within this large group. It is this group that could potentially benefit from further risk stratification with endothelial function testing. If prospective studies confirm the predictive nature of endothelial markers for cardiovascular outcomes, then incorporation of these measures into risk factor models would lead to more effective prevention. A positive test in a subject at low to moderate risk would identify an individual whose risk would warrant pharmacological treatment. Other proposed markers of risk, including coronary calcium score (electron beam computed tomography), carotid intimal-medial thickness, ankle-brachial index, and stress testing.

5.1.3 Value of noninvasive screening

Improved noninvasive assessment of global cardiovascular risk is valuable in many situations. First, it would improve routine cardiovascular risk estimation and allow for better selection of high-risk individuals for additional exams and for institution of preventive measures. Furthermore, individualized risk assessment would be possible for asymptomatic individuals with multiple risk factors who are categorized as being at "intermediate risk." Because treatment decisions in patients at intermediate risk for CHD can be difficult, further risk stratification by noninvasive tests to assess atherosclerotic burden may be particularly useful within this risk category. Intermediate-risk patients could benefit most from further risk stratification testing, if such testing is feasible, practical, targeted, and effective at further defining risk or in motivating effective behavioral changes. In these, recommendations for lifestyle changes may be better

justified and possibly more convincing if atherosclerosis is documented objectively. Lastly, this method might improve patient selection for primary prevention of atherosclerosis by objectively documenting clinically silent atherosclerosis with a favorable impact on cost effectiveness in cardiovascular prevention. Accordingly, the Prevention V Conference examined whether current techniques or a combination of tests can optimize or improve risk assessment for primary prevention of CHD and suggested the potential for more routine use of office-based risk assessment for initial patient stratification (Grundy et al., 2000). Noninvasive quantification of atherosclerosis severity will probably enhance, but not replace, conventional risk factor assessment, as these analyses offer complementary information.

The "gold standard" for many manifestations of vascular disease, especially arterial occlusive disease, is conventional x-ray angiography, an invasive, costly, and potentially hazardous procedure. Atherosclerosis can be quantified non-invasively using the increasingly reliable and precise modalities described herein, which include ultrasound and magnetic resonance imaging. While each modality assesses "atherosclerosis", the particular morphological entities captured may reflect different aspects of atherogenesis with different biological determinants. For instance, among carotid ultrasound determinations, intima-media thickness (IMT) may reflect medial hypertrophy from hypertension, while plaque volume and stenosis and calcium deposition may additionally reflect foam cell proliferation, scarring or thrombosis. Clarifying the biological and clinical correlates of images may guide the choice of modality for specific applications. In addition, these tools are presently used to assess structures at a single time point. However, using them to follow temporal changes may further enhance their value. In this regard, certain modalities, such as ultrasound assessment of carotid plaque area or volume, may be more sensitive than others, such as assessment of IMT, for detecting temporal changes in

atherosclerosis. Combining modalities - and adding new biomarkers of disease - may be necessary to grasp the full complex vascular phenotypic picture of both individual subjects and groups of patients. In evaluating new determinants and novel therapies, it will be important to consider the biology and clinical correlates of a specific measured atherosclerosis phenotype in order to select the most appropriate modality.(Spence et al., 2004)

5.2 Noninvasive techniques for evaluation of atherosclerosis

Waiting until patients have symptomatic vascular disease is problematic because about half of patients who experience a stroke or myocardial infarction have no warning symptoms. It would be desirable, therefore, to have noninvasive methods for identifying patients at higher risk by the presence of preclinical atherosclerosis. Noninvasive detection of atherosclerosis should ideally involve methods that are safe, inexpensive, noninvasive, reliable, and reproducible. Additionally, their results should correlate with the extent of atherosclerotic disease and have high positive and negative predictive value for clinical events. Here, the major noninvasive methods of testing for atherosclerotic disease are described.

5.2.1 Ankle/Brachial Index (ABI)

Individuals with peripheral arterial disease of the lower extremities are among the highest-risk vascular patients. The presence of peripheral arterial disease is an indicator of widespread atherosclerosis in other vascular territories such as the coronary, carotid, and cerebrovascular arteries. There is substantial evidence that peripheral arterial disease is a

predictor of future cardiovascular outcomes such as myocardial infarction, stroke, and death. Patients with symptomatic peripheral arterial disease, that is, those with intermittent claudication, are well known to have markedly increased risks of CHD events and of other cardiovascular disease (CVD) events - up to 15-fold increased in one study (Criqui et al., 1992).

The ankle–brachial index (ABI) is the ratio of the ankle to brachial systolic blood pressure, and a value of <0.90 indicates the presence of flow-limiting arterial disease affecting the limb. An illustration is shown in Figure 3. The ABI is used in the diagnosis of peripheral arterial disease of the extremities in symptomatic patients and in the assessment of vascular risk in asymptomatic patients. The ABI is a simple and noninvasive test that can be performed in the office or clinic setting. The intraobserver variability of the test in trained observers is low at ≈7%. The validity of the ABI for detecting ≥50% stenosis in the leg arteries is high (90% sensitivity and 98% specificity). (Greenland et al., 2000)

An abnormal ABI, in an otherwise asymptomatic patient, provides incremental prognostic information, especially in people older than 60 years of age or in smokers. The ABI could therefore be useful in refining risk prediction in these categories of intermediate-risk patients. The ABI can be measured in a vascular laboratory or physician's office with inexpensive equipment, which consists of an ordinary blood pressure cuff and a Doppler ultrasonic sensor. Systolic blood pressure is measured by Doppler ultrasonography in each arm and in the dorsalis pedis (DP) and posterior tibial (PT) arteries in each ankle. The higher of the two arm pressures is selected, as is the higher of the two pressures in each ankle. The right and left ankle–brachial index values are determined by dividing the higher ankle pressure in each leg by the higher arm pressure. An ABI <0.90 in either leg is considered evidence of PAD, and progressively lower ABI values indicate more severe obstruction.

