Thermal-Based Probe for Testing Endothelial Dysfunction and Possible Implications for Diagnosing Atherosclerosis

by

Muyinatu A. Lediju

SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE IN MECHANICAL ENGINEERING
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

JUNE 2006

ARCHIVES

Signature of Author: _____
Department of Mechanical Engineering
May 12, 2006

Certified by: _____
H. Frederick Bowman, Ph.D.
Harvard-MIT Division of Health Sciences & Technology
Senior Academic Administrator
Thesis Supervisor

Accepted by: _____
John H. Lienhard, V
Professor of Mechanical Engineering
Chairman, Undergraduate Thesis Committee

Thermal-Based Probe for Testing Endothelial Dysfunction and Possible Implications for Diagnosing Atherosclerosis

by

Muyinatu A. Lediju

SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING ON MAY 12, 2006 IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE IN MECHANICAL ENGINEERING

Abstract

Endothelial dysfunction is a precursor to atherosclerosis. Thus, the vascular health of an individual can be assessed if endothelial dysfunction can be readily and unambiguously quantified. A thermal-based approach using temperature and blood perfusion measurements in conjunction with an arterial challenge has the potential to quantitatively assess endothelial dysfunction. This report includes a detailed review of previous attempts to characterize endothelial dysfunction and a preliminary evaluation of a thermal-based approach that relies on temperature and perfusion measurements. Two simple thermal models are used to contextualize results obtained from this technique. Results reveal that this thermal-based method serves as a valid indicator of endothelial assessment while at the same time reducing some of the mitigating factors of existing approaches to identifying endothelial dysfunction. More testing must be performed in order to optimize this thermal-based approach.

Thesis Supervisor:
H. Frederick Bowman, Ph.D.

Title:
Senior Academic Administrator
Harvard-MIT Division of Health Sciences & Technology

Acknowledgements

I would like to thank Dr. Fred Bowman for his unending support and guidance in this project. I came to him as an eager undergraduate, searching for an appropriate senior thesis topic. He allowed me to assist him with the background research and preliminary testing of this thermal-based probe for identifying endothelial dysfunction. Throughout the course of the project, he has worked closely with me and helped me to produce the work that appears within these pages.

I also extend my gratitude to the many professors in the Mechanical Engineering Department at MIT who have helped me to build a solid foundation of basic mechanical engineering principles. Without such knowledge, it would not have been possible for me to formulate the thermal models, solve the accompanying differential equations, and present the solutions in a clear, neat, graphical form. All of which were necessary for understanding, analyzing, and drawing conclusions about the phenomena at hand.

Last but certainly not least, many thanks and appreciation to my dear mother who was always quick to offer more than enough love, encouragement, and support during my first two and a half years at the Institute. I long for the day to see her again. May she rest in peace until that day when "the Lord himself shall descend from heaven with a shout, with the voice of the archangel, and with the trump of God: and the dead in Christ shall rise first." (1 Thessalonians 4:16). In the meantime, I thank my loving brother, Abdul-Rahman Lediju, and many other supportive family members and friends who have done their part to fill in the gap.

Table of Contents

List of Figures

1 Introduction

Atherosclerosis is a disease that affects many people, both the young and the old. The disease is typically defined as hardening of the arteries. Dysfunction of the endothelial cells that line the artery is seen as a pre-cursor to atherosclerosis. As a result, a standard method for assessing endothelial dysfunction can lead to earlier assessment of atherosclerosis. This report includes an investigation of previous and existing methods for identifying endothelial dysfunction, such as flow-mediated dilation, brachial artery ultrasound, strain gauge plethysmography, arterial tonometry, and assessment using temperature. All of these existing approaches have certain limitations, such as operator-dependent variability, inconsistent reproducibility, and complex data analysis methods. A novel thermal-based approach that relies on evaluation of blood perfusion in addition to temperature seems to offer the most promise, especially since this approach does not have any of the aforementioned limitations.

Two simple models for the thermal interactions governing this thermal technique have been proposed. One model describes the temperature profiles within unperfused tissue when a constant temperature probe is placed in contact with the surface of the skin. The second model describes the temperature profile for perfused tissue.

To explore the proposed theory of assessing endothelial function via temperature and perfusion, a prototype of the thermal probe was built and tested. The thermal-based approach relies on temperature and perfusion measurements in response to reactive hyperemia. Therefore, an experiment was designed to measure temperature and perfusion in response to reactive hyperemia induced b arterial occlusion. Experimental data was evaluated to infer the status of a subject's vascular health. The results show that measuring temperature and perfusion can prove to be a useful tool in identifying endothelial dysfunction.

2 Endothelial Dysfunction

2.1 Function of Endothelial Cells

Endothelial cells make up the lining of all blood vessels. They have a number of functions, some of which include[1,2]:

- Regulating vasoconstriction and vasodilation, and hence the control of blood pressure
- Regulating vascular smooth muscle tone, local blood flow, vascular growth and repair
- Balancing blood clotting and clot dissolution: maintaining a dynamic barrier between tissue and the bloodstream
- Regulating immune function and inflammation, including inflammatory factors that underly plaque formation and instability in occlusive (atherosclerotic) vascular disease
- Controlling the passage of white blood cells, lipid particles, and foreign bodies through the vascular wall, into and out of the blood stream
- Regulating tissue nourishment, ion transport, and electrolyte balance

Based on the list above, endothelial cells are responsible for a number of bodily functions. Focusing on the relationship between endothelial cells and the vascular system, a healthy endothelium plays a central role in cardiovascular control.[3] For example, the healthy endothelium dilates in response to an increase in blood flow.[4] This vasodilation can be induced by a blood pressure cuff squeezing the forearm to block off circulation, followed by an immediate release to increase the blood flow.

There is also abundant evidence that the endothelium plays a crucial role in the maintenance of vascular tone and structure. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO), an endogenous messenger molecule formed in healthy vascular endothelium from the amino acid precursor L-arginine.[5] Other

endothelium-derived substances are prostacyclin (PGI2) and C-type natriuretic peptide (CNP).[6]

2.2 Characteristics of Endothelial Dysfunction

Any deviation from the normal function of endothelial cells is classified as endothelial dysfunction. For example, endothelial dysfunction is known to promote abnormal vascular growth.[6] In endothelium dysfunction the blood vessels' ability to dilate is impaired and its ability to constrict is paradoxically increased; as such, endothelium-derived dilating and constricting factors are reduced and increased, respectively.[7] Furthermore, impaired dilation is directly related to the ability of arterial endothelial cells to produce and release the vasodilator nitric oxide; this impairment is reflected in reduced production of NO. [8] Thus, abnormal vascular growth, impaired dilation, and reduced levels of nitric oxide in the blood stream can all be regarded as important markers of endothelial cell dysfunction. Additionally, there is also literature which supports the notion that endothelial dysfunction is a reversible disorder.[7]

To understand the effect of nitric oxide on endothelial dysfunction, the biological and biochemical processes must be explored. Decrease in the availability of nitric oxide is associated with increased platelet adhesion, increased plasminogen activator inhibitor, decreased plasminogen activator, increased tissue factor, decreased thrombomodulin, and alterations in heparan sulfate proteoglycans. The consequences include a procoagulant milieu and enhanced platelet thrombus formation. Furthermore, oxidized low-density lipoprotein (LDL) activates inflammatory processes at the level of gene transcription by up-regulation of nuclear factor kappa-B, expression of adhesion molecules, and recruitment of monocytes and macrophages. Elevated serum levels of LDL cholesterol overwhelm the antioxidant properties of the healthy endothelium and result in abnormal endothelial metabolism of this lipid moiety. Oxidized LDL is capable of a wide range of toxic effects and cell/vessel wall dysfunction, including impaired endothelium-dependent dilation and paradoxical vasoconstriction. These dysfunctions are the result of direct inactivation of nitric oxide by the excess production of free radicals, reduced transcription of nitric oxide synthase messenger RNA (mRNA), and posttranscriptional destabilization of mRNA.[4]

Furthermore, endothelial damage also triggers platelets to adhere and aggregate at the site of the damage, this enhances monocytes to enter the tunica intima, and proliferation within the tunica-media junction of the artery. This effect causes the arterial wall to herniate at this site. With increased monocyte invasion into arteries, and continual herniation the lumen of the artery can become processively reduced. This combination of biochemical and anatomical alterations contributes to oxidative stress and increased vascular damage; the so-called precursors for atherogenic changes within arteries.[9]

2.2.1 Endothelial Dysfunction as a Precursor to Atherogenesis and a Predictor of Vascular Disease

Studies have shown that endothelial dysfunction is significantly related to atherogenesis.[10] In fact, endothelial dysfunction is an early event in experimental studies of atherogenesis, and it precedes the formation of plaques. Furthermore, oxidative stress and increased vascular damage are also precursors to atherogenic changes within the arteries.[11] Therefore, endothelial dysfunction is the earliest measurable functional abnormality of the vessel wall in atherogenesis. [3]

Endothelial dysfunction reflects a vascular phenotype prone to atherogenesis and may therefore serve as a marker of the inherent atherosclerotic risk in an individual. In line with this hypothesis, dysfunction of either the coronary or peripheral vascular endothelium is an independent predictor of cardiovascular events, providing valuable prognostic information additional to that derived from conventional risk factor assessment. [7] Furthermore, in patients with diabetes mellitus, dysfunction of endothelial cells has been postulated as an initial trigger of atherosclerosis and is believed to play a pivotal role in the progression and/or development of vascular disease.[6]

2.2.2 Causes of Endothelial Dysfunction

One study stated that endothelial dysfunction is caused by various cardiovascular risk factors, metabolic diseases, and systemic or local inflammation.[5] Another study corroborates this notion by stating, "Endothelial dysfunction is a consequence of the harmful effects of the risk factors of atherosclerosis on the vessel wall and is more or less important depending upon the number of risk factors, their intensity and their duration."[3]

Endothelial dysfunction has been demonstrated in subjects with hypercholesterolemia, diabetes, hypertension, in patients who smoke, and in patients with atherosclerotic disease (coronary, peripheral arterial).[12(cited in 3), 13 (cited in 3)] Since endothelial dysfunction is seen as a precursor to atherogenesis and other types of vascular disease, many of the risk factors for these types of diseases are seen as risk factors for endothelial dysfunction. These risk factors include: [14]

- Elevated levels of oxidized serum cholesterol, triglycerides, fibrinogen, homocysteine, or insulin
- High blood pressure
- Obesity
- Lifestyle factors such as physical inactivity and tobacco smoking

In addition to risk factors for atherosclerotic diseases, possible causes of damage to the endothelium also include: free-radical reactions, chronic inflammation, and diabetes.[14]

Endothelial dysfunction also occurs in children. There is considerable evidence that the pathogenesis of endothelial dysfunction and atherogenesis in childhood is related to hypercholesterolemia and oxidation-sensitive mechanisms during early stages of human development. These mechanisms can affect the subsequent fate of vascular lesions in adult and elderly life.[15]

From the biological perspective, dysfunction of endothelial cells causes loss of the endothelium-derived substance NO, which induces an inflammatory response and recruits oxidized LDL to activate this response. "Oxidized LDL is capable of a wide range of toxic effects and cell/vessel wall dysfunctions that are characteristically and consistently associated with the development of atherosclerosis."[4]

3 Atherosclerosis

Atherosclerosis comes from the Greek words *athero*, which means gruel or paste, and *sclerosis*, which means hardness. It is typically defined as hardening or thickening of an artery. A normal artery has many layers. The *adventitia* is the outermost wall, made up of a fibrous material as well as smooth muscle cells. The *media* is the central layer of the artery, consisting of multiple layers of smooth muscle cells. The *intima* is an innermost layer of the artery and is made up of connective tissue, with endothelial cells acting as a lining inside the arteries. It is on this innermost layer where deposits of fats (lipids), cholesterol (lipoproteins), calcium, cellular waste products, and other substances build up to cause atherosclerosis. This build-up is called plaque.

