A STUDY ON SKIN BLOOD FLOW CONTROL MECHANISMS USING WAVELET ANALYSIS: IMPLICATIONS FOR ALTERNATING PRESSURE SUPPORT SURFACES

by

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ABSTRACT

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A STUDY ON SKIN BLOOD FLOW CONTROL MECHANISMS USING WAVELET ANALYSIS: IMPLICATIONS FOR ALTERNATING PRESSURE SUPPORT SURFACES

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University of Pittsburgh, 2004

The benefits of using alternating pressure (AP) support surfaces for pressure ulcer prevention are associated with providing periodic pressure relief and stimulating a protective increase in skin blood flow (SBF). The physiological mechanisms responsible for the increase in SBF are not well understood; however, these protective mechanisms may be assessed through study of SBF oscillations.

A computer-controlled system has been designed to apply various stimuli and loading conditions on the sacrum with simultaneous measurement of physiological responses (i.e. SBF and skin temperature). The results indicate that the system provides reliable data for use in the study of the relationship between tissue biomechanical and physiological characteristics and loading methods.

A time-frequency approach using wavelets was used to decompose SBF into frequency bands reported to be associated with metabolic, neurogenic, myogenic,

respiratory and cardiac SBF control mechanisms. The method was used to differentiate blood flow control mechanisms responding to indentation from those responding to heating. Incremental heat (35-45°C, 1°-step/minute) and pressure (0-60 mmHg, 5-mmHg step/3 min) were applied to the sacrum in ten healthy subjects. The results suggest that skin blood flow is mediated by myogenic control after application of incremental external pressure and is mediated by metabolic control after incremental local heat exposure ($p<0.01$).

The study of blood flow responses is confounded by temporal variability in blood flow measurement. This study investigated the effectiveness of our wavelet analysis technique in reducing week-to-week variability in SBF. The results show that coefficients of variation for the power in each frequency band are smaller than that of baseline SBF or maximal blood flow ratio ($p<0.05$).

Ten healthy participants were subjected to both constant loading for 20 min at 30 mmHg and AP for 20 min (5-min cycle x 4) at either 60 or 0 mmHg on their sacrum. The results indicate AP stimulates an increase in SBF compared to constant loading ($p<0.01$). SBF during the high-pressure phase of four AP cycles shows an increasing trend. Our study suggests that optimization of AP parameters to compensate for impaired control mechanisms in pathological populations may be possible using wavelet analysis of blood flow oscillations.

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1.0 INTRODUCTION

1.1 Statement of the Problem

Lesions produced in weight-bearing tissues subjected to unrelieved pressure are identified by their etiology as pressure ulcers (Agency for Health Care Policy and Research, 1992). Pressure ulcer prevalence varies by population, care setting, and ulcer site. Wheelchair users and others with mobility impairments tend to be at greatest risk for development of pressure ulcers (Allman, 1997) with reported annual prevalence rates of 50-80% for individuals with spinal cord injuries (SCI) and up to 28% for elderly nursing home residents (Smith, 1995; Salzberg et al., 1998). Pressure ulcers exact a devastating loss of function, increase the risk of death in geriatric populations, and increase healthcare costs. More than 60,000 people die from complications of pressure ulcers each year in the United States (Thomas et al., 1996). Conservative estimates of the spinal cord injury population in the US range between 219,000 and 243,000 (National Spinal Cord Injury Statistical Center, 2003) with annual pressure ulcer treatment costs of $1.3 billion, 25% of the total cost of SCI treatment (Byrne and Salzberg, 1996). Similarly, conservative estimates of the costs associated with the management of pressure ulcer of all etiologies exceed $6.4 billion annually (Marwick, 1992). The Agency for Healthcare Research and Quality (AHRQ), formerly AHCPR, lists pressure ulcers as one of the seven most important health issues in the United States (Agency for Health Care Policy and Research, 1994), and the Department of Veterans Affairs' Quality Enhancement Research Initiative (QUERI) reports that significant gaps still exist in the pressure ulcer

prevention and treatment knowledge base (Weaver et al., 2000). It is clear that research regarding the prevention and treatment of pressure ulcers remains a priority.