There is a considerable overlap of persons with ABI-detectable PAD and clinical cardiovascular disease. In population studies, persons with a low ABI have been found to have considerably higher prevalence of cardiovascular disease (CVD) (defined as history of myocardial infarction, coronary artery bypass graft, stroke, or stroke surgery, or other measures of clinical CVD such as angina or congestive heart failure) than persons with a normal ABI. These data confirm that atherosclerosis is a diffuse (ie, systemic) disease and that an abnormal ABI test will often indicate significant atherosclerosis in other vascular beds. Several studies have demonstrated that after adjusting for conventional cardiovascular risk factors, a low ABI is an independent predictor of cardiovascular risk. However, among population-based cohorts, the ABI is found to be highly specific but not sensitive for predicting cardiovascular disease. In other words, a low ABI helps to "rule in" a high-risk patient, but a normal ABI does not "rule out" a high-risk patient.(Doobay *et al.*, 2005) Because of its low sensitivity (yet high specificity), the ABI cannot be used as a generic screening test. Rather, it must be used in a focused manner, choosing individuals for whom the yield of the test is expected to be higher.

The American Heart Association (AHA) Prevention Conference V described the ABI as a strong and independent risk factor for cardiovascular mortality and recommended it be used to detect subclinical disease in the prevention of cardiovascular mortality and stroke. AHA recommended that the ABI might be a useful addition to the assessment of CHD risk in selected populations, especially among people ≥50 years of age or among those who appear to be at intermediate or higher risk for cardiovascular disease on the basis of traditional risk factor assessment, such as cigarette smokers or individuals with diabetes mellitus. Their recommendation takes into account that the prevalence of a low ABI increases significantly with

age and is 4 to 5x higher among individuals >70 years of age compared with those <50 years of age.

Despite convincing evidence from population-based studies showing increased ABI to predict a wide range of cardiovascular end points, few physicians currently use ABI in clinical screening, and the financial incentives have not been established. This may be partly due to the absence of advertising and commercialization of this test, which limits its current use to primarily a research tool and not to widespread use as a self-referred test by the public. The relatively low cost of equipment and performance of the required measures for determination of ABI, and its ability to detect subclinical peripheral arterial disease, suggest there may be benefit for self-referral in certain populations at potential risk, such as persons aged 50 years and over or those with multiple risk factors.

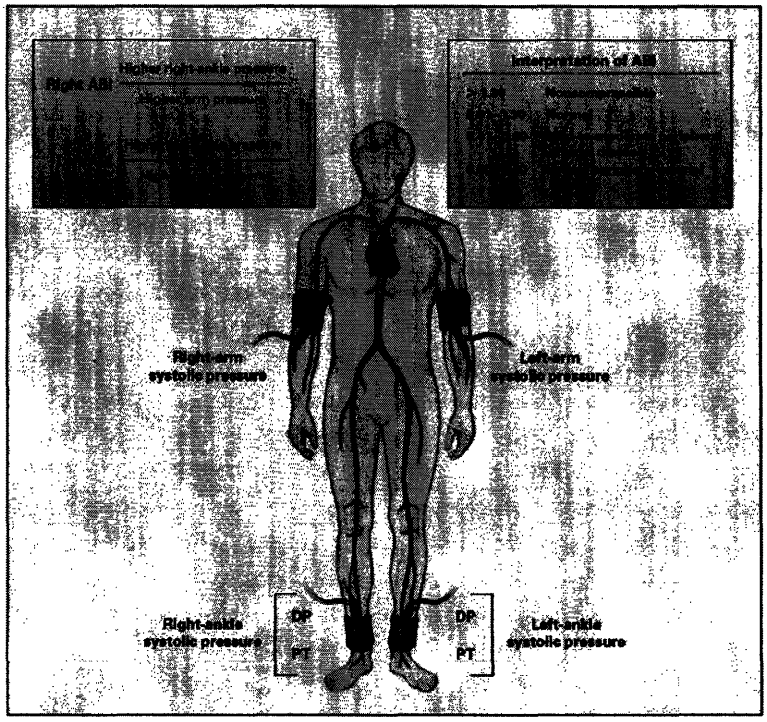

Figure 3: Measurement of the Ankle–Brachial Index (ABI). (Hiatt, 2001)

5.2.2 Endothelium-dependent flow-mediated vasodilatation (FMD)

A widely used noninvasive technique has evolved to evaluate flow-mediated vasodilatation (FMD), an endothelium-dependent function, in the brachial artery using high-resolution ultrasound imaging. The capacity of blood vessels to respond to physical and chemical stimuli in the lumen confers the ability to self-regulate tone and to adjust blood flow and distribution in response to changes in the local environment. Many blood vessels respond to an increase in flow, or more precisely shear stress, by dilating (lowering the vascular resistance). This phenomenon is designated FMD. A principal mediator of FMD is endothelium-derived NO. By creating a shear stress stimulus that produces an NO-dependent response, the FMD measurements can be used as an assay of NO bioavailability (a combination of NO production by the endothelium and destruction by reactive oxygen species). In humans FMD is typically assessed in the large peripheral conduit arteries (brachial, radial and femoral) and is often taken to represent the response in the more clinically relevant coronary circulation (Anderson et al., 1995).

The reactive hyperemia test is the most popular technique (Celermajer et al., 1992). Typically, forearm or hand ischemia is induced and maintained for 5 minutes by interrupting arterial blood supply with a blood pressure cuff inflated to suprasystolic pressure. When the pressure is released, reactive hyperemia occurs caused by dilation of the distal microvasculature. The reactive hyperemic response can be characterized by two distinct phases: a maximal, or peak, response that occurs within a few seconds after removal of the occlusion and a more prolonged total hyperemic period that is thought to represent the blood flow debt repayment. Theory

suggests that shear stress and alterations in hydrostatic pressure during reactive hyperemia result in the local release of nitric oxide (NO) with subsequent vasodilatation that can be imaged and quantitated by ultrasound as an index of vasomotor function. NO is of particular interest to researchers as it is an antiatherogenic molecule, and a reduction in its bioavailability may play a role in the pathogenesis of vascular disease (Cooke et al., 1997). A small FMD response is interpreted as indicating a low NO bioavailability and possibly an associated increased risk of vascular disease or cardiac events.