3.1 Progression of Atherosclerosis

Injury to the endothelial cells is the first step toward atherosclerosis. Atherosclerosis begins when lipoproteins, or cholesterol, penetrate the endothelium and accumulate in the intima. Gradually, these lipoproteins undergo a series of chemical changes, one of which being oxidation. Oxidation occurs when excess amounts of unstable oxygen-free radicals are available to interact with and alter nearby molecules. The oxidized lipoproteins release a number of toxins into the arterial wall in response to oxidation and the area becomes inflamed.[16] The immune system is then signaled to release macrophages at the site of inflammation. This initiates a process called the inflammatory response. Macrophages literally "eat" the oxidized cholesterol leaving behind foamy cells that attach to the artery's smooth muscle cells. The foamy cells then build up within the artery. After the immune system senses the foamy cells, it releases other factors called cytokines, which attract more macrophages and perpetuate the whole cycle. This cycle usually repeats itself forming atherosclerotic lesions.[17]

The next step involves the recruitment of white blood cells, or leukocytes, to the site of the injury. These cells also migrate into the arterial wall, and over time, the site begins to accumulate more white blood cells and blood lipids, including various forms of cholesterol (LDL, VLDL and triglycerides). Eventually, a plaque forms. [16]

As fatty material accumulates, some of the plaque formations acquire a relatively thick covering. These are considered to be stable plaques and are a primary cause of narrowed arteries. Other plaques have a thinner, more volatile coating. These are called unstable plaques, because the coating can be stripped off, releasing small fatty particles into the bloodstream and causing plaque rupture.[16]

The site of the plaque rupture attracts blood platelets. When platelets find an injury, they rapidly gather and initiate a cascade of events that results in a blood clot. If this happens inside the coronary artery, the resulting blood clot can cause a heart attack. If it blocks a blood vessel that feeds the brain, it causes a stroke. And if it reduces blood supply to the arms or legs, it can cause difficulty walking and eventually gangrene.[18]

In short, atherosclerosis involves the slow buildup of deposits of fatty substances, cholesterol, body cellular waste products, leukocytes, and fibrin on the inside lining of an artery. The plaque that results can partially or totally block the flow of blood through the artery. If the plaque ruptures, this can lead to the formation of a blood clot on the surface of the plaque. The occurrence of either event can block the entire artery and cause a heart attack, a stroke, or even gangrene.

3.2 Mechanical Interactions during Atherosclerosis

From a mechanical point of view, the lesions of atherosclerosis do not occur in a random fashion. Hemodynamic factors interact with the activated vascular endothelium. Fluid shear stresses generated by blood flow influence the phenotype of the endothelial cells by modulation of gene expression and regulation of the activity of flow-sensitive proteins. Atherosclerotic plaques characteristically occur in regions of branching, marked curvatures, and geometric irregularities; it is in these regions where blood velocity undergoes sudden changes in magnitude and direction of flow. The decreased shear stress and turbulence has the potential to promote atherogenesis at these locations. Primary sites for these distinct geometric changes are the coronary arteries, the major branches of the thoracic and abdominal aorta, and the large conduit vessels of the lower extremities.[4]

3.3 Risk Factors for Atherosclerosis

There are certain risk factors for atherosclerosis, some of which include:

- Excess amounts of oxygen free radicals. Oxygen-free radicals are usually released as part of normal bodily processes, but environmental toxins, such as viruses or smoking, can hinder the body from releasing them and produce excess amounts.[17]
- High concentrations of LDL. It has been shown that the greater the concentration of LDL, the greater the risk of developing atherosclerosis.[9]
- Artery size. Atherosclerosis usually affects large and medium-sized arteries.[18]
- Age. Fatty deposits of plaque usually develop over many years.[17] Some hardening of arteries often occurs when people grow older.[18]
- High blood cholesterol, overweight, little exercise, smoking[19]

3.4 Atherosclerosis and Aging

It was once thought that atherosclerosis affected only middle- aged and elderly people. Since then, researchers have learned that atherosclerosis typically begins in childhood with fatty streaks that develop on the interior wall of an artery. These streaks lay the foundation for a buildup of plaque and also cause the artery to gradually lose its elasticity. This is compounded by the fact that the arteries naturally become less flexible with age. Although the process may take many years, it is greatly accelerated by the presence of saturated fat and cholesterol in the bloodstream.[16] Therefore, atherosclerosis progresses rapidly in some people and signs are prevalent even in their third decade.[18]

3.5 Systemic Nature of Atherosclerosis

Atherosclerosis can affect the arteries of the brain, heart, kidneys, arms and legs.[20] Typically, atherosclerosis affects medium and large sized arteries. It most frequently affects the aorta (the largest blood vessel in the body), the coronary arteries, the cerebral arteries, and sometimes arteries in the legs and abdomen.[17] As mentioned earlier, a blocked coronary artery can cause a heart attack, and blocked carotid arteries in the neck

or cerebral arteries in the brain can lead to a stroke. And, inadequate blood flow to the lower extremities can cause peripheral arterial disease – a condition that can lead to poor circulation, leg pain with walking (claudication), non-healing leg ulcers, and gangrene.[16]

3.6 Testing for Atherosclerosis

Atherosclerosis may not be diagnosed until symptoms develop. Prior to complications, atherosclerosis may be noted by the presence of a "bruit" (a whooshing or blowing sound heard over the artery with a stethoscope). The affected area may have a decreased pulse. Tests that indicate atherosclerosis (or complications) include:[21]

- An abnormal difference between the blood pressure of the ankle and arm (ankle/brachial index, or ABI)
- Doppler study of the affected area
- Ultrasonic Duplex scanning
- CT scan of the affected area
- Magnetic resonance arteriography (MRA)
- Arteriography of the affected area
- Intravascular ultrasound (IVUS) of the affected vessels
- Cardiac stress testing

Atherosclerosis involving the heart may also be diagnosed through:[16]

- Electrocardiogram, or EKG
- Exercise stress test
- Nuclear stress test, a form of stress testing that involves the injection of a radionucleotide contrast agent such as thallium, *myoview*, *sestamibi*
- Ultrafast computed tomography, or ultrafast CT
- Coronary Angiogram (more invasive)

Additionally, researchers are studying new tools to help find cardiovascular disease in earlier stages, before symptoms appear. For example, the National Heart, Lung, and Blood Institute is sponsoring a ten-year study called the Multi-Ethnic Study of Atherosclerosis (MESA). The MESA study will help show which risk factors are the best predictors of future heart disease in men and women and in certain ethnic groups. For now, individuals should suspect that they are at a high risk for atherosclerosis if they have high blood cholesterol, are overweight and get little exercise, smoke, or have any other risk factors.[19]

4 Identifying Atherosclerosis via Measurement and Assessment of Endothelial Dysfunction

4.1 Implications of the Connection between Endothelial Dysfunction and Atherosclerosis

Given the relationship between endothelial dysfunction and atherosclerosis, it is likely that the status of endothelial function may reflect the propensity of an individual to develop atherosclerotic disease, and thus, the presence of endothelial dysfunction may serve as a marker of an unfavorable cardiovascular prognosis.[7] For example, the brachial arterial dilator response to increased blood flow during reactive hyperemia has been shown to be caused mainly by an endothelial release of NO, to correlate significantly with endothelial function, as well as with the extent and severity of atherosclerosis. [3] Additionally, endothelial dysfunction induces disruption of the balance between vasoconstrictive factors and vasodilatory factors secreted from endothelial cells. Among these factors, NO and angiotensin II are especially important factors, and have been shown to exert various direct effects on the endothelial functions that are closely related to the pathogenesis of atherosclerosis. Endothelial dysfunction induces decreased NO bioactivity and increased angiotensin II expression, which increases oxidative stress and expression of adhesion molecules, cytokines, and chemokines. These conditions mediate inflammation, proliferation, and thrombogenesis in vessel wall and promote atherosclerotic lesions.[22]

Therefore, endothelial dysfunction is a key variable in the pathogenesis of atherosclerosis and its complications. With the important role of endothelial dysfunction for the development and progression of atherosclerosis, it seems attractive to consider endothelial dysfunction, aside from the treatment of established cardiovascular risk factors, as a primary therapeutic target in the prevention of atherosclerotic disease.[7]

Less-invasive or noninvasive techniques for the assessment of endothelial function include: strain-gauge forearm plethysmography in conjunction with intra-arterial infusion of endothelium-dependent vasodilators, such as methacholine or acetylcholine; a high-resolution external vascular ultrasound to measure flow-mediated endothelium-dependent dilation (FMD) of the brachial artery during reactive hyperemia; brachial artery ultrasound; arterial tonometry; and temperature assessment of the certain locations on the hand before, during, and after reactive hyperemia. These techniques are based on the fact that endothelial dysfunction is not confined to the coronary arteries but rather represents a systemic disorder that also affects peripheral vascular beds, including both conduit arteries and small resistance vessels in the extremities.[7]

4.2 Flow Mediated Dilation

Flow Mediated Dilation (FMD) is a technique that was developed in the 1990s; it uses ultrasound images to asses vasomotor dilation due to a stimulus, which is usually an inflated cuff placed around the bicep area. An image of the brachial artery is taken before and after the stimulus is applied to determine percent dilation. Essentially, FMD measures the ability of an artery to relax in response to the increase in blood flow, or blood velocity, caused by the stimulus. The technique also provokes the release of nitric oxide, resulting in vasodilation, which can be regarded as an index of vasomotor function.[23,24,25,26]

Looking at the technique from a biological perspective, the endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear stress. Although FMD is known to involve these specialized ion-channels within the endothelial cells, the precise mechanisms for the acute detection of shear forces and subsequent signal transduction to modulate vasomotor tone are not fully understood. However, the effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry (there are no voltage-gated calcium channels in endothelial cells). Calcium activates an enzyme, endothelial nitric oxide synthase (eNOS), and the subsequent generation of NO appears to account for FMD. Furthermore, endothelial denudation or treatment with a nitric oxide synthase (NOS) inhibitor abolishes FMD in a variety of arterial vessels.[26]

4.2.1 FMD Subject Preparation

The subject preparation for this technique must adhere to a specific protocol. This protocol takes into account the fact that numerous factors affect flow-mediated vascular reactivity, such as temperature, food, drugs and sympathetic stimuli. The protocol is as follows:[26]

- Subjects should fast for at least 8 to 12 h before the study.
- Subjects should be studied in a quiet, temperature-controlled room.
- All vasoactive medications should be withheld for at least four half-lives, if possible.
- Subjects should not exercise.
- Subjects should not ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.
- The investigator should be cognizant of the phase of the subject's menstrual cycle, as it too may affect FMD.

All of these confounding factors must be considered in preparing a subject in studies that seek to determine the impact of a single intervention. For observational cohort studies, data must be collected on those factors known to affect the measurement of FMD, and analysis should address their impact.

4.2.2 Measurement Methods for FMD

4.2.2.1 Equipment

Certain pieces of equipment must be available to evaluate FMD. Ultrasound systems must be equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler, an internal electrocardiogram (ECG) monitor and a high-frequency vascular transducer. A linear array transducer with a minimum frequency of 7 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images

with sufficient resolution for subsequent analysis. Image resolution is enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers. Timing of each image frame with respect to the cardiac cycle is determined with simultaneous ECG recording on the ultrasound system video monitor.[26]

4.2.2.2 Image Acquisition and Assessment[26]

The image acquired with this equipment should be standard for all test subjects. In order to ensure that this is the case, the subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging. Currently, cross-sectional imaging of the brachial artery cannot be used to determine maximum diameter or area of the lumen because of inadequate image definition of the lateral walls. Also, skew artifacts from cross-sectional imaging limit accurate diameter determination. In addition to 2D grayscale imaging, both M mode and A mode (wall tracking) can be used to continuously measure the diameter, yet these techniques may be more subject to error owing to tracking drift. No direct comparison has been made of diameter determinations from continuous recording using grayscale images versus wall tracking. During image acquisition, anatomic landmarks such as veins and fascial planes are noted to help maintain the same image of the artery throughout the study. A stereotactic probe-holding device can be helpful. Accurate analysis of brachial artery reactivity is highly dependent on the quality of ultrasound images.

Several studies have suggested that the maximal increase in diameter occurs approximately 60 s after release of the occlusive cuff, or 45 to 60 s after peak reactive hyperemic blood flow. The increase in diameter at this time is prevented by the NOS inhibitor N^G-monomethyl-L-arginine, indicating that it is an endothelium-dependent process mediated by NO. Other measures of vasodilator response include time to maximum response, duration of the vasodilator response and the area under the dilation curve.

Timing of the measurement during the cardiac cycle is also done in a precise manner. Brachial artery diameter should be measured at the same time in the cardiac

cycle, optimally achieved using ECG gating during image acquisition. The onset of the R-wave is used to identify end diastole, and the peak of the T-wave reproducibly identifies end systole. Peak systolic diameter is larger than end systolic diameter, because the vessel expands during systole to accommodate the increase in pressure and volume generated by left ventricular contraction. The magnitude of systolic expansion is affected by the vessel compliance, and it may be reduced by factors such as aging and hypertension (possibly by reduced bioavailability of NO). Thus, functional characteristics of the brachial artery may obfuscate the measurement of FMD if diameter is measured during end systole; however, this concern has not been tested in a rigorous trial.

Although it may seem simple, ultrasonographic assessment of brachial artery reactivity is technically challenging and has a significant learning curve. Ideally, an individual trained in the principles and technical aspects of 2D and Doppler ultrasonography would perform the technique. The learning curve typically requires several months and depends both on the technical skill of the individual and the frequency with which the technique is performed. Optimal training in the technique requires hands-on training by an experienced individual who can demonstrate the pitfalls and ultrasound artifacts and who can delineate manual techniques and optimal ultrasonography system parameters.