Pressure ulcer prevention strategies attempt to minimize the effects of interrelated factors known to be associated with their etiology. Specific strategies to prevent sitting-acquired pressure ulcers have been focused on periodic un-weighting of the ischial area and the provision of support surfaces designed to maintain tissue integrity (Brienza et al., 1991; Brienza et al., 1993; Brienza and Karg, 1998; Brienza et al., 2001). The support surface's role in maintaining tissue integrity is to distribute weight as evenly as possible over the surface, thus reducing pressure gradients, preventing extreme heat transfer rates, controlling wetness, and minimizing shear forces. Product developers have focused their efforts on reducing pressure and pressure gradients. However, pressure is but an indirect measure of the detrimental effects of a person's body weight pressing through soft tissue to the underlying support surface. Blood flow measurements provide a more direct measure of risk because it is the blood that provides the tissue with the critical nutrients necessary for its survival.

The interaction between interface pressure and microvascular blood flow appears to be complex. Different pathologies result in various deficits of normal physiological responses to mechanical stresses. For one example, loss of neurogenic control over the cardiovascular system following spinal cord injury impairs blood flow regulation (Byrne and Salzberg, 1996; Teasell et al., 2000). For another, loss of elastin and/or degradation of the collagen matrix in aging skin reduces the body's ability to withstand external pressure without excessive deformation of the blood vessels (Bader, 1990; Ballas and Davidson, 2001). In some circumstances, it may be impossible to reduce and hold

interface pressure below critical levels. Alternating pressure support surfaces - surfaces that provide cyclic changes in interface pressure - appear to reduce risk of tissue damage by increasing microvascular blood flow. However, the underlying physiological mechanisms responsible for the increases are not understood (McLeod, 1997; Mayrovitz and Smith, 1999; Brienza and Geyer, 2000; Rithalia and Gonsalkorale, 2000).

Laser Doppler flowmetry (LDF) has been used extensively to quantify skin perfusion responses (ml_{LD}/min/100g tissue or au) to compressive loading (Bennett et al., 1981; Xakellis and Frantz, 1990; Xakellis et al., 1993; Schubert et al., 1994; Colin and Saumet, 1996; Sanada et al., 1997; Mayrovitz and Smith, 1999; Stefanovska et al., 1999). LDF is noninvasive, requires no heating of the skin (as $TcPO_2$ does) and can detect microcirculatory changes at a depth of ~1mm below the surface of the skin. Traditionally, LDF blood flow has been reported only in the time domain. Because the physiological rhythms associated with blood flow control mechanisms are imbedded in the blood flow signal, decomposing the signal via spectral analysis reveals various characteristic frequencies that may contribute to our understanding of these complex mechanisms. Unfortunately, few investigators have used spectral analysis to date and a systematic methodology appears to be lacking (Meyer et al., 1989; Breit and Intaglietta, 1994). To this end, we designed a series of experiments using this method to investigate skin microcirculatory control mechanisms in response to various stimuli and loading conditions.

Fourier transform-based power spectrum analysis has been used to study blood flow that is characterized by two peaks (Kastrup et al., 1989). However, Fourier transform analysis does not provide sufficient time resolution for analysis of non-

stationary physiological signals, such as heart rate and myoelectric signals (Stefanovska and Bracic, 1999; Karlsson et al., 2000; Lotric et al., 2000). Although windowed Fourier transform method permits time-frequency analysis, obtaining adequate precision in both domains requires selection of a proper window that balances time and frequency resolution. For complex signals with several mixed frequency components (i.e. skin blood flow), this balance is not possible (Stefanovska and Bracic, 1999). To overcome the limitations of the Fourier method, Morlet first conceptualized wavelet analysis in 1983. Later Grossman and Morlet laid the mathematical foundation for the wavelet transform permitting multi-resolution, time-frequency analysis (Grossmann and Morlet, 1984) of the blood flow signal.

Stefanovska and Bracic used the wavelet transform to analyze respiration, electrocardiogram, blood pressure, and LDF signals. They recorded responses at rest and during exercise in subjects with normal, well-conditioned and pathological cardiovascular systems. According to their eloquently reported results, wavelet analysis of blood flow reveals various peaks in the power spectrum corresponding to specific origins: 1) heart rate (0.4-2.0 Hz), 2) respiratory activity (0.15-0.4 Hz), 3) vascular myogenic responses (0.06-0.15 Hz), 4) neurogenic responses (0.02-0.06 Hz) and 5) metabolic responses (0.0095-0.02 Hz) (Bracic and Stefanovska, 1998; Kvernmo et al., 1998b; Stefanovska and Bracic, 1999; Stefanovska et al., 1999; Kvandal et al., 2003; Soderstrom et al., 2003). Since Stefanovska et al. demonstrated that wavelet analysis permits examination of the contributions of the myogenic, neurogenic and metabolic components of vasomotion relative to an exercise stimulus, we theorized that this method might also be useful in the study of mechanisms and beneficial responses associated with alternating pressure.