Although the brachial artery circulation is most commonly interrogated to determine changes in blood vessel diameter during reactive hyperemia, other peripheral arteries may be evaluated, including the carotid, superficial femoral, and radial arteries. This technique is attractive because it is noninvasive and allows repeated measurements. Brachial artery reactivity has been shown to accurately reflect coronary reactivity and therefore can be used as a surrogate marker of coronary endothelial function (Celermajer et al., 1994). This observation is based on the principle that endothelial dysfunction actually precedes clinical manifestations of coronary artery disease and is thus a better prognostic indicator of events than coronary lesions themselves. FMD is associated with several risk factors and can be improved by the correction of many classic risk factors such as hypercholesterolemia and arterial hypertension (Corretti et al., 2002).

A potential advantage of testing flow-mediated dilation is the unique information gained about vascular function. In contrast, the other modalities largely provide information about the presence and severity of fixed anatomic disease, which may be less relevant to the pathogenesis of events. Brachial FMD measurement is one of the few noninvasive techniques that can measure peripheral endothelial dysfunction, even in subjects as young as 5 years of age. Given the long preclinical phase of atherosclerosis and the rarity of clinical cardiovascular events

before the age of 30, FMD of the conduit arteries is emerging as an important surrogate marker of vascular risk in the young. The major limitations of this technique are the need for ultrasonographic expertise and a significant day-to-day variability (about 25%) due to biological circadian rhythms (Verma et al., 2003). Furthermore, despite widespread use in clinical research, the methods being used for the noninvasive assessment of peripheral endothelial function are insufficiently standardized. For example, in brachial artery reactivity studies, the occluding cuff is placed above the elbow by some, whereas others place it below the elbow. The time after cuff deflation when measurements are taken also varies between laboratories. Finally, there is considerable operator dependence in several aspects of how the test is performed. Therefore, this tool is limited in clinical utility until standard methodology is developed that will provide more consistency for longitudinal follow-up and allow comparisons of data from various laboratories.

Under specific conditions this stimulus has been shown to elicit a primarily NO dependent FMD response (Joannides et al., 1995) although later evidence reveals that certain shear stress stimulus profiles illicit mechanisms primarily independent of NO (Mullen et al., 2001). Different occlusion durations, cuff positions, degrees of ischemic dilatation (with the addition ischemic forearm exercise) or areas of the circulation examined (upper *versus* lower limb) result in distinct shear stress profiles created upon occlusion release (Betik et al., 2004). This suggests that there are multiple mechanisms involved in FMD and that the mechanisms involved in any given response are highly sensitive to the nature of the stimulus imposed (stimulus response specificity). Unfortunately, guidelines in the current literature provide an inadequate description of and rationale for these critical conditions that are required to evoke a reliably NO-dependent FMD response.

In addition to a lack of standardization in endothelial function testing, the role of non-NO FMD mechanisms in the development of vascular disease has been largely ignored in human studies (Pyke et al., 2005). The common clinical view, as stated in the technique report by the International Brachial Artery Reactivity Task Force (Corretti et al., 2002), is that vascular endothelial NO production accounts for FMD. However, evidence suggests that this not entirely true, challenging the notion that FMD specifically reflects NO-mediated endothelial function. In particular, it is well-researched that an increase in shear stress results in the endothelial production of at least three vasodilators: NO, prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF). Shear stimulus has also been demonstrated to elicit production of endothelin-1 (ET-1), a potent vasoconstrictor with pro-atherogenic properties (Yoshizumi et al., 1989). Thus, it would seem that vascular tone is the net result of interaction between simultaneously active vasoregulatory factors. The idea of multiple interacting mechanisms in FMD is also supported by evidence of sympathetic modulation of FMD. Many pathologic characteristics associated with endothelial dysfunction as evidenced by blunted FMD also demonstrate sympathetic hyperactivity such as in aging, obstructive sleep apnea, heart failure, and hypertension (Tschakovsky et al., 2005). Nonpathological states associated with elevated sympathetic activation also demonstrate blunted FMD such as diurnal variations in FMD (Otto et al., 2004) and mental stress, particularly in persons characterized by high levels of hostility (Gottdiener et al., 2003). The notion of sympathetic activation in reducing FMD is still controversial. It has recently been shown that not all means of sympathetic arousal blunts FMD (Dyson et al., 2006), adding further support to the notion that multiple cascades are activated by reactive hyperemic shear stress. Future studies must be geared towards identifying exactly what dilatory mechanisms are at work, in what proportions and over what time courses, in response to

specific shear stress stimulus profiles. This will allow experimenters and clinicians to design tests that are better able to isolate and investigate specific vasodilatory pathways associated with FMD.

Abnormalities in peripheral endothelial function, detected by methods such as brachial artery ultrasound, correlate with the presence of coronary vasomotor dysfunction. Furthermore, as with the coronary circulation, peripheral vasomotor function is diminished in subjects at risk for atherosclerosis, and medical interventions and lifestyle changes that reduce atherosclerotic risk are also associated with improved peripheral vascular function. The correlation in endothelial function in both the coronary and the peripheral vasculature suggests that a common pathway contributes to endothelial dysfunction in both vascular beds. On the basis of the literature, which demonstrates an association between endothelial dysfunction and the presence of either risk factors for atherosclerosis or atherosclerosis itself, endothelial function testing has rapidly grown into a widely used research tool. In the area of endothelial dysfunction measurement, there is a striking need to determine the prognostic value of the approach in a cohort of patients for whom cardiovascular risk prediction is more tenuous than in most of the populations studied to date. However, the lack of appropriate data demonstrating that vascular function testing contributes specific and independent prognostic value continues to limit its clinical utility. Virtually none of studies performed up to this time pertain solely to populations without manifest cardiovascular disease or without diseases that have a high a priori likelihood of underlying vascular disease (Gokce et al., 2002). Furthermore, the previously mentioned limitations in standardization and lack of research towards dissecting out the multiple mechanisms underlying FMD in response to shear stress must be addressed.