4.2.2.3 FMD Data Analysis[26]

Several approaches exist to describe the differences in any two sets of measurement results. One is the *correlation coefficient*, which is derived from data that represent the entire range of measurements anticipated in the setting in which the technique will be employed. A second metric is simply the *mean and range of differences between the measures*, which gives an intuitive understanding of the lower limits of differences that can meaningfully be ascribed to variation between subjects or secondary to intervention. The third metric, the *coefficient of variation*, is intended to communicate the size of the variance of a measure relative to the mean value of what is being measured. Because FMD is a percentage-ratio measure, small differences between observers appear very large.

There is no single ideal measurement to assess reproducibility of this technique. A scatterplot showing results obtained at time one and time two along with the line of identity, accompanied by the results of the three metrics described in the previous text, is likely the most complete way to describe reproducibility of FMD of the brachial artery. Rigorous attention to protocol standardization, training and ongoing quality improvement is critical to generating valid, reproducible data.

4.2.3 Studies Involving FMD

This section contains abstracts of three specific studies involving FMD: (1) Local Shear Stress and Brachial Artery Flow-Mediated Dilation; (2) Variability of Flow-Mediated Dilation Measurements with Repetitive Reactive Hyperemia; and (3) Noninvasive Assessment of Vascular Function in the Posterior Tibial Artery of Healthy Humans.

Local Shear Stress and Brachial Artery Flow-Mediated Dilation:

The first study recognizes that flow-mediated dilation is a homeostatic response to short-term increases in local shear stress. It focuses on the effect of risk factors on evoked shear stress caused by the stimulus for dilation. The risk factors included: age, sex, mean arterial pressure, pulse pressure, heart rate, body mass index, lipid medication use, and hormone replacement therapy. These risk factors were correlated with percent dilation in two separate models: one excluded the effect of shear stress and the other included it. The study agrees with the notion that evoked hyperemic shear stress is a major correlate of brachial artery flow-mediated dilation, but concludes that the associations between many risk factors and FMD may be a result of a reduced stimulus for dilation, rather than a result of impaired local conduit artery response during hyperemia.[23]

Noninvasive Assessment of Vascular Function in the Posterior Tibial Artery of Healthy Humans:

The second study sought to assess FMD in the lower legs of humans. Six healthy subjects (27 ± 6 yrs) were tested. Doppler ultrasound images of the posterior tibial artery

were taken before, during, and after 5 minutes of proximal cuff occlusion. FMD was measured as the percent increase in diameter after cuff release. Vascular tone was calculated using the resting diameter as a percentage of the vessel's vasoactive range. Minimum diameter occurred during ischemia and maximal diameter occurred following reactive hyperemia with local heating. The lower leg was heated with 10 minutes of immersion in 44°C water. Mean diameters at rest, cuff, and during release were 0.267 ± 0.062, 0.162 ± 0.036, 0.302 ± 0.058 cm, respectively. FMD was 13.5 ± 6.6 % and vascular tone was 29 ± 16.3%. The researchers also found that retesting on a second day produced mean diameter values within 8% of the first day. Larger resting diameter (decreased tone) correlated with decreased FMD ($r^2 = 0.73$). This study concluded that FMD and vascular tone can be measured in the posterior tibial artery, and this is a potentially powerful tool to non-invasively measure vascular health in the lower legs of people at risk for vascular disease.[25]

Variability of Flow-Mediated Dilation Measurements with Repetitive Reactive Hyperemia:

The third study set out to examine the effect of repetitive reactive hyperemia on brachial artery FMD as well as to determine whether brachial artery FMD is stable during a 2-h morning period. FMD was investigated in 20 apparently healthy college students on three randomized treatment days every 30 min, 60 min, and 120 min throughout a 2-h morning period (08AM to 10AM). The study found that repetitive reactive hyperemia over a 2-h period has no effect on FMD measurements in apparently healthy college students. There was also a stable FMD response throughout the 2-h morning period.[24]

4.2.4 Limitations of FMD

The development of FMD as a non-invasive measurement of brachial artery reactivity has increased the ability to identify and detect cardiovascular disease before the symptoms arise.[24] Despite its widespread use as a research tool and the development of guidelines to standardize its use,[26] protocols for assessing FMD of the brachial artery still vary among different laboratories and are operator dependent. This decreases the feasibility of this noninvasive technique as a valuable screening tool for endothelial

dysfunction in clinical practice. [7] Furthermore, the smaller baseline artery diameter seen in women, leading to an increased and possible false-negative FMD, has also questioned the reliability and sensitivity of this measurement; although, it is accepted that FMD varies among subjects. An additional limitation to this technique is the burdensome necessity of performing multiple post intervention measurements of flow-mediated dilation in order to capture the response of a single acute intervention. There are also interpretive limitations to this technique.[26] Thus, there is growing interest in developing a noninvasive, reliable method to assess endothelial function.

4.3 Brachial Artery Ultrasound

Brachial artery ultrasound is also used widely as a noninvasive measure of endothelial cell function. In this method, the forearm blood flow is occluded for 5 minutes using a blood pressure cuff maintained at a standard pressure. When the pressure is released, reactive hyperemia occurs. This results in shear stress-induced NO release and subsequent vasodilatation (flow-mediated vasodilatation). This technique has the advantage of being noninvasive and can readily identify populations with attenuated endothelial function. 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. Nonetheless, at present, this approach is widely used to assess vasomotion function.[27]

4.4 Strain Gauge Plethsmography

Strain gauge plethysmography is a standardized technique to measure flow and vascular resistance by using a special transducer placed on the forearm and use of two inflated blood pressure cuffs proximal and distal to the site for the temporary occlusion of either venous or arterial flow. This technique also has been used to assess endothelial dysfunction by measurements of blood flow volume and rate of flow into the forearm.[28] Strain gauges employed in plethysmography for determination of limb blood flow tend to counter the expansion of the limb during venous occlusion. Traditionally a mechanical calibration is performed in situ to compensate for tissue compressibility.[29]

Blood flow in the human forearm or calf can be monitored non-invasively by venous occlusion plethysmography with an optional PowerLab system. Venous occlusion plethysmography involves tying a strain gauge around the limb. The strain gauge could be a stretchable tube containing a liquid metal such as mercury or an indium-gallium alloy. Changes in limb circumference alter the cross-sectional area of the tube and hence the electrical resistance of the liquid metal. [30] This method samples the swelling at one point in the length of the limb, as opposed to measuring the possibly uneven swelling all down the limb. Hence the absolute results should be treated with caution, but comparison between treatments and subjects is legitimate and shows reasonable consistency in practice.[30] Changes in limb circumference can be monitored by a suitable meter, which can output an analog voltage signal direct to a PowerLab system. A sample strain gauge is illustrated in Figure 4-1.

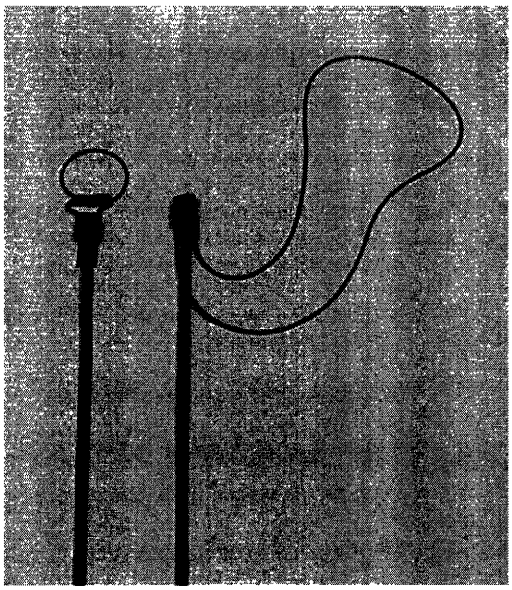

Figure 4-1: Hokanson strain gauge. This strain gauge is used to take quantitative bloodflow measurements noninvasively. It uses a four-wire construction to eliminate errors caused by lead resistance. [29]

Forearm blood flow is measured by stopping the flow out of the limb at an instant while not changing the rate of arterial inflow. This is done by temporarily occluding the venous occlusion cuff placed just above the elbow and inflated to about 60 mmHg. This pressure is sufficient to shut the veins but not the arteries. The slight swelling of the distal

portion of the limb due to continued arterial inflow is then measured. As blood flows into the limb, it cuases the limb to swell and the rate of swelling is a measure of the arterial flow rate at that instant. The assumption is made that flow is similar throughout the limb so the volume flow rate is equal throughout the measurement volume. Flow in the hand is quite variable so it is usually stopped during the test by inflating a wrist cuff above systolic pressure.[29] In theory, the limb swelling will plateau after a while as the venous pressure rises sufficiently to cause renewed venous outflow past the obstruction. However, the initial rate of swelling should represent arterial inflow under relatively normal conditions.[31]

Since blood flow varies continuously in response to various physiological effects, such as thermoregulation, psychological state, and exercise (coming up the stairs to the lab), there isn't a unique value for forearm blood flow. A rested subject in a stable environment is likely to give the most consistent results, but there is still likely to be some more-or-less cyclical variation over periods of minutes to tens of minutes.[31]

4.4.1 Measurement Methods for SGP

4.4.1.1 SGP Basic Equipment

To make blood flow measurements with the SPG technique, the basic equipment includes: a strain gauge, output recording device, and an occluding cuff. The strain needs to be selected according to the limb circumference. Along with the strain guage, there needs to be a system that provides easy recording and analysis of the SPG signal, or plethysmograph output. The occluding cuff is used to provoke a hyperemic response. Either the upper arm cuff can be used alone or both the upper arm and the wrist can be used as sites for the occlusion of blood flow. Rapid inflation of these cuffs is desirable in order to measure the initial slope of the inflow curve, and an automatic cuff inflator is often recommended for this task.[31]

4.4.1.2 SGP Technique

The rested subject is made comfortable with the arm supported by foam blocks or slings at the wrist and elbow. The cuff is fitted, but not yet inflated. Once the cuff is inflated, initial flow is measured by determining the initial slope of the volume curve

after any cuff artifact which might exist, where the cuff artifact is a rapid rise of the curve immediately after inflation of the venous occlusion cuff.[31] Flow rates are commonly measured in the cc's of flow per 100 cc's of tissue. This type of normalized measurement makes it possible to compare flow rates regardless of the subject's size.[29]

4.4.1.3 SGP Data Analysis

There are two methods by which data can be anaylyzed: manually and electronically. To manually determine blood flow, a line is drawn tangent to the first pulses after the cuff artifact. Drawing the line requires some judgement, but with practice, consistent results are possible. To determine the slope of the line, the change in volume per unit time must be calculated. Examples of manual results are shown in Figure 4-2.

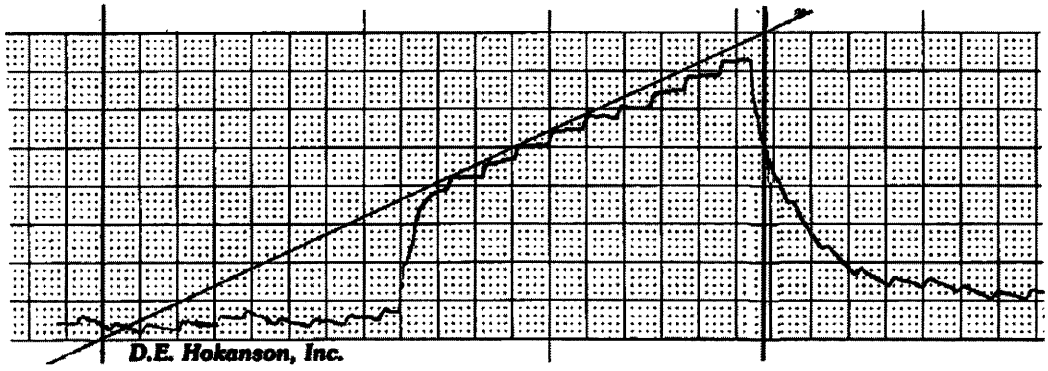

Figure 4-2: Manual data analysis of a strain gauge plethsmograph. This chart was recorded from a TL400 Totalab strain gauge plethysmograph. The tangent line is then drawn and the time for this line to cross the chart is 17.8 seconds. The volume change across the chart is 0.8% and the flow rate is therefore 2.70 %/min.[31]

This measurement can also be performed with the aid of a computer program. A gauged is selected and fitted to the limb at a standardized fraction of the forearm length. The system is then electronically calibrated to give exact % stretch for that particular gauge and initial limb circumference. (The calibration method depends on the electronics used. The output is actually % increase in limb cross-sectional area, which is twice the % circumference increase.) The cuff is inflated to a standard pressure (usually about 60 mm Hg) while the SPG signal is recorded. If required, this step is repeated a number of times. On the recording, the initial slope is measured and noted. On a Biopac system with the AcqKnowledge software, this consists of dragging a cursor over the relevant period of the

waveform and transferring the resulting least-squares slope to a journal with a single keystroke.[29] Figure 4-3 shows a snapshot window of a typical result:

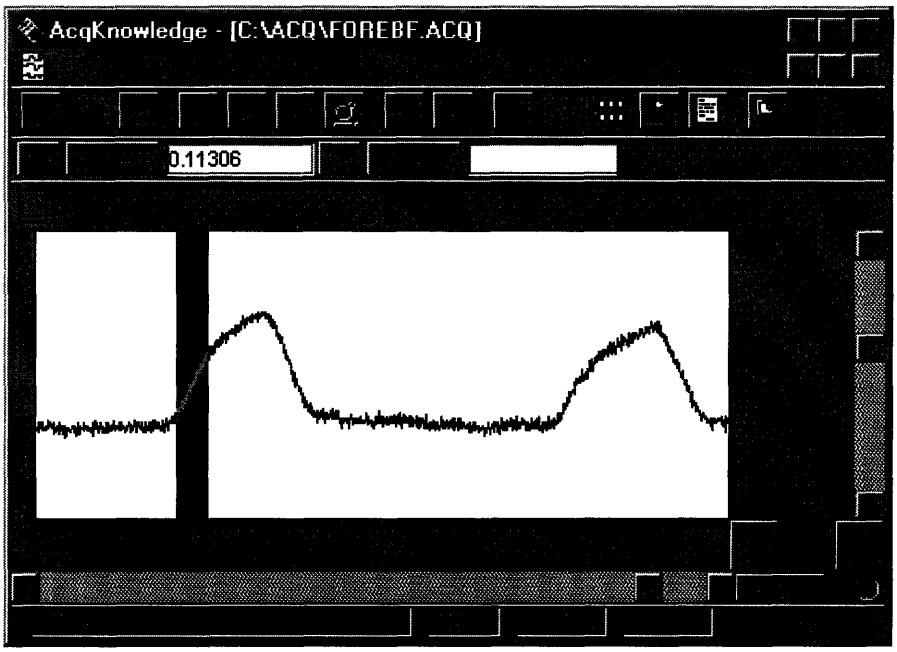

Figure 4-3: Electronic data analysis of a strain gauge plethsmograph using the AcqKnowledge software with a Biopac system.[29]

With a calibrated electronic system, the result will be in %/s. Based on an assumption of uniform swelling along the limb, the units of the result can be interpreted as mls blood / 100 ml tissue / second. A typical result for forearm flow is of the order of 0.1 ml/100 ml/s (or 5 ml/100 ml/minute).[29]

4.5 Arterial Tonometry

Arterial tonometry was first developed in 1963 by G.L. Pressman and P.M. Newgard. Arterial tonometry allows non-invasive and continuous registration of the arterial pressure waveform, by applanating, or flattening, a superficial artery supported by bone with an external transducer. This technique provides the clinician with numeric values for systolic, mean, and diastolic pressures, and a numeric value for pulse rate.[32,33,34] The sensor used for tonometry is placed on the wrist over the radial artery as shown in the Figure 4-4.

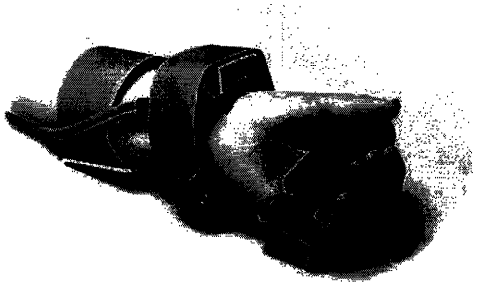

Figure 4-4: Tonometry sensor device[32]

The sensor contains piezoelectric pressure transducers separated by 0.2 mm. A pneumatic pump and bellows press the transducer array against the skin and tissue above the artery. A schematic of this description is shown in Figure 4-5.

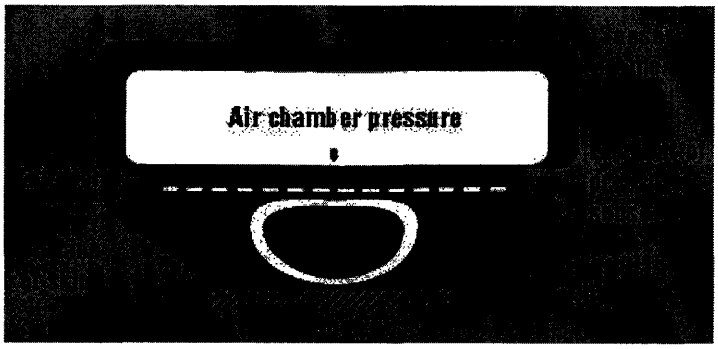

Figure 4-5: Schematic of how the tonometry sensor works[32]

The pressure recorded when the transducer is pressed against the skin is called the hold down pressure (HDP). To determine optimal HDP, the monitor searches through a range of pressure values until it measures a signal indicating that the artery is of the form shown in the figure below. When the artery is partially flattened, a graph, called a tonogram, can be plotted to show sensor pulse amplitude versus transducer number. The individual sensor elements whose pulse amplitudes are near the maximum pulse amplitude are calibrated to the systolic and diastolic values obtained in the oscillometric cuff measurement.[32] The accuracy of this method depends on the performance of both the piezoelectric crystal array and the oscillometric cuff.[33] A schematic of the plot is shown in Figure 4-6.

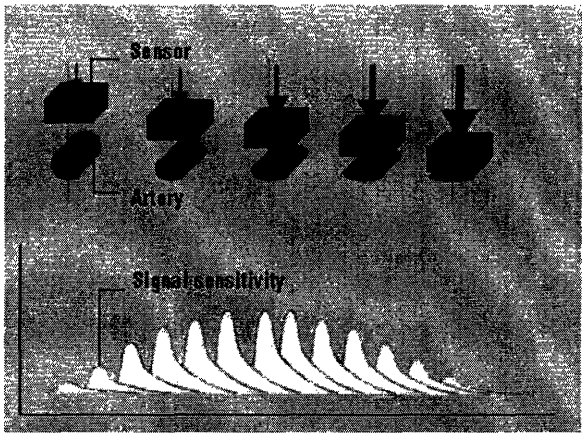

Figure 4-6: Schematic of the tonogram produced with variations in hold-down pressure[32]

4.5.1 Advancements in Tonometry

Although G.L. Pressman and P.M. Newgard built the first arterial tonometer in 1963, accuracy remained poor until the onset of silicon technology and the development of new sensor production techniques. G.M. Drzewiecki et al. published a second, more elaborate theoretical model for tonometer positioning in 1983. A few years thereafter, the first modern tonometers were commercialized. Although the problems of sensor positioning, motion artefacts and calibration still exist, the tonometer has proven its usefulness in arterial compliance and hypertension studies. Attention should now go to analysis of the arterial pressure waveforms, and the combination with other signals (e.g. flow wave morphology) to allow a complete non-invasive haemodynamical description of the heart and the arterial tree.[34]

4.5.2 Studies Using Arterial Tonometry

This section contains abstracts of three studies that use arterial tonometry: (1) Comparison of Arterial Tonometry with Radial Artery Catheter Measurements of Blood Pressure in Anesthetized Patients; (2) Peripheral Arterial Tonometry: A Diagnostic Method for Detection of Myocardial Ischemia Induced during Mental Stress Tests: A

Pilot Study; and (3) Estimation of Central Aortic Pressure Waveform by Mathematical Transformation of Radial Tonometry Pressure.

Comparison of Arterial Tonometry with Radial Artery Catheter Measurements of Blood Pressure in Anesthetized Patients:

The first study seeks to evaluate the overall performance of arterial tonometry in terms of the performance of the piezoelectric crystal array and the oscillometric cuff by comparing it with simultaneous recording of blood pressure from an intraarterial catheter. Seventeen adult patients were studied during general anesthesia. Blood pressure was measured with an intraarterial catheter and with an arterial tonometry system. Analog pressure waveforms were sampled at 100 Hz. Blood pressure measurements obtained by oscillometry were recorded by computer. Comparisons of mean blood pressure on a beat-by-beat basis were made with and without correction for the calibration error introduced by oscillometry. The difference between pairs of blood pressure determined by arterial tonometry and intraarterial measurement was 1.3 +/- 9.4 mmHg (mean +/- SD, bias +/- precision) with 88,158 pairs of measurements. The difference between blood pressure determined by oscillometry and intraarterial measurement was 2.4 +/- 7.5 mmHg (mean +/- SD) with 401 comparisons. After correcting for calibration error, the difference between the tonometry measurements and intraarterial measurements was -1.0 +/- 5.6 mmHg. Continuous episodes of discrepancy from intraarterial measurements in excess of 10 mmHg and lasting 5-60 s occurred 4.6 +/- 0.8 times per hour with tonometry and 12.6 +/- 1.4 times per hour with oscillometry. The study concluded that discrepancies in blood pressure readings by arterial tonometry versus intraarterial measurement result from both the piezoelectric crystal array and the oscillometry used for calibration. Accuracy for individual measurement is inferior to oscillometry alone. Additionally, the ability to detect significant changes of blood pressure more rapidly than with oscillometry alone is limited by the accuracy of the piezoelectric crystal component but is enhanced by the reduced interval between measurements.[33]

Peripheral Arterial Tonometry: A Diagnostic Method for Detection of Myocardial Ischemia Induced during Mental Stress Tests, A Pilot Study:

The second study was undertaken to test the diagnostic capability of peripheral arterial tonometry (PAT) to detect peripheral arterial vasomotor changes. This study is based on the assumption that arterial tonometry is a valid indicator of vasomotor changes. The researchers monitored pulsatile finger blood volume changes using a unique finger plethysmograph, PAT designed to detect peripheral arterial vasomotor changes. Since it has been shown that myocardial ischemia induced during mental stress tests is specifically associated with peripheral arterial vasoconstriction, equilibrium radionuclide angiography (ERNA) performed simultaneously with PAT in 18 male patients. PAT and ERNA were performed when the patients were at rest as well as during a mental arithmetic stress test with harassment. All patients had previously diagnosed coronary disease and positive exercise tests. Peripheral arterial tonometry tracings were considered abnormal when the pulse wave amplitude decreased by ≥20% from baseline. (Myocardial ischemia was diagnosed by ERNA when global ejection fraction fell ≥8% during mental stress or when new / worsened focal wall motion abnormalities occurred.) There were only 16 usable studies out of the 18 subjects tested. Both ERNA and PAT were abnormal in 8 patients. The tests were negative by both methods in 6 patients, and in 2 cases, the results were discordant. Therefore, when considering an abnormal PAT tracing as indicative of mental stress-driven myocardial ischemia, concordance of the two methods was 88%. This means that there was an 88% correlation between peripheral arterial vasoconstriction and mental stress testing. Thus, the use of peripheral arterial tonometry has the potential to facilitate both clinical testing and research during mental stress, which is assumed to be directly associated with peripheral arterial vasoconstriction.[35]

Estimation of Central Aortic Pressure Waveform by Mathematical Transformation of Radial Tonometry Pressure:

In the third study, researchers developed mathematical transfer functions that use peripheral tonometry data to predict central aortic pressures and waveforms, which convey important information about a person's health but can only be measured invasively. Peripheral pressures can be measured noninvasively, and although they often

differ substantially from central pressures, they may be mathematically transformed to approximate the latter. By examining intersubject and intrasubject variability and the validity of using a single averaged transformation (which enhances its applicability), central aortic pressures were accurately estimated from radial tonometry with the use of a generalized transfer function. The reconstructed waveform provided arterial compliance estimates but underestimated the augmentation index because the latter requires greater fidelity reproduction of the wave contour. More specifically, invasive central aortic pressure by micromanometer and radial pressure by automated tonometry were measured in 20 patients at steady state and during hemodynamic transients (Valsalva maneuver, abdominal compression, nitroglycerin, or vena caval obstruction). For each patient, transfer functions (TFs) between aortic and radial pressures were calculated by parametric model and results averaged to yield individual TFs. A generalized TF was the average of individual functions. TFs varied among patients, with coefficients of variation for peak amplitude and frequency at peak amplitude of 24.9% and 16.9%, respectively. Intrapatient TF variance with altered loading (>20% variation in peak amplitude) was observed in 28.5% of patients. Despite this, the generalized TF estimated central arterial pressures to ≤0.2±3.8 mm Hg error, arterial compliance to 6±7% accuracy, and augmentation index to within -7% points (30±45% accuracy). Individual TFs were only marginally superior to the generalized TF for reconstructing central pressures.[36]

4.6 Thermal-Based Assessment via Temperature

There are many limitations to the existing methods for identifying endothelial dysfunction, some of which include operator-dependent variability, inconsistent reproducibility, and complex analysis of data. A thermal based approach that can reduce or eliminate these limitations will prove to be a more useful tool for assessing vascular health.