Increases in skin blood flow in response to mechanical stress have been considered a protective response by other investigators (Sacks et al., 1988; Frantz and Xakellis, 1989; Xakellis and Frantz, 1990; Xakellis et al., 1993; Sanada et al., 1997; Mayrovitz and Smith, 1999; Patel et al., 1999), and may be impaired in at-risk populations. The physiological mechanism responsible for increases in skin blood flow is not well understood; however, the autoregulation of local microcirculation may be associated with this protective response. Autoregulation is the tendency of the local microcirculatory system to maintain constant blood flow. It is considered a local protective mechanism independent of neurogenic control (Levick, 2000). We postulated that autoregulation is the underlying physiological mechanism responsible for the stress dependent protective increase in skin blood flow.

Both metabolic and myogenic factors have been considered to be associated with autoregulation of the local microcirculatory system. Both factors are mechanical stress dependent (Lewis and Grant, 1926; Duff and Shepherd, 1953; Aulick et al., 1977; Freund et al., 1981; Osol and Halpern, 1988; Meininger and Davis, 1992; Achakri et al., 1995; Butler et al., 2000). Vascular smooth muscle cells receive information from these interrelated blood flow control mechanisms thereby setting the contraction rhythm to provide sufficient blood flow. These interrelated control mechanisms from the neurological system and local mechanisms work together to respond to different stimuli. By using wavelet analysis, the understanding of interactions of metabolic, myogenic and neurogenic responses to different stimuli (e.g. heating and various loading patterns) may be advanced. Improved knowledge of blood flow control mechanisms may allow

assessment of the effectiveness of alternating pressure therapy and thereby contribute to the determination of optimal alternating pressure parameters.

Prevention of pressure ulcers and enhanced tissue integrity may be possible using alternating pressure support surfaces. To this end our investigation was developed to study blood flow control mechanisms and their interrelated interactions with alternating pressure. Sacral skin blood flow responses to incremental heating and incremental loading in frequency band reported to be associated with metabolic, myogenic and neurogenic control mechanisms are identified first. The protective physiological mechanisms associated with alternating pressure are then characterized. Improved understanding of beneficial response to alternating pressure could provide rationale for the optimization of alternating pressure support surfaces for enhancing blood flow and reducing pressure ulcer risk.

1.2 Purposes of the Study

The purpose of this study is to develop a method for characterizing skin blood flow control mechanisms response to alternating pressure as well as to validate the effectiveness of using alternating pressure. The long-term goal is to improve the effectiveness of alternating pressure support surfaces by optimizing loading parameters (i.e. cycle and pressure parameters and configuration of air cells). The specific hypothesis of this study is that the applications of alternating pressure stimulate myogenic responses as measured from wavelet analysis of blood flow oscillation, thereby enhancing skin blood flow.

A series of studies investigating the skin's microvascular response to various stimuli and loading conditions as measured by laser Doppler flowmetry has been conducted to study blood flow control mechanisms with implications for the study of protective physiological mechanisms associated with alternating pressure. The specific aims of this research are to:

1. Develop a computer-controlled system to apply various stimuli and loading conditions on the sacrum with simultaneous measurement of physiological responses (i.e. skin blood flow and temperature);
2. Characterize skin blood flow control mechanisms from laser Doppler flowmetry measurements by implementing wavelet analysis (time-frequency analysis);
3. Propose a normalization method for studying relative contributions of the various blood flow control mechanisms (i.e. metabolic, neurogenic, myogenic, respiratory, and cardiac control mechanisms) responding to various stimuli;
4. Differentiate blood flow response associated with local heating from those associated with indentation (nonnoxious externally applied pressure);
5. Identify blood flow control mechanisms responsible for pressure-induced vasodilation and reactive hyperemia;

6. Compare the effectiveness of wavelet analysis to traditional methods used by other researchers for reducing blood flow variability (e.g. maximal blood flow ratio);
7. Study the repeatability of blood flow control mechanisms' response to local heating;
8. Compare mean skin blood flow under constant loading and alternating pressure at the sacrum; and
9. Identify protective physiological mechanisms associated with alternating pressure based on above methods and findings.