5.2.3 Duplex Ultrasound Measurement of Carotid Intima-Media Thickness (IMT) and Plaque Area

High resolution ultrasound examination of the carotid arteries can provide determinations of intima-media thickness (IMT), total cross-sectional area of carotid plaques, or severity of arterial stenosis (by Doppler ultrasound). CIMT uses ultrasound to measure the combined thickness of the intimal and medial layers of the carotid artery walls. Originally, this method had been used primarily in clinical research, in epidemiologic studies and in clinical trials evaluating the effects of different interventions to slow the anatomic progression of atherosclerosis. Current ultrasound instrumentation with transducers ≥8 MHz are most capable of identifying the 2 arterial interfaces (lumen-intima and media-adventitia) necessary for measuring IMT. The screening examination is performed bilaterally on the extracranial carotid artery segments. These segments are the distal straight 1 cm of the common carotid arteries, the carotid bifurcations, and the proximal 1 cm of the internal carotid arteries. Circumferential longitudinal scans can identify IMTs that are >1.3 mm on the near and far walls of each segment (total of 6 walls per side). A template can be used to identify these IMT values. If the IMT value is >1.3 mm, the actual thickness of each lesion is measured with ultrasound instrument calipers. IMT is an operational measurement definition of a single characteristic of atherosclerosis based on considerable information documenting that both the intima and media are involved in atherogenesis and the anatomical progression of lesions. Several pathological studies have demonstrated that increases in intimal thickness (fibromuscular hyperplasia) are associated with aging and that medial thickness (smooth muscle hypertrophy) is associated with hypertension, even in the absence of atherosclerotic plaque.

Several clinical intervention or prevention trials have illustrated the ability of carotid B-mode ultrasound imaging to monitor changes in IMT over time. Many epidemiological and clinical studies have documented that the average annual IMT progression rates are ≤0.03 mm. In such plaque monitoring studies, quantitative quality control of sonographers who perform the examinations and readers who make the measurements was found to be critical. Although serial measurements can be standardized in well-controlled research settings, protocols for sonographers to monitor IMT over time in a valid and reliable manner have not yet been implemented in clinical practice environments. This represents a barrier to routine use of IMT for serial assessment of plaque progression/regression in medical practice. Provided that technical issues of this type can be resolved by using standardized protocols for scanning and monitoring of IMT, this method would be useful in follow-up of patients treated for plaque progression or regression.

Across studies, different laboratories employ different scanning protocols, different methods for measurement of the carotid arterial wall thickness from the scans performed, and also different statistical methods for summarizing the numerous measurements performed and providing an overall estimate of atherosclerotic burden and disease progression. For example, scanning protocols can vary, from fixed-angle scanning to circumferential scanning aimed at defining the thickest region of the carotid artery wall. The site of scanning can vary greatly -- being confined to a point measurement or involving a larger region close to the flow divider of the carotid arteries. The instrumentation used for image acquisition also varies significantly and may affect the measurements obtained. Reading protocols vary, from online measurements that are less precise to more sophisticated offline digitizing of images and measuring by manual tracking or, more recently, by use of computerized automated border-detection techniques. Some

laboratories concentrate on defining the thickest region in the carotid arterial wall for a given segment or use an index derived from the thickest arterial wall regions across a number of segments, while others provide an estimate of mean carotid arterial wall thickness.

Thus, the use of "normal" reference values -- for baseline measurements and progression rates of atherosclerosis as well as for estimating cardiovascular risk -- are somewhat suspect. While such reference values may provide an overall framework, they may vary quite significantly in different laboratories. Such variation is important, since even small deviations from "normal" values may be of great clinical significance. Differences in measurements of carotid artery IMT at one point in time as well as in the progression rates derived from serial measurements may vary only by fractions of millimetres between patients with a multitude of risk factors who are at high risk for adverse events and patients without major risk factors who have only a low risk for cardiovascular events.

An additional exciting new perspective is the combined use of carotid artery IMT measurements with functional tests, which can also be performed noninvasively by ultrasonography. Brachial artery flow-mediated vasodilatation, a surrogate marker of vascular endothelial function, also involves ultrasonographic techniques and is being used increasingly in clinical research. Combining these two ultrasonographic methods can provide data on the anatomic extent of atherosclerosis and on its functional aspects, resulting in important and extensive information acquired noninvasively by relatively simple and inexpensive means. However, the carotid ultrasound examination for IMT measurement is not routinely performed in clinical ultrasound examinations, and the best-validated predictive value of carotid IMT derives exclusively from highly quality-controlled research laboratories (Greenland *et al.*, 2001). It is not

clear that a similar quality of IMT measurements can be derived in the majority of clinical ultrasound laboratories.

5.2.4 Stress Testing

Patients with typical angina/symptoms of coronary heart disease normally undergo routine noninvasive tests such as exercise ECG, echocardiography, myocardial scintigraphy, or pharmacological stress tests. These tests are used when patients are symptomatic with far-advanced disease, are based on indirect signs of atherosclerosis that result from inadequate myocardial perfusion, and have a high pretest probability of being positive. The exercise ECG – also called a stress test or treadmill test– is a test used to provide information about how the heart responds to exertion. It usually involves walking on a treadmill or pedaling a stationary bike at increasing levels of difficulty, while an electrocardiogram, heart rate and blood pressure are monitored. The vast majority of exercise testing is performed in adults with symptoms of known or suspected ischemic heart disease (Gibbons et al., 1997). The majority of positive stress tests are false positives in unselected asymptomatic people because of the low pre-test probability of disease (Pasternak et al., 2003). Hence, the absolute risk of cardiac events in these populations remains low; consequently, the routine use of exercise ECG testing in completely unselected asymptomatic populations is not recommended.