4.6.1 Research at the University of Texas

In 2003 and 2004, researchers at the University of Texas were exploring a temperature based technique for measuring endothelial dysfunction. This technique takes advantage of the response of endothelial cells to changes in pressure. Pressure changes

cause the cells to produce nitric oxide which in turn causes blood vessels to dilate. This vasodilation can be induced by temporarily blocking blood flow and causing reactive hyperemia – an increase in blood flow resulting from the restoration of temporarily blocked flow. Valvano and his colleagues are developing a technique to measure endothelial dysfunction by causing reactive hyperemia in the arm and measuring the temperature variations of the hand region. They create reactive hyperemia by occluding the arm by blood pressure cuff at about 200 mmHg for 5 minutes, and then suddenly releasing the pressure. Using a computer-based data acquisition system, the temperature variations of the distal palmer pad/middle finger, during the entire procedure are continuously monitored.[37] A sample of results is shown in Figure 4-7.

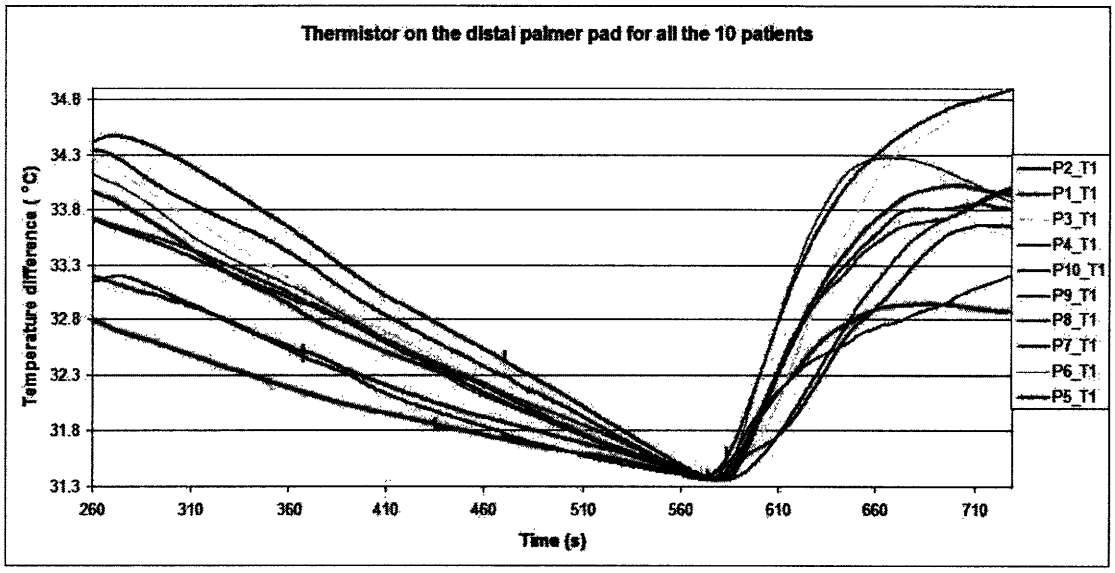

Figure 4-7: Valvano's preliminary results for predicting endothelial dysfunction using temperature variations.[37]

To analyze data and make it useful for assessing endothelial dysfunction, Kharalkar and Valvano study the rate of temperature fall during occlusion and the rate of temperature rise after release. Initial studies on normal subjects have indicated that the rate of temperature increase is significantly higher than the rate of temperature decrease.[38]

4.6.2 Naqvi, Shah, Kaul, and Naghavi Present Data for Assessment via Temperature

A temperature based approach to assessing endothelial dysfunction is also described in a PowerPoint presentation given by Principal Investigator, Tasneem Z Naqvi, MD on December 16, 2003 at the University of California Los Angeles.[39] Naqvi's co-investors are P.K. Shah, MD, Sanjay Kaul, MD and the founder of a vascular health company named Endothelix, Morteza Naghavi, MD.

The team describes the assessment of endothelial function by this novel temperature-based method. Their hypothesis is that digital temperature changes in response to hyperemia is a marker of endothelial function, and their overall aim is to determine if digital temperature change in response to hyperemia correlates with brachial artery reactivity as measured by ultrasound. The technique utilizes the change of temperature as an indicator of change in flow, where the slope and pattern of temperature rise at the finger tips after release of occlusion correlate with brachial vasoreactivity. They claim that the benefits of this device are that it is cheap, can be self-administered, and can be easily adapted into home-based blood pressure monitoring device.[40]

This team performed an experiment to monitor brachial artery flow-mediated vasodilation, as depicted in Figure 4-8.

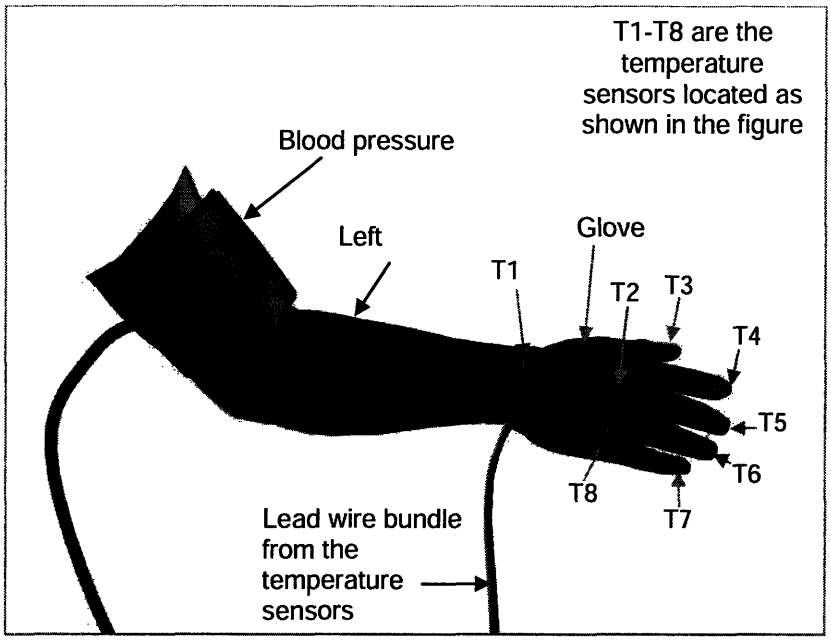

Figure 4-8: Schematic diagram for the temperature-based test procedure[40]

Sample results for endothelial function measured by digital thermal monitoring at the tip of the index finger are shown in Figure 4-9.

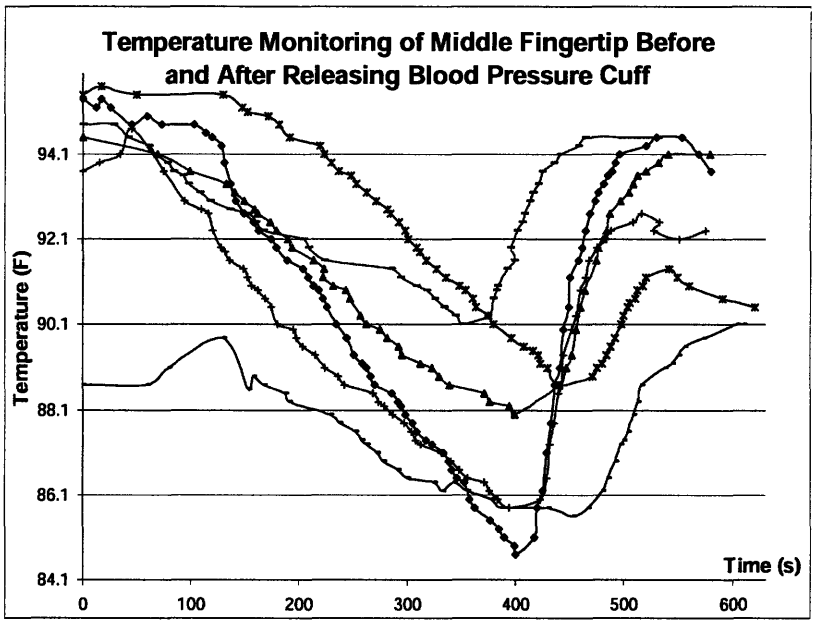

Figure 4-9:Temperature monitoring results[40]

The parameters that can be measured from the graph in Figure 4-9 are the rate of temperature fall after the cuff is inflated, the rate of temperature rise after the cuff is deflated, and the percent increase in temperature post hyperemia.

4.6.3 Thermal Method Is Used by Endothelix

Endothelix is a Delaware corporation based in Houston, Texas and was formed in December 2003. The company is dedicated to promoting vascular endothelial health; their products are intended to help practicing physicians to regularly monitor their patient's endothelial function and use appropriate therapies for treatment of endothelial dysfunction. On January 16, 2006, a press release stated that Endothelix received an exclusive license from the University of Texas Health Science Center and the Texas Heart Institute for the thermal detection of endothelial dysfunction. The technology measures a marker of vascular endothelial dysfunction by monitoring temperature changes at the fingertip. Dr. Morteza Naghavi, the founder of Endothelix, inventor of the technology, and former director of the Vulnerable Plaque Research Center at the UT Houston Health Science Center and the Texas Heart Institute, says "Endothelial function monitoring can fill the gap between heart attack risk factor measurements, such as blood tests, and advanced imaging technologies like heart scans." Dr. Michael Jamieson, who sits on Endothelix's Advisory Board, says "An accurate, automated (operator-independent), and inexpensive method of monitoring endothelial function for clinical practice can make as great an impact on today's medicine as automated blood pressure monitoring devices have done for blood pressure monitoring in the past decade." Essentially, Endothelix is positioning itself as a leader in endothelial function monitoring.[41]

5 A Novel Thermal-Based Approach: Assessment via Temperature and Perfusion

5.1 The Advantage of Monitoring Perfusion

The existing thermal based approaches use changes in temperature during reactive hyperemia as an indicator of the health of endothelial cells. The temperature at the finger tips reflects changes in the level of blood perfusion to the fingers. In other words, the source of these temperature changes is a change in the balance between heat loss to the environment, heat gain from perfusion, and local tissue metabolism (which may not vary much during the short occlusion). When blood flow to the arm is temporarily occluded, the temperature of the tissue downstream of the occlusion decreases with time as the warmer arterial blood coming from the body's core is removed from the overall tissue heat balance. After occlusion, the blood is allowed to rush back into the arterial blood vessels, leading to a rapid increase in measured tissue temperature. The more vasodilation there is, the greater the perfusion following occlusion. Thus, a method to accurately measure perfusion allows the quantification of reactive hyperemia, which can provide valuable insight into the health of endothelial cells. In addition to looking at temperature as an indicator of vascular health, this approach is also concerned with the source of the temperature changes, which is perfusion.

5.2 Simple Thermal Analyses

To analyze this phenomenon of assessing vascular health using temperature and perfusion measurements, two simple thermal models were employed. One model describes the temperature profiles within unperfused tissue when a constant temperature probe is placed in contact with the surface of the skin. The second model describes the temperature profile for perfused tissue.

5.2.1 Heated Disk In Contact with Skin

When the constant temperature electrode comes into contact with the skin, the surface of the skin is assumed to be instantaneously set to the same temperature as the probe. The underlying layers of tissue experience the temperature gradient and isotherms shown in Figure 5-1.

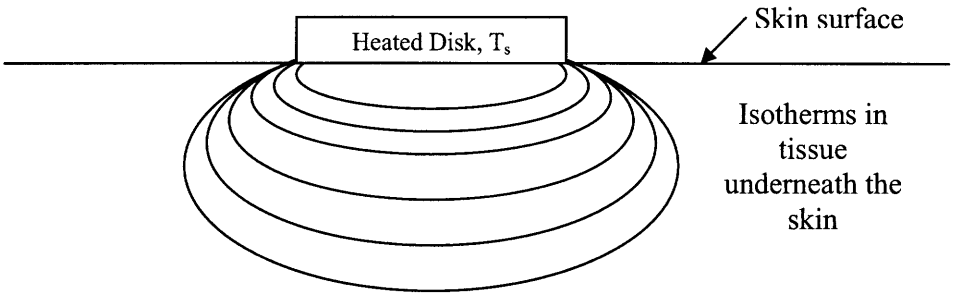

Figure 5-1: Heated disk at constant temperature, T_s, and the corresponding isothems in the underlying skin tissue.

In this analysis the skin and underlying tissue is modeled as a semi-infinite solid with heat diffusivity α=0.1 mm^2/s. Focusing on the local heating caused by the disk in contact with the skin, Figure 5-2 depicts a schematic representation of this phenomenon. In this figure, temperature, T, is graphed as a function of distance into the skin, x, at different instances in time, t. T_i is the initial temperature of the skin; in other words, it is the skin's temperature before the thermal probe comes into contact with it.