To best of our knowledge this is the first study using wavelet analysis to characterize skin blood flow responses to thermal stress or mechanical stress. Skin blood flow control mechanisms are attributable to metabolic, neurogenic, myogenic, respiratory and cardiac control mechanisms (Kvernmo et al., 1998b; Kvernmo et al., 1999; Stefanovska and Bracic, 1999; Stefanovska et al., 1999; Kvandal et al., 2003; Soderstrom et al., 2003). In order to validate the use of spectral analysis and to advance understanding of interrelated reactions of metabolic, myogenic and neurogenic control mechanisms, we designed experiments using local heat and incremental loading to test responses of blood flow control mechanisms. Based on the findings, the beneficial physiological mechanisms associated with alternating pressure may be identified.

1.3 Potential Outcomes and Benefits

Prevention of pressure ulcers and enhanced tissue integrity may be possible using alternating pressure support surfaces. Alternating pressure may stimulate a protective increase in skin blood flow, but the physiological mechanism by which this occurs needs closer examination. This series of studies was designed to provide insight into the control mechanisms by which SBF is affected by alternating pressure operating parameters. With improved understanding it may be possible to optimize these parameters for enhancing blood flow in populations at-risk for developing pressure ulcers (i.e. individuals with SCI, elderly population or individuals with endothelial dysfunction). Our method may serve as an objective assessment of alternating pressure support surfaces. If successful, this method would ultimately translate into improved wheelchair seat cushions and full-body support systems for these populations.

Our study may also advance the knowledge base related to pressure ulcer prevention. Pressure ulcer prevention strategies attempt to minimize the effects of interrelated factors known to be associated with their etiology. Product developers have focused their efforts on reducing pressure and pressure gradients. However, pressure is but an indirect measure of the detrimental effects of a person's body weight pressing through soft tissue to the underlying support surface. Blood flow measurements provide a more direct measure of risk because it is the blood that provides the tissue with the critical nutrients necessary for its survival. The interaction between interface pressure and microvascular blood flow appears to be complex. Different pathologies result in various deficits of normal physiological responses to mechanical stresses. Characterization of skin blood flow responses to various stimuli in pathological populations could improve

the effectiveness of risk assessment in clinical practice. Improved understanding of impaired mechanisms associated with pressure ulcer may facilitate the development of new treatment and prophylactic support surfaces.

1.4 Outline of the Dissertation

The remaining chapters of this dissertation are outlined as follows:

Chapter 2 describes the development of a computer-controlled system to apply various stimuli and loading patterns to the soft tissues with simultaneous measurement of physiological responses (e.g. skin blood flow & temperature). A nonlinear contact axisymmetric finite element model was established to select the curved edge radius for minimizing edge effect.

Chapter 3 describes using wavelet transform with quantification methods to characterize frequency components embedded in blood flow oscillations which were then designated to the proposed corresponding physiological mechanisms: metabolic (0.008-0.02 Hz), neurogenic (0.02-0.05 Hz), myogenic (0.05-0.15 Hz), respiratory (0.15-0.4 Hz) and cardiac (0.4-2.0 Hz). Skin blood flow responses to incremental heating (35 to 45°C) are discussed.

Chapter 4 compares the skin blood flow responses to incremental heating and incremental loading (0 to 60 mmHg, 5 mmHg step/3 min). Blood flow control mechanisms are compared, and the mechanisms associated with heating or indentation are discussed.

Chapter 5 describes variability of baseline blood flow and power within characteristic frequency bands at baseline for measurements taken once per week for

three consecutive weeks. The repeatability of blood flow control mechanisms' response to local heating for three consecutive weeks is studied.

In Chapter 6 skin blood flow responses to alternating pressure (20 min (5 min cycle × 4) at either 60 mmHg or 3 mmHg) are compared to the responses to constant loading (20 min at 30 mmHg). The theoretical basis and physiological rationale of using alternating pressure for enhancing tissue viability are discussed.

Chapter 7 summarizes the findings of this dissertation and offers recommendations for future research.

2.0 DEVELOPMENT OF A COMPUTER-CONTROLLED SYSTEM FOR EVALUATING DERMAL BLOOD FLOW RESPONSE TO ALTERNATING PRESSURE

Abstract – The use of alternating pressure (AP) support surfaces to prevent pressure ulcers is based on the premise that AP loading reduces ischemia in load bearing soft tissue. However, evidence to support this is weak. A computer-controlled system with a compound sensor head was developed to assess the effect of AP loading patterns on physiological parameters (i.e. skin perfusion, temperature, blood pressure). Test protocols were developed and tested on ten healthy volunteers to optimize controlled test parameters for future studies on AP. The results indicate that the system provides reliable data for use in the study of the relationships between tissue biomechanical and physiological characteristics and loading methods. Such comparisons may be useful for determining risk of tissue injury and assisting in the design and assessment of prophylactic support surfaces.