In addition to the standard exercise stress test, other methods of cardiovascular stress testing include scintigraphy and echocardiography. Exercise stress scintigraphy uses a radioactive tracer to enhance abnormal areas of myocardial blood flow. Stress scintigraphy can be performed with pharmacologic agents dobutamine or dipyridamole instead of exercise if the patient's condition does not allow sufficient physical activity for performing the study.

Echocardiography has recently been used in combination with exercise or pharmacologic stress testing as yet another form of noninvasive cardiac evaluation although it adds cost and complexity to the exam. The rationale for its use is that cardiovascular stress will result in ischemia, which in turn is manifested as a regional wall motion abnormality (WMA) distal to an obstructive coronary lesion.

Certain clinical circumstances render a lower than acceptable sensitivity and specificity to routine exercise stress testing (e.g. female gender, LVH, digitalis effect, conduction defects, etc.), and in these cases, the use of myocardial perfusion single-photon emission computed tomography (SPECT), as an adjunct to exercise testing, significantly improves on test reliability (Hachamovitch et al., 1996). They are performed to detect the possibility of flow-limiting lesions found in far-advanced disease but, when negative, give no information as to the presence of significant plaque burden and do not identify patients with subclinical atherosclerosis who may be at risk for future myocardial events, thereby alerting the patient/and physician to vigorously pursue preventive measures. Because few prognostic studies have included adequate numbers of asymptomatic people, data are scarce regarding the prognostic utility of noninvasive measures for the detection of myocardial ischemia in apparently asymptomatic people. With the exception of exercise ECG testing in asymptomatic men with increased cardiovascular risk profiles, few data exist to support the use of the noninvasive testing modalities (Armstrong et al., 2005).

5.2.5 Coronary Calcium Scoring (CCS)

Calcification within the coronary arterial wall is a recognized marker of atherosclerosis. It is a noninvasive measure of the calcified component of atherosclerotic plaque of the coronary

arteries and has been shown to be strongly related to the extent of atherosclerosis and to be an independent predictor of cardiac events (Arad *et al.*, 2005; Greenland *et al.*, 2004; Kondos *et al.*, 2003; Pletcher *et al.*, 2004; Taylor *et al.*, 2005). Electron Beam Computed Tomography (EBCT) and helical CT are highly sensitive and fast imaging methods for measuring the amount of coronary calcium in blood vessels and are being intensively evaluated as a noninvasive means of defining coronary atherosclerotic disease and identifying the asymptomatic but high-risk coronary artery disease (CAD) patient. EBCT uses an electron sweep of stationary tungsten target rings to generate x-ray images that can detect small amounts of calcium with considerable accuracy, whereas helical CT uses a continuously rotating x-ray source. Both allow quantification of calcium area and density, and both are relatively high cost. Histologic studies support the association of tissue densities ≥130 Hounsfield units with calcified plaque. EBCT calcium scores also correlate with pathological examination of the atherosclerotic plaque.

An important caveat is that vulnerable plaque can be present in the absence of calcium. That is, a CCS of zero does not eliminate the possibility of future cardiovascular events, nor does it rule out the existence of underlying significant coronary stenoses or myocardial ischemia (Brook et al., 2006). The pathological progression of atherosclerosis explains why vulnerable lipid-rich coronary lesions may not be calcified in the earlier stages of disease. It is likely that the prognostic strength of a high CCS is caused primarily by its correlation with the extent of underlying atherosclerotic burden throughout the coronary vasculature and not to the identification of calcium within any particular individual lesion. The inability of CCS testing to identify patients (particularly young people) with clinically significant minimally or noncalcified (yet potentially vulnerable) coronary plaques is a significant limitation. The value of a CCS measurement likely lies in its ability to correlate with the extent of underlying coronary

atherosclerosis by histopathology and invasive angiography. However, myocardial infarctions and significant coronary disease can occur in patients with little to no coronary calcium. A CCS of zero is not an infallible marker of low absolute coronary risk and it does not meaningfully lower event rates in high-risk patients with elevated risk scores. Other limitations are test cost, and the limited availability of equipment. When considering the risk benefit of subclinical CCS as an atherosclerosis screening tool, it is also important to note that CAC assessment using CT involves exposure to ionizing radiation. Although discovery of subclinical atherosclerosis in the coronary bed may change the CVD treatment strategy and be a valuable addition to an individual's care, the radiation exposure from this test should be considered as we determine the appropriateness of using this test in low-risk individuals or as part of a nationwide screening program. (Wilson *et al.*, 2003) The greatest potential for coronary calcium scores appears to be in the detection of advanced coronary atherosclerosis in patients who are apparently at intermediate risk.

5.2.6 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging is a potentially useful tool for assessing early atherosclerosis, as not only is it non- invasive and safe, but it yields information on flow as well as tissue characterization. Magnetic resonance imaging is used routinely in clinical practice to assess arteries in the peripheral circulation, in particular the cerebral arteries and the aorta; however, the application of magnetic resonance imaging to the coronary circulation has been limited by the relatively small size of these arteries and the rapid movements they undergo during the cardiac and respiratory cycles. Application of magnetic resonance imaging to the study of the coronary circulation is particularly attractive, as it would allow simultaneous

acquisition of information on the anatomy and ultrastructure of coronary arteries, as well as cardiac perfusion, metabolism and function. Through cardiac magnetic resonance, the dynamics of cardiac function can be appreciated in minute detail, and pathophysiological mechanisms became evident, forming the basis for clinical or surgical management of patients with known or suspected coronary artery disease.