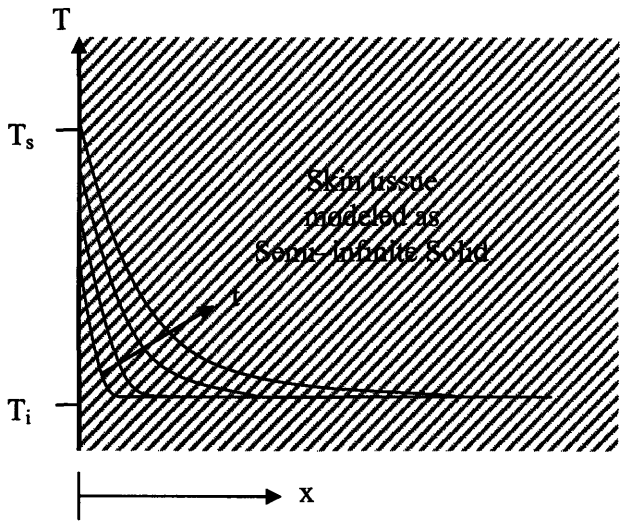

Figure 5-2: Temperature profile in semi-infinite solid

The governing equation for this model is the one-dimensional form of the heat diffusion equation

$$\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2} \tag{1}$$

The solution to this equation for the case at hand is given by[42]

$$\frac{T - T_i}{T_s - T_i} = erfc \frac{x}{2\sqrt{\alpha t}} \tag{2}$$

Consider the case where the difference between skin temperature and initial temperature, T-T_i, is within 10% of the difference between surface temperature and initial temperature, T_s-T_i. For this case the equation reduces to

$$0.1 = erfc \frac{x}{2\sqrt{\alpha t}} \tag{3}$$

According to computation tables for the complementary error function, the argument of this function equals 1.16 when the value of the function is 0.1009. Therefore, for the case being considered,

$$1.16 = \frac{x}{2\sqrt{\alpha t}} \tag{4}$$

which leads to the solution,

$$x(t) = 2.32\sqrt{\alpha t} \tag{5}$$

When x is plotted as a function of time, the following graph is achieved

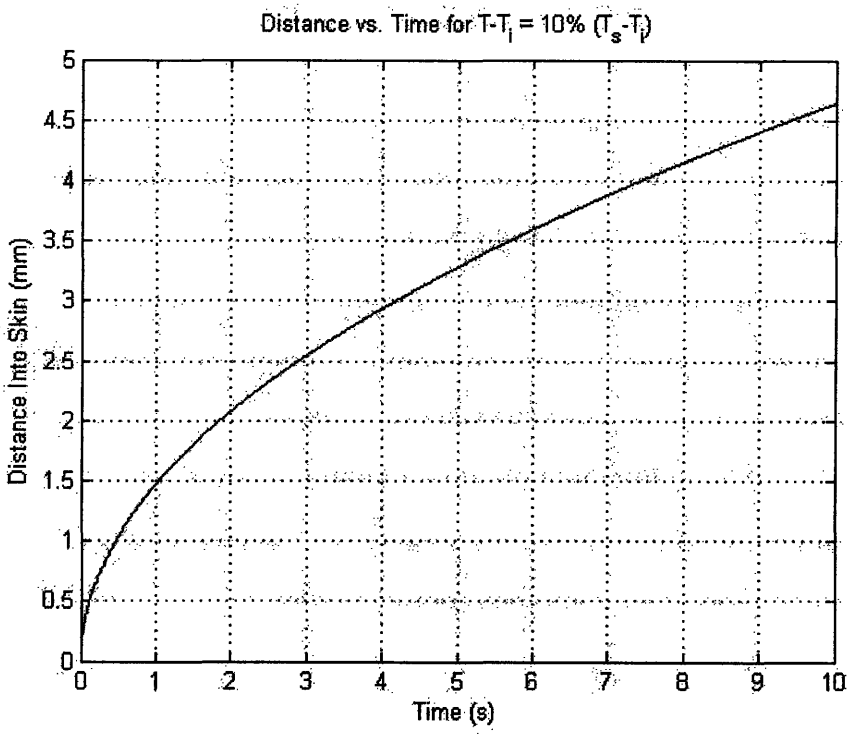

Figure 5-3: Plot of distance underneath skin where the difference between temperature under skin surface and initial temperature is within 10% of the difference between surface temperature and initial temperature, as a function of time.

In the model and solutions obtained thus far the effect of perfusion has been ignored. Due to this neglect of perfusion, the graph in Figure 5-3 never reaches a steady state. Nevertheless, this model is suitable for estimating the temperature in the skin, where there is relatively little blood perfusion. When looking at depths that involve the tissue where there are physiologic levels of perfusion, this perfusion must be accounted for.

5.2.2 Accounting for Blood Perfusion

The bioheat transfer equation is used to describe the temperature field in a continuous, homogenous, perfused tissue and is given by[43]

$$\alpha_m \frac{\partial^2 T_a}{\partial x^2} + \alpha_m \frac{q_m}{k_m} - \frac{\rho_b c_b}{\rho_m c_m} \varpi_b (T - T_a) = \frac{\partial T_m}{\partial t} \tag{6}$$

where α is tissue diffusivity, T is temperature, x is distance into the tissue, q is local tissue metabolic heat generation, k is conductivity, ρ is density, c is heat capacity, and ω is blood perfusion. The subscript m refers to these parameters in the tissue while the subscript b is used to identify properties of the blood. In order to reduce this equation into a simple analytical form, the following assumptions are made:

- The density and heat capacity of blood is comparable to that of tissue. In otherwords, $\rho_b c_b \sim \rho_m c_m$
- Metabolic heat generation, q_m, is small and serves to establish the tissue baseline temperature, T_i
- The probe operates under quasi-steady state conditions

Using these assumptions, the bioheat transfer equation reduces to

$$0 = \frac{\partial^2 T}{\partial x^2} - \frac{\omega}{\alpha}(T - T_i) \tag{7}$$

A solution to this differential equation can be obtained by noticing that equation 7 has the same form as the fin equation.[44] Following a similar approach to that used to solve the fin equation and applying the boundary conditions $T=T_s$ at $x=0$ and $T=T_i$ at $x=L$ (where L is the thickness of the tissue), yields the solution in equation 8.

$$\frac{T - T_i}{T_s - T_i} = \frac{\sinh m(L - x)}{\sinh(mL)}, \text{ where } m = \sqrt{\frac{\omega}{\alpha}} \tag{8}$$

The tissue diffusivity, α, is taken to be 0.1 mm^2/s. To determine suitable values for perfusion, the clinical perfusion units of mL/100g-min must be converted into local perfusion with units of 1/s. The appropriate conversion factor is 1/6000 such that local perfusion, ω, is equal to (1/6000) x Clinical Perfusion. Typical values for clinical perfusion range from 0-50 mL/100g-min, thereby resulting in local perfusion values ranging from 0-0.0083 s^{-1}.

For the temperature profile where the difference between the temperature in the tissue and the initial temperature of the tissue, T-T_i, is 10% of the difference between the temperature at the surface of the skin and the initial temperature of the tissue, T_s-T_i, the left-hand expression of equation 8 simply becomes $\frac{T - T_i}{T_s - T_i} = 0.1$. Solving for perfusion as a function of the distance into the tissue where this temperature profile occurs yields equation 9.

$$x = \frac{mL - \sinh^{-1}(0.1 \sinh mL)}{m} \tag{9}$$

Figure 5-4 contains a graphical representation of equation 9 for different values of tissue thicknesses, L.

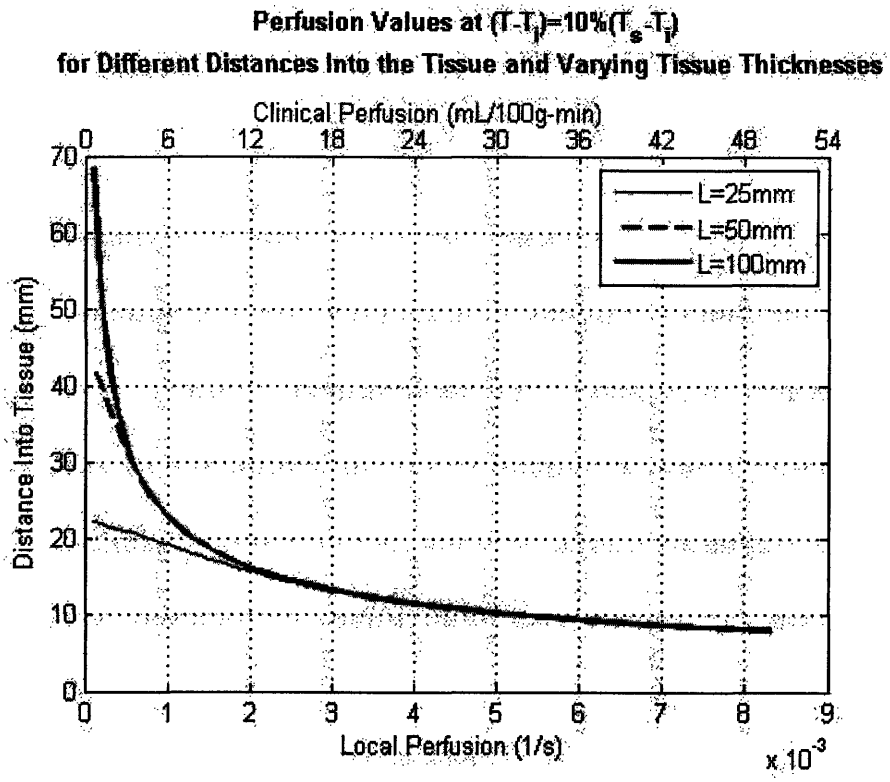

Figure 5-4: Perfusion as a function of the distance into the tissue where the difference between temperature under skin surface and initial temperature is within 10% of the difference between surface temperature and initial temperature, for different values of tissue thicknesses.

The chart in Figure 5-4 can be used to predict the thermal interrogation depth – that is the depth into the tissue at which the probe is measuring and registering perfusion

values. This distance will correspond with the location in the tissue where the difference between tissue temperature and initial temperature, T-T_i, is within 10% of the difference between surface temperature and initial temperature, T_s-T_i. The curve for a tissue thickness of 75mm is very close to that for 100mm, and therefore it is not shown. For tissue thicknesses greater than 100mm, the curves are essentially the same as that of L=100mm. For all thicknesses, the depth into the tissue where the temperature curve settles within 10% is between 9mm and 16mm for clinical perfusion values between 50mL/100g-min and 12mL/100g-min, respectively. Essentially, the higher the perfusion level, the smaller the depth of interrogated tissue.

5.3 Prototype Probe Design

The probe used for testing was a simple modification of an existing invasive temperature probe that is currently used to measure blood perfusion in the brain during and after neurosurgery. The existing probe and its accompanying modification are shown in Figure 5-5.

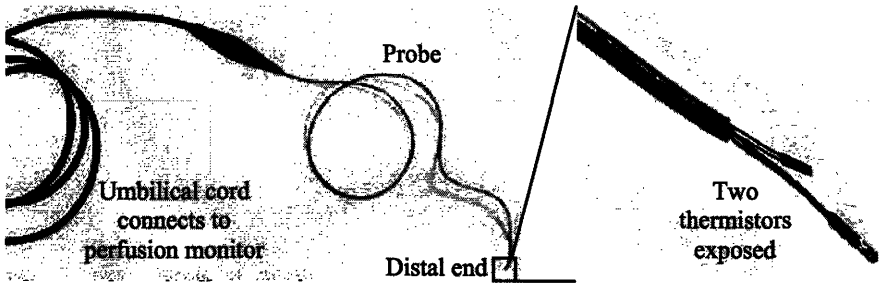

Figure 5-5: Thermal diffusion probe[45] with modified distal end.

The distal end of the probe has two exposed thermistors. The surface of the thermistor is controlled to a small increment (~2 °C) above the tissue baseline temperature. The power dissipated in the thermistor provides a measure of the ability of the tissue to carry heat by both thermal conduction within the tissue and by thermal convection due to tissue blood flow. The passive, proximal thermistor is used to monitor and compensate for temporal tissue baseline temperature changes.[45]

This modification was created by removing approximately 0.5 inch of catheter to expose the two thermistors. The thermal probe with exposed thermistors was connected to the perfusion monitor via an umbilical cord.

5.4 Preliminary Testing

The umbilical cord of the probe is inserted into the multi-pin connector on the left-hand side of the perfusion monitor shown in Figure 5-6.