Cardiac magnetic resonance methods allow assessment of global and regional cardiac function, cardiac volumes, myocardial perfusion, cardiac valves, pericardium, myocardial viability, and proximal coronary anatomy, among others. For example, constrast-enhanced magnetic resonance angiography is now routine for imaging of large vessels such as the aorta, carotid, and femoral arteries and replaces conventional angiography. MR can also differentiate plaque components with reasonable accuracy and detect morphologic changes in atherosclerotic arteries, such as arterial remodeling with increases in wall thickness, vessel wall area, and total vessel area. Individually, these methods have shown promise as alternatives to the established tools for the noninvasive detection of coronary stenosis and myocardial infarction. A unique advantage of cardiac magnetic resonance imaging is that several of its methods can be combined in a single scanning session.

The direct visualization of the atherosclerotic plaques will enhance our understanding of the natural history of this disease. Several invasive imaging techniques, such as x-ray angiography, intravascular ultrasound, angioscopy, and optical coherence tomography, as well as noninvasive imaging techniques, such as surface B-mode ultrasound and ultrafast computed tomography, can be used to assess atherosclerotic vessels. Most of the standard techniques identify luminal diameter or stenosis, wall thickness, and plaque volume. However, none of these

imaging methods can characterize completely the composition of the atherosclerotic plaque and therefore are incapable of identifying vulnerable or high-risk plaques.

High-resolution magnetic resonance imaging (MRI) has emerged as the potential leading noninvasive in vivo imaging modality for atherosclerotic-plaque characterization. MRI differentiates plaque components on the basis of biophysical and biochemical parameters such as chemical composition and concentration, water content, physical state, molecular motion, or diffusion. MRI provides imaging without ionizing radiation and can be repeated sequentially over time.

High-resolution MRI relies on the same principles as other MRI techniques. During the examination, the patient is subjected to a high, local magnetic field, usually 1.5 T, which aligns the protons in the body. These protons (or spins) are excited by a radiofrequency pulse and are subsequently detected with receiver coils. The detected signals are influenced by the relaxation times (T1 and T2), proton density (PD), motion and flow, molecular diffusion, magnetization transfer, changes in susceptibility, etc. There are 3 additional magnetic fields (gradient fields) that are applied during the MRI procedure: 1 field to select the slice and 2 fields to encode spatial information. The timing of the excitation pulses and the successive magnetic field gradients determine the image contrast. The ability to obtain images of the atherosclerotic vessels is dependent on the amount of available signal, contrast, and the lack of noise.(Fayad *et al.*, 2001)

5.2.7 Skin Sterol

A noninvasive test that provides a quantitative assessment of cholesterol in the skin has been shown to provide an independent assessment of risk for coronary artery disease. The test, called PREVU* Point of Care (POC) Skin Sterol Test, is conducted by placing a drop of

synthetic copolymer conjugated with digitonin and horseradish peroxidase. The digitonin binds selectively to the unesterified cholesterol or phytosterols in the skin, on the palm of the hand. After a one-minute incubation period, the area is blotted dry to remove any unbound digitonin solution. A second drop of indicator solution containing a substrate for the horseradish peroxidase enzyme is then added. When combined, a blue color from the hydrolysis product occurs in direct proportion to the amount of digitonin that is bound to skin sterol. After two minutes, a hand-held spectrophotometer is placed over the drop to measure the precise blue color, which indicates the skin sterol value.

Zawydiwski et al. (2001) reported a correlation between the magnitude of the skin cholesterol test result and the presence or absence of an abnormal treadmill stress test in asymptomatic patients with no known history of CAD. Furthermore, the association between the skin cholesterol and the treadmill results were not related to the ambient, serum cholesterol values, suggesting that the skin test may be affected by other determinants, suggesting skin tissue cholesterol is an independent risk factor. Follow-up studies also show skin sterol to be associated with angiographic disease (Sprecher et al., 2003) and Framingham risk score (Mancini et al., 2002). When measured in the Johns Hopkins site of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, skin cholesterol correlated with coronary calcium in Caucasian, but not African-American patients (Vaidya et al., 2005). Thus, this test provides the ability to evaluate the amount of cholesterol accumulated in skin tissue, which has been shown to parallel cholesterol build-up in coronary arteries. Proposed benefits of this method are that it is quick, noninvasive, painless, cost-effective and does not require any patient preparation or dietary changes.

In November of 2005, PreMD, Inc (formerly IMI International Medical Innovations, Inc.) began a 600-person multi-site study called Predictor of Advanced Subclinical Atherosclerosis

(PASA) to examine the relationship between skin sterol and carotid intima media thickness (CIMT), an established predictor of cardiovascular disease. Results from this study are expected to provide data for broader regulatory clearance for the PREVU* test in identifying asymptomatic at-risk patients for primary prevention.

5.2.8 Peripheral arterial tonometry (PAT)

This relatively new technique has been studied by a group of researchers from the Mayo Clinic in Rochester, Minn., and Tufts University Medical Center in Boston (Bonetti et al., 2003) and involves a finger plethysmographic device that allows the isolated detection of pulsatile arterial volume changes. RH-PAT (reactive hyperemia peripheral arterial tonometry) permits the noninvasive assessment of peripheral vascular reactivity by measuring digital pulse volume at rest and during reactive hyperemia. The device, made by Itamar Medical Ltd., Caesarea, Israel, consists of two finger-mounted probes which a system of inflatable latex air-cushions within a rigid external case. The probe design is able to envelop the finger up to and beyond its tip and allows the application of a constant and evenly distributed near-diastolic counterpressure within the entire probe, which increases sensitivity by unloading arterial wall tension, and prevents venous blood pooling to avoid venoarteriolar reflex vasoconstriction. Pulsatile volume changes of the fingertip are sensed by a pressure transducer and transferred to a personal computer where the signal is band pass-filtered (0.3 to 30 Hz), amplified, displayed, and stored.

Used originally to measure peripheral vasoconstriction during REM sleep (Lavie et al., 2000), this technology has been found to demonstrate impaired digital hyperemic response corresponding to coronary artery disease (Bonetti et al., 2004). Based upon the technique of measuring endothelial function through NO-mediated vasodilatation, the extent of reactive

hyperemia was calculated as an RH-PAT index, defined as the ratio of the average amplitude of the PAT signal over a 1-min time interval divided by the average amplitude of the PAT signal of before cuff inflation (baseline). In this study, the RH-PAT device was tested on 94 consecutive patients who were referred for coronary angiography. The average RH-PAT index was lowered in individuals with known coronary artery disease and demonstrated a correlation with coronary blood flow response to acetylcholine.

The FDA-cleared PAT technology can be applied to measure myocardial ischemia, obstructive sleep apnea, congestive heart failure, endothelial dysfunction, stress and others ("Itamar Medical Ltd."). The RH-PAT device for measuring endothelial dysfunction is integrated into the Endo-PAT2000 system and is a noninvasive tool with potential to identify patients during early stages of cardiovascular disease using a simple test based on digital reactive hyperemia (FMD). Further research must be done to explore the possibility of applying such a screening index in the general population and to investigate whether the RH-PAT index augments conventional risk scores.

5.2.9 Thermal monitoring

VENDYS™, a proprietary technology which was developed jointly by the UT Houston Health Science Center and Texas Heart Institute, measures a marker of vascular endothelial dysfunction by monitoring temperature changes at the fingertip. The test is conducted by inflating a cuff around the arm, forearm or wrist for two minutes. Fingertip temperature usually drops one to two degrees during this period. When the cuff is deflated, fingertip probes continuously monitor the temperature changes and feed data to software for analysis (Dvorin, 2005). The rate of temperature fall, recovery, and overshoot reflects the state of normal or

abnormal vascular reactivity (Kharalkar et al., 2004). Temperature that struggles to reach baseline indicates an impaired endothelium, while patients with normal endothelial function overshoot the baseline temperature before settling.

Endothelix's (www.endothelix.com) first clinical study was completed in 2004 at Cedars Sinai Hospital in Los Angeles where investigators compared digital thermal monitoring with ultrasound imaging for assessment of brachial artery endothelial function. Changes in temperature at the index fingertip of 30 healthy volunteers were measured before, during and after brachial artery occlusion. Temperature changes measured at the fingertip using DTM showed strong correlation with the conventional method of brachial artery endothelial function measurement using ultrasound imaging of brachial artery. Thus, this technique relies on the previously described correlation between brachial artery reactivity, coronary atherosclerosis, and digital microcirculation.

In contrast to the Endo-PAT2000 technology, this form of gauging endothelial function is proposed as being a more rapid form of endothelial testing and provides simultaneous data output. With this technology, the founders of Endothelix hope to provide a noninvasive, non-imaging, low-cost tool for detection of endothelial dysfunction in routine clinical practice.

5.3 Looking ahead: Early Detection and Primary Prevention

The goal of cardiovascular disease (CVD) screening is to accurately determine risk early in the natural history of disease. Adding subclinical disease markers to traditional CVD risk-factor screening has the potential to facilitate more appropriate, targeted interventions that will further reduce CVD morbidity and mortality in clinical and population-based settings. Various

studies have determined that subclinical disease markers of atherosclerosis improved the ability to identify the subset of individuals who are at increased risk for CVD outcomes. Examples of specific markers that have been shown to provide additional information beyond traditional CVD risk factors include ankle brachial index and carotid intima-media thickness (IMT). It is therefore logical to anticipate that the addition of noninvasive markers of atherosclerosis may enhance our ability to diagnose the amount and potential severity of early/asymptomatic CVD. Other atherosclerosis markers (magnetic resonance imaging, coronary artery calcium, and brachial artery vasoreactivity) also appear to have potential but may require additional scientific evidence documenting their validity, reproducibility, and value in predicting CVD events beyond conventional risk factors.

The American College of Cardiology (ACC) and the American Heart Association (AHA) endorse the global risk assessment based on the Framingham risk prediction model, which includes the traditional risk factors of age, gender, smoking, blood pressure, total and high-density lipoprotein (HDL) cholesterol. Once the patient's absolute CHD risk is assessed, the physician then determines whether simple reassurance, further lifestyle or pharmacologic intervention, or diagnostic testing may be warranted (Greenland *et al.*, 2001). The goal of additional noninvasive imaging for atherosclerosis is to improve identification of individuals at a high or low risk for CHD (i.e., optimize risk stratification so as few patients as possible are classified as intermediate risk). This presumes that such classification can aid physicians in prescribing a management strategy for prevention, in that patients assigned into a "high-risk" category will likely benefit from aggressive risk-factor modification, while those at low risk will less likely benefit. It is important to recognize that the outcome of efforts to better detect risk is dependent upon the effectiveness of the risk reduction therapies that ensue (Wilson *et al.*, 2003).

In addition to early detection, the effectiveness of therapeutic intervention or lifestyle changes can be tracked over time, lending strength to the goals of treatment. For example, measuring carotid plaque area by ultrasound has been presented as a way to evaluate and track severity of cardiovascular burden. Measuring plaque as a continuous variable appears to be more powerful than simply detecting the presence or absence of plaque at extracoronary sites or counting the number of sites involved. Regression of plaque is also used as the therapeutic goal so that follow-up measurements of plaque can determine whether therapy is successful. In practice, showing patients the pictures and measurements of their plaque progression often seems to help motivate them to implement lifestyle changes such as smoking cessation and dietary change. Conversely, plaque regression validates and encourages persistence with successful lifestyle changes (Spence et al., 2002).