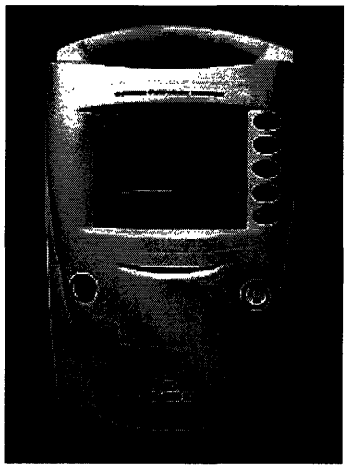

Figure 5-6: Perfusion Monitor[45]

This probe was tested on three subjects. The two exposed thermistors of the temperature probe, wetted with glycerol to increase thermal communication, were placed on the subject's thumb pad and covered with an adhesive-based insulating patch. The monitor is designed to wait for temperature stability of approximately 0.020°C over a 30 second time period. Tissue thermal conductivity was computed from the initial power vs. time record and displayed on the screen; perfused skin thermal conductivity values ranged from 2.8 to 3.5 mW/°C-cm. Thereafter, power and temperature data was collected at a sampling rate of 1 Hz. Perfusion was computed from the solution of a coupled probe-tissue thermal model.[46]

5.5 Results and Discussion

Thermal stabilization took longer than desired due to the tight stability settings appropriate for an implanted neurological perfusion probe. Following thermal stabilization, the tissue thermal conductivity was calculated from power time measurements; and as a final step, the value of perfusion was extracted. The calculated perfusion values of all three subjects tested was zero. Consequently, of the three subjects tested, there was no usable data for evaluation and no time to collect additional data. The calibration parameters of the sensors also may have been out of specification. Nonetheless, an interesting phenomenon was discovered in the process. Whenever pressure was applied to the probe, the machine output clinical perfusion values ranging from 0-48mL/100g-min. This could suggest poor thermal coupling between the probe and tissue.

Despite the fact that the prototype system used had an unknown glitch, past data corroborates the phenomenon that perfusion measurements can be used to assess endothelial health. This data is presented in Figures 5-7 to 5-10 and used with permission from Dr. Bowman.

Figure 5-7 shows the results of continuous perfusion measurements (including vasomotor activity and reactive hyperemia) during repeated occlusion of a rabbit epigastric flap. The expected vasomotor activity is witnessed throughout the course of the experiment. Although this activity looks like noise, it in fact reflects the arteries undergoing continual rapid contraction and dilation as part of the normal regulation of flow; endothelial cells regulate this vasomotor activity. After release of the arterial occlusion, the reactive hyperemia results in a perfusion measurement that is above the baseline measurement. For successive arterial occlusions followed by release, the resulting perfusion value is considerably increased as the reactive hyperemia is not allowed to return to baseline before the next occlusion takes place.

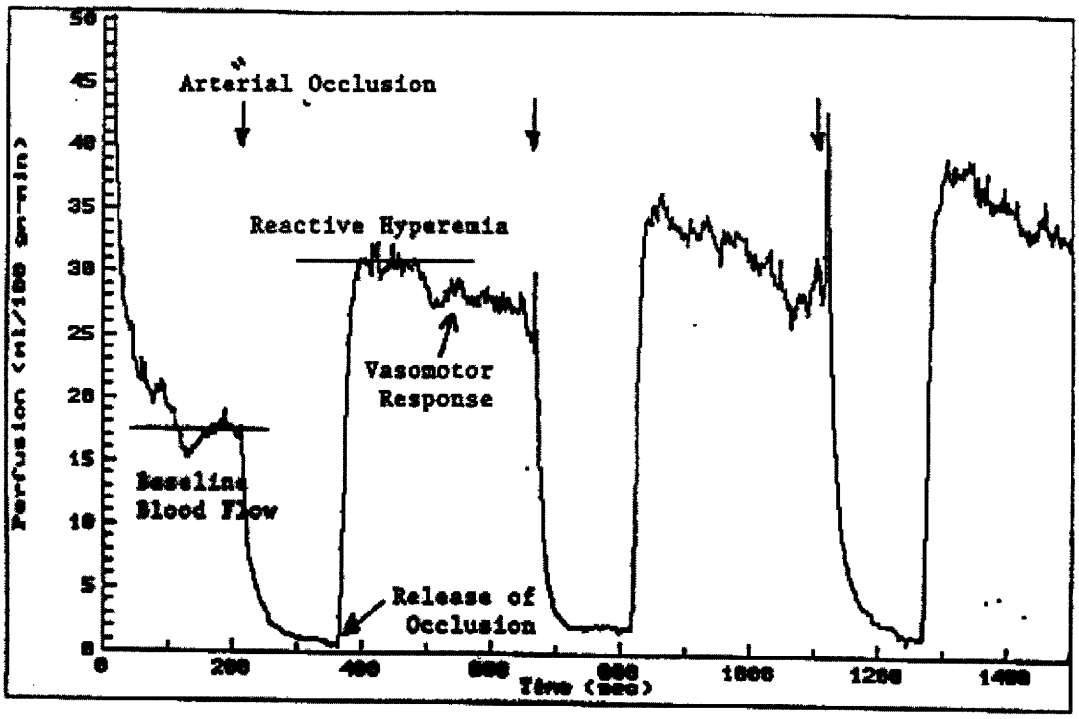

Figure 5-7: Invasive thermal diffusion probe measurements in a rabbit epigastric flap subjected to repeated arterial occlusions. Note vasomotor response and reactive hyperemia.

In Figure 5-8, the results of successive application of an arm tourniquet to a 54-year-old male who had never been diagnosed any form of vascular disease is shown. This method of arterial occlusion was followed by release 300s later with both temperature and perfusion recorded. Similar reactive hyperemia and vasomotor response results were obtained. Furthermore, notice that more information about vasomotor activity is obtained from the perfusion graph as compared with that of the temperature graph. This graph serves as supporting evidence that measuring perfusion along with temperature can provide more insight into a person's vascular health when compared with measuring temperature alone.

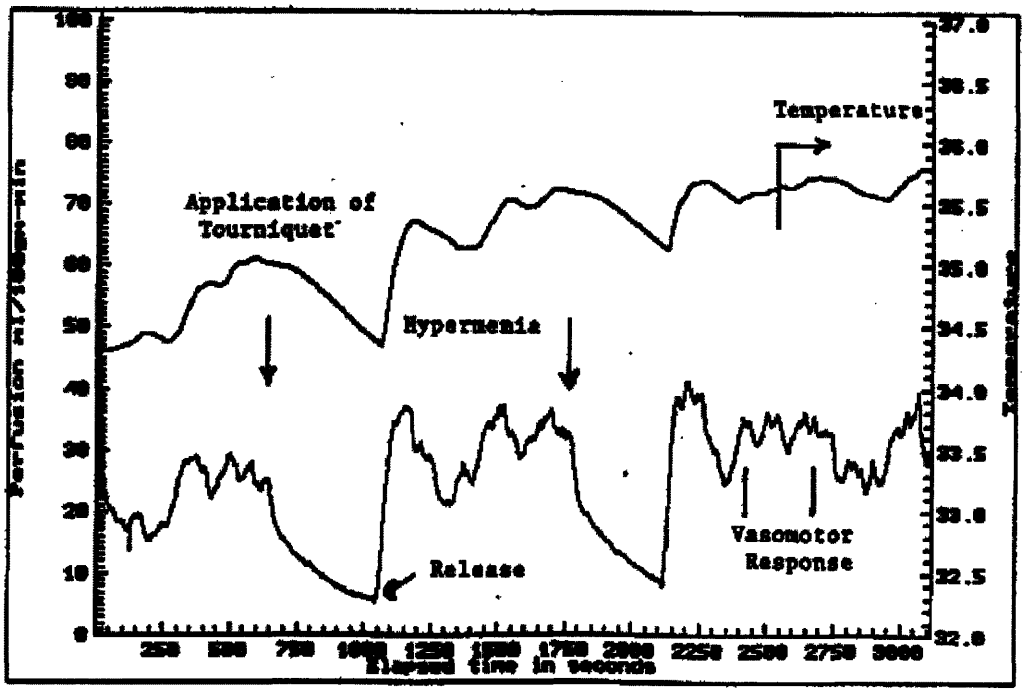

Figure 5-8: Non-invasive thermal diffusion probe measurements of blood flow in a finger of a normal 54-year-old male volunteer in response to successive applications of arm tourniquet. Note vasomotor response and hyperemia.

In Figure 5-9 the results for a 21-year-old female who had never been diagnosed with any type of vascular disease is shown. In comparison to the male in Figure 5-8, this female volunteer returns to her baseline perfusion and temperature more readily. Once again the perfusion plot conveys more information about vasomotor activity than the temperature plot does. Also, for some reason, this female experiences two distinct perfusion peaks for every trial of occlusion followed by immediate release. This detail can not be easily discerned from the temperature plot.

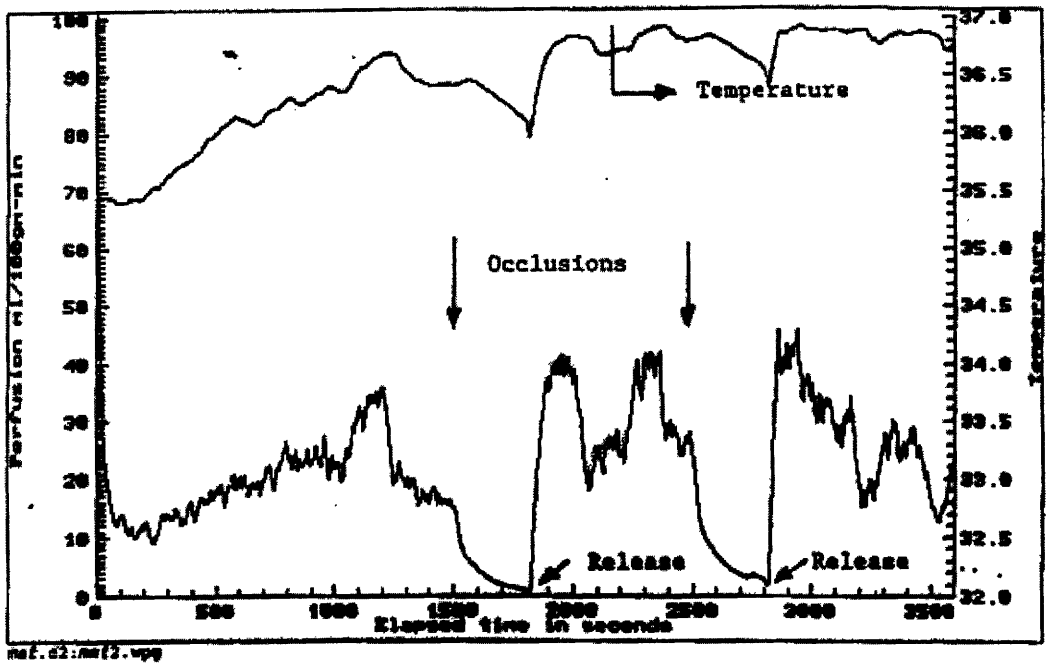

Figure 5-9: Non-invasive thermal diffusion probe measurements of perfusion in a finger of a normal 21-year-old female volunteer in response to successive applications of arm tourniquet. Note vasomotor response and reactive hyperemia.

Figure 5-10 shows the results for a 60-year-old male who was being treated for hypertension. The most outstanding difference between these results and those shown in the other volunteers is the lack of vasomotor activity. Additionally, after occlusion followed by immediate release, the reactive hyperemia peak is not as pronounced.

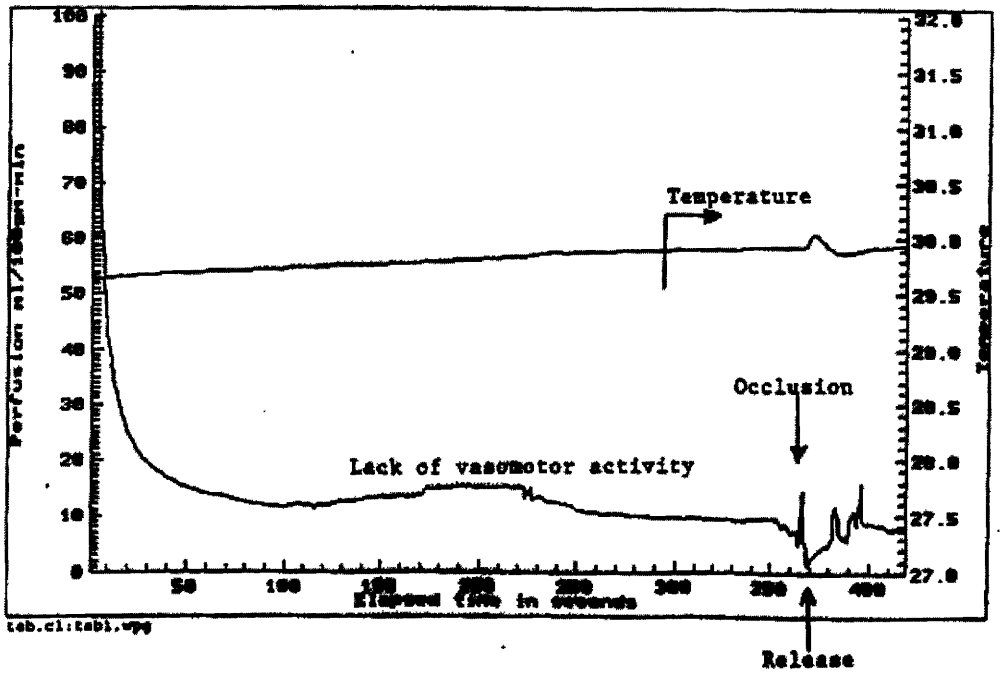

Figure 5-10: Non-invasive thermal diffusion probe measurements of perfusion in thumb pad of a 60-year-old male volunteer being treated for hypertension. Note lack of vasomotor activity.