5.4 Epidemiological considerations

Cardiovascular diseases are the single largest killer of men and women in the United States, accounting for the underlying cause of 37.3% of all deaths in 2003. CVD claims more lives than the next four leading causes of death (cancer, chronic lower respiratory disease, accidents and diabetes mellitus) combined (Thom et al., 2006). According to the National Health and Nutrition Examination Survey [NHANES 1999–2002] conducted by the CDC, one in three adult men and women has some form of CVD. More troubling, about two thirds of unexpected cardiac deaths occur without prior recognition of cardiac disease (Myerburg et al., 1993). The current methods of diagnosis and treatment have had little effect on the outcome of a disease that is largely preventable with institution of strict risk factor modification and statin therapy if discovered early.

Several important factors will serve to accelerate the rate of cardiovascular disease and its complications in the coming years. First, aging of the population will undoubtedly result in a concomitant increase in the incidence of chronic diseases, including coronary artery disease, heart failure, and stroke. The US Census Bureau estimates that there will be 40 million individuals aged >65 years in the United States in 2010 (*Projected Population of the United States, by Age and Sex: 2000 to 2050*, 2004). Second, there is currently an explosive increase in the prevalence of obesity and type II diabetes with their related complications of hypertension, hyperlipidemia, and atherosclerotic vascular disease. For CDC data from 1999-2002, 65.1% of adults in the US were overweight or obese, 30.4% were obese, and 4.9% were extremely obese (Hedley et al., 2004). In almost every age and racial/ethnic group, the prevalence of overweight or obesity exceeded 50%. In addition, there are presently 17 million individuals in the United States with diabetes mellitus (Grundy et al., 2002; Mokdad et al., 2001). This represents a doubling in disease prevalence over the past decade, and the number continues to increase sharply. Finally, there is an alarming increase in unattended risk factors in the younger population that will continue to fuel the cardiovascular epidemic for years to come (Williams et al., 2002). Pathological data have shown that atherosclerosis begins in childhood and that the extent of atherosclerotic change in children and young adults can be correlated with the presence of the same risk factors relevant in adults. The younger population is increasingly overweight, stemming from physical inactivity and poor dietary habits. "Adult-onset" type II diabetes is appearing with increasing frequency in children. Teenage smoking continues to be a major health hazard. Nearly 30% of high school students are present smokers and almost 6000 teenagers take their first cigarette every day, over 57% of whom will become chronic smokers (Giovino, 2002).

The cost of cardiovascular diseases and stroke in the United States for 2006 is estimated at $403.1 billion. This figure includes health expenditures (direct costs, which include the cost of physicians and other professionals, hospital and nursing home services, the cost of medications, home health care and other medical durables) and lost productivity resulting from morbidity and mortality (indirect costs). By comparison, in 2004 the estimated cost of all cancers combined was only $190 billion ($69 billion in direct costs, $17 billion in morbidity indirect costs and $104 billion in mortality indirect costs) (Thom et al., 2006).

Both BMI and weight gain are major risk factors for type II diabetes. Diabetes alone is the sixth leading cause of death in the United States and about 65% of deaths among people with diabetes are due to heart disease and stroke. While both type I and type II diabetes manifest cardiovascular complications, type II diabetes may account for about 90% to 95% of all diagnosed cases, and it is increasingly being diagnosed in children and adolescents. According to the CDC, the direct and indirect costs of diabetes total to $132 billion. Importantly, research studies have found that lifestyle changes can prevent or delay the onset of type II diabetes among high-risk adults. Lifestyle interventions included diet and moderate-intensity physical activity (such as walking for 2 1/2 hours each week). In the Diabetes Prevention Program, a large prevention study of people at high risk for diabetes, the development of diabetes was reduced 58% over 3 years. Additionally, a common complication of diabetes is lower limb amputation due to atherosclerotic occlusion and limb ischemia. This area of diabetes treatment would directly benefit from a more accessible measure of blood flow to the extremities, resulting in early detection of ischemic symptoms. Indeed, simply instituting more comprehensive foot care programs is projected to reduce amputation rates by 45-85%.

Early detection of endothelial dysfunction and subsequent lifestyle modifications could greatly increases survival from cardiovascular complications and reduce CAD risk. Numerous factors contribute to reducing cardiovascular risk, including exercise, dietary modifications, blood pressure reduction, and smoking cessation. Also shown to be associated with improved endothelial function are interventions such as antioxidant therapy, lipid-lowering therapy, ACE inhibitors, hormone replacement therapy, drug therapy to increase insulin sensitivity in diabetics, others.

Conclusion

There is a clear need for a safe and objective method of measuring perfusion in peripheral tissues, both for use as a diagnostic tool and also as a clinically important parameter of circulatory health. The utility of a noninvasive, continuous, real-time monitor of perfusion is important for key clinical goals and can be used either singly or in combination with other methods to (1) provide indirect information on the generalized microvascular 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. The amount of information available on a microvascular level should not be underestimated as emerging targets include atherosclerosis, diabetes, hypertension, peripheral arterial disease, complications including chronic skin ulcer development and healing potential. Assessment of adequate local microcirculation will potentially aid in minimizing tissue loss, support wound healing ability, and predict suitable amputation levels when necessary. Furthermore, improved understanding and testing of functional microcirculatory deficits and their relationship to macrocirculatory dysfunction will undoubtedly aid in diagnosis, prognosis, and treatment of large artery diseases and cardiac dysfunction. The challenge lies in doing so without involving additional cost or risk, such as that engendered by complicated and/or invasive procedures. Preliminary data indicates that thermal diffusion technology is an acceptable method of obtaining absolute measures of perfusion from skin in a safe and reliable way. By reviewing the existing literature on related technology, a clear context of proper design constraints can be assessed. Finally, knowledge of the clinically relevant areas that such a technology could be widely applied to supports the motivation for

designing a device which is relatively simple to operate, safe, continuous, and has sufficient resolution for measuring the exceedingly important biological parameter of tissue perfusion.

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