Based on the observations in Figures 5-7 to 5-10, there appears to be a highly promising application for identifying endothelial dysfunction using perfusion measurements. The use of perfusion along with temperature also has advantages over using temperature alone. Furthermore, this approach reduces some of the problems associated with non-thermal methods. Once standard set-up procedures are performed, the results are not operator dependent. Additionally the results can be reproduced with a fair amount of consistency, as shown in the successive measurements for occlusion followed by release for each test subject. Finally, a computer program can be written to automatically analyze resulting data. Maybe the program can analyze peaks, characterize vasomotor activity, and calculate slopes for the decreasing perfusion after occlusion and release.

6 Conclusion and Recommendations

There are many methods available for measuring and assessing vascular health. Each method has its own unique limitations. A thermal based approach has the potential to eliminate some of the limitations seen with other measurement techniques. Perfusion and temperature serve as promising parameters for identifying endothelial dysfunction. The method of using a thermal diffusion probe could be standardized to test the vascular health status of individuals. An immediate next step is to debug the perfusion technique such that meaningful results could be obtained from the prototype probe. One might also want to consider using a blood pressure cuff to apply a constant pressure on the probe as data is being recorded.

The data returned from the perfusion monitor should be analyzed to extract important measurable data such as peaks, slopes, and vasomotor activity. A computer program could be written for these peak analysis, vasomotor activity characterizations, and slope calculations. This type of data analysis program could then be incorporated in the perfusion monitor to avoid the limitations due to variations in operator-dependent data analysis.

Other additional work would include increasing the accuracy and reproducibility of data collection and identifying body sites that are most revealing of general vascular health.

References

[1] Endothelix, Inc. <http://www.endothelix.com/science.html>. Accessed 2006 May 5

[2] Endothelium Encyclopedia Topic <http://www.absoluteastronomy.com/reference/endothelium>. Accessed 2006 Feb 12.

[3] Poredos P. 2003. Endothelial Dysfunction and Cardiovascular Disease. E-Journal of Cardiology Practice. <http://www.escardio.org/knowledge/cardiology_practice/ejournal_vol1/Vol1_no16.htm>. Accessed 2006 Feb 12.

[4] Orford JL, Selwyn AP. 2005 Nov 2. Atherosclerosis. <http://www.emedicine.com/med/topic182.htm>. Accessed 2006 May 5.

[5] Boger RH, Ron ES. 2005 March. L-arginine improves vascular function by overcoming the deleterious effects of ADMA, a novel cardiovascular risk factor. Alternative Medicine Review 2005;10(1):14-23. <http://www.findarticles.com/p/articles/mi_m0FDN/is_1_10/ai_n13557315>. Accessed 2006 Mar 6.

[6] Nakagami H, Kaneda Y, Ogihara T, Morishita R. Endothelial Dysfunction in Hyperglycemia as a Trigger of Atherosclerosis. Current Diabetes Reviews 2005;1:59-63. < http://www.bentham.org/cdr/samples/cdr1-1/D0006D.pdf>. Accessed 2006 May 5

[7] Bonetti PO, Lerman LO, Lerman A. 2002 December 12. Endothelial Dysfunction: A Marker of Atherosclerotic Risk. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23:168. <http://atvb.ahajournals.org/cgi/content/full/23/2/168/>. Acessed 2006 May 5.

[8] American Heart Association. 2004 Oct 26. Endothelial dysfunction linked with early signs of atherosclerosis in young adults. <http://www.americanheart.org/presenter.jhtml?identifier=3025948>. Accessed 2006 May 5.

[9] Banning M. The Pathogenesis of Atherosclerosis. <http://www.continuingeducation.com/nursing/atherosclerosis/atherosclerosis.pdf>. Accessd 2006 May 5.

[10] Hashimoto M, Eto M, Akishta M, Kozaki K, Ako J, Iijima K, Kin S, Toba K, Yoshizumi M, Ouchi Y. Correlation Between Flow-Mediated Vasodilatation of the Brachial Artery and Intima-Media Thickness in the Carotid Artery in Men. Arteriosclerosis, Thrombosis, and Vascular Biology. 1999;19:2795. <http://atvb.ahajournals.org/cgi/content/full/19/11/2795>. Accessed 2006 May 5.

[11] Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992 Nov 7;340(8828):1111-5. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=1359209&dopt=Citation>. Accessed 2006 May 5.

[12] PoredoÅi P, Orehek M, Tratnik E. Smoking is associated with dose-related increase of intima-media thickness and endothelial dysfunction. Angiology 1997; 50: 201-7.

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10088799&dopt=Abstract >

[13] Mombouli JV, Vanhoutte PM. Endothelial dysfunction: from physiology to therapy. J Mol Cell Cardiol 1999; 31: 61-74. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10072716&dopt=Abstract>

[14] Life Extension[SM]. 2006 Mar 22. Atherosclrosis. <http://www.lef.org/protocols/prtcl-015.shtml>. Accessed 2006 May 5.

[15] Napoli C, Pignalosa O, de Nigris F, Sica V. Childhood Infection and Endothelial Dysfunction: A Potential Link in Atherosclerosis? American Heart Association Circulation. 2005;111:1568-1570. <http://circ.ahajournals.org/cgi/content/full/111/13/1568>. Accessed 2006 May 5.

[16] Prewitt K, Shappell S. 2005 Oct 11. Atherosclerosis. <http://heart.healthcentersonline.com/cholesterol/atherosclerosis.cfm>. Accessed 2006 May 5.

[17] Atherosclerosis. <http://www.mamashealth.com/atherosclerosis.asp>. Accessed 2006 May 5.

[18] American Heart Association. Atherosclerosis. <http://www.americanheart.org/presenter.jhtml?identifier=4440>. Accessed 2006 May 5.

[19] Women'sHealth.gov. <http://www.4woman.gov/faq/atheroscle.htm>. Accessed 2006 Feb 21.

[20] National Heart, Lung, and Blood Institute: Diseases and Conditions Index. 2006 Mar. Atherosclerosis. <http://www.nhlbi.nih.gov/health/dci/Diseases/Atherosclerosis/Atherosclerosis_WhatIs.html>. Accessed 2006 May 5.

[21] Keller S. 2004 Jul 6. Medical Enyclopedia: Atherosclerosis. <http://www.nlm.nih.gov/medlineplus/ency/article/000171.htm>. Accessed 2006 May 5.

[22] Kitamoto S, Egashira K. Endothelial Dysfunction and Coronary Atherosclerosis. Current Drug Targets - Cardiovascular & Hematological Disorders, 2004 March;4(1):13-22. <http://www.ingentaconnect.com/content/ben/cdtchd/2004/00000004/00000001/art00003>. Accessed 2006 May 5.

[23] Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney Jr. JF, Keyes MJ, Levy D, Vasan RS, Benjamin EJ. 2004 July 12. Local Shear Stress and Brachial Artery Flow-Mediated Dilation: The Framinham Heart Study. American Heart Association Hypertension. 2004;44:134 <http://hyper.ahajournals.org/cgi/content/abstract/44/2/134>. Accessed 2006 May 5.

[24] Harris RA, Padilla J, Rink LD, Wallace JP. Variability of flow-mediated dilation measurements with repetitive reactive hyperemia. Vascular Medicine 2005;10:1–6 <http://www.indiana.edu/~afp/cep/manuscripts/harris.pdf>. Accessed 2006 May 7.

[25] Black CD, Vickerson B, McCully KK. 2003 February 11. Noninvasive assessment of vascular function in the posterior tibial artery of healthy humans. Dynamic Medicine.

2003;2:1. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=151670>. Accessed 2006 May 7.

[26] Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita JV, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology. 2002 January 16;39(2):257-65. <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T18-44VDM75-D&_coverDate=01%2F16%2F2002&_alid=371669526&_rdoc=1&_fmt=&_orig=search&_qd=1&_cdi=4884&_sort=d&view=c&_acct=C000022659&_version=1&_urlVersion=0&_userid=501045&md5=c9894317a7f00127f325182fd530cdea>. Accessed 2006 May 7.

[27] Verma S, Buchanan MR, Anderson TJ. Endothelial Function Testing as a Biomarker of Vascular Disease. American Heart Association Circulation. 2003;108:2054. <http://circ.ahajournals.org/cgi/content/full/108/17/2054 - R10-134992#R10-134992>. Accessed 2006 May 5.

[28] Cardiovascular Imaging and Clinical Research Core Laboratory. 2002. <http://circl.wustl.edu/tests/testsdetails.htm#Strain%20Gauge%20Plethysmography>. Acessed 2006 May 7.

[29] Brengelmann GL, Savage M. Influence of tissue compressibility on calibration for strain-gauge plethysmography. Journal of Applied Physiology. 1986;61:1210-1216. <http://jap.physiology.org/cgi/content/abstract/61/3/1210>. Acessed 2006 May 7.

[30] ADinstruments. <http://www.adinstruments.com/applications/full.php?exp_id=55&name_id=37&template=research>. Accessed 2006 May 7.

[31] SDR Clinical Technology. <http://www.sdr.com.au/forebf.html>. Accessed 2006 May 7.

[32] Colin. <http://colin-europe.com/pages/tonometry.html>. Accessed 2006 May 7.

[33] Siegel LC, Brock-Utne JG, Brodsky JB. Comparison of arterial tonometry with radial artery catheter measurements of blood pressure in anesthetized patients. Anesthesiology. 1994 Sep;81(3):578-84. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8092502&dopt=Abstract>. Accessed 2006 May 7.

[34] Matthys K, Verdonck P. Development and modelling of arterial applanation tonometry: A review. Technology and Health Care. 2002;10(1):65-76.

[35] Goor D, Sheffy J, Schnall RP, Arditti A, Caspi A, Bragdon EE, Sheps D. Peripheral Arterial Tonometry: A Diagnostic Method for Detection of Myocardial Ischemia Induced during Mental Stress Tests: A Pilot Study. Clinical Cardiology. 2004;27, 137–141 <http://www.clinicalcardiology.org/productcart/pc/briefs/0403/march_CI_2.pdf>. Accessed 2006 May 7.

[36] Chen C, Nevo E, Fetics B, Pak PH, Yin CPF, Maughan WL, Kass DA. Estimation of Central Aortic Pressure Waveform by Mathematical Transformation of Radial Tonometry Pressure:Validation of Generalized Transfer Function. American Heart

Association Circulation. 1997;95:1827-36. <http://circ.ahajournals.org/cgi/content/full/circulationaha;95/7/1827>. Accessed 2006 May 7.

[37] Valvano JW. Electro-Thermal Bioinstrumentation Laboratory, Novel temperature based technique for measurement of endothelial dysfunction. 2003 Dec 19. <http://www.ece.utexas.edu/~valvano/research/electro.htm>. Accessed 2006 May 7.

[38] Kharalkar N, Valvano JW. Analysis of a thermal method for assessing endothelial dysfunction. Biomed Sci Instrum. 2004;40:86-92. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=15133940&dopt=Abstract>. Accessed 2006 May 7.

[39] K30@UCLA, Graduate Training Programs in Translational Investigayion. 2003. <http://149.142.238.229/k30/meetings.asp>. Accessed 2006 May 7.

[40] Naqvi TZ, Shah PK, Kaul S, Naghavi M. Assessment of Endotheilal Function by a Novel Method. <http://149.142.238.229/k30/reading/12.16.03%20Naqvi%20RI.ppt>. Accessed 2006 May 7.

[41] PRWeb Press Release Newswire. Beyond Blood Pressure: Endothelix Licenses Technology for Thermal Monitoring of Endothelial Function. 2006 January 16. <http://www.prweb.com/releases/2006/1/prweb332379.htm>. Accessed 2006 May 7.

[42] Cravalho EG, Smith Jr. JL, Brisson Jr. JG, McKinley GH. Thermal-Fluids Engineering: An Integrated Approach to Thermodynamics, Fluid Mechanics, and Heat Transfer. Oxford University Press; 20042. p 50-2.

[43] Bowman HF. Estimation of Tissue Blood Flow. In: Shitzer A, Eberhart RC, editors. Heat Transfer in Medicine and Biology, Analysis and Applications, Volume 1. New York: Plenum Press; 1985. p 203.

[44] Cravalho EG, Smith Jr. JL, Brisson Jr. JG, McKinley GH. Thermal-Fluids Engineering: An Integrated Approach to Thermodynamics, Fluid Mechanics, and Heat Transfer. Oxford University Press; 20042. p 24-7.

[45] Hemedix. 2002. <http://www.hemedex.com/bpmonitor.html>. Accessed 2006 May 8.

[46] Personal communication with HF Bowman. 2006 May 11.