Key Words: alternating pressure, computer-controlled device, laser Doppler flowmetry, finite element model, skin blood flow, vasomotion.

Abbreviations: AP = Alternating Pressure; BPM^2 = Blood Perfusion Monitor 2; FEM = Finite Element Model; I/R = Ischemia-Reperfusion; LDF = Laser Doppler Flowmetry; SCI = Spinal Cord Injury; $TcPO_2$ = Transcutaneous Oximetry; TSI = Thermal Stress Index.

2.1 A Conceptual Design of the System

The use of alternating pressure (AP) support surfaces to prevent pressure ulcers is based on the premise that AP loading reduces ischemia in load bearing soft tissue (Gardner, 1948; Kosiak, 1961). However, evidence to support this is weak (McLeod, 1997; Rithalia and Gonsalkorale, 2000). In the absence of strong evidence, developers and manufacturers of AP support surfaces have produced a variety of designs in need of validation. The need to identify rationale for determining AP parameters for enhancing tissue viability is well acknowledged by both clinicians and manufactures. Several studies have attempted to provide insight on the beneficial mechanisms associated with AP (Bader, 1990; Mayrovitz et al., 1993; Mayrovitz and Smith, 1999; Rithalia and Gonsalkorale, 2000); however, the results from these studies have been inconclusive. Recent progress in soft tissue mechanics and microvascular physiology has the potential to advance understanding of protective mechanisms associated with AP. The purposes of this study are to provide a conceptual design for investigation of the beneficial mechanisms associated with AP and to develop a system to evaluate these parameters.

2.1.1 Selection of a key physiological response

There is broad consensus among researchers that enhanced tolerance is related to reduced externally applied pressure and improved microvascular response to mechanical stress (Dinsdale, 1974; Daniel et al., 1981; Crenshaw and Vistnes, 1989; Bader, 1990). Oxygen and nutrients needed by cells are exchanged in the microcirculation. If externally applied pressure occludes blood flow, the resulting insufficient supply of oxygen and nutrients to cells may lead to tissue necrosis (Dinsdale, 1974; Daniel et al., 1981). Thus,

monitoring perfusion of the compressed tissues is important for assessing efficacy of support surfaces (AP support surfaces in our case). There are several methods providing non-invasive measurement of perfusion, for example, laser Doppler flowmetry (LDF), photoplethysmography, thermography, and transcutaneous oximetry ($TcPO_2$). Photoplethysmography is sensitive to motion artifact (Webster, 1998), thus is not be an ideal candidate to study perfusion responses to AP. Thermography has been shown unreliable for predicting tissue viability (Sprigle et al., 2001). Another disadvantage of $TcPO_2$ monitors is that they require the skin to be heated to enhance its permeability to O_2. Studying perfusion responses to mechanical stresses at this temperature may not be representative of clinical use (Patel et al., 1999).

LDF provides a non-invasive, real-time evaluation of skin microcirculation, and has been shown to be reliable (Shepherd and Oberg, 1990). The advantage of using LDF to investigate the beneficial mechanisms associated with AP is that it is capable of monitoring blood flow oscillations reflecting vasomotion patterns (Colantuoni et al., 1994; Lossius and Eriksen, 1995; Bertuglia et al., 1996). Vasomotion is the periodic constriction and dilation of blood vessels (Nicoll and Webb, 1955), and is attributed to local metabolic needs, neurogenic control and vascular myogenic responses (Salerud et al., 1983; Kastrup et al., 1989; Ursino and Fabbri, 1992; Schubert and Mulvany, 1999; Stefanovska and Bracic, 1999; Stefanovska et al., 1999; Soderstrom et al., 2003). Vasomotion changes in response to various stimuli for maintaining sufficient blood supply (Intaglietta, 1991). Thus AP parameters may be optimized using vasomotion patterns monitored indirectly through blood flow oscillation measurements.

Recently, Stefanovska and colleagues demonstrated the possibility of using wavelet analysis to characterize frequencies embedded in LDF blood flow corresponding to specific control mechanisms (Stefanovska and Bracic, 1999; Stefanovska et al., 1999). We theorized this method has great potential to advance the understanding of the beneficial mechanisms associated with AP thereby providing rationale for optimization of AP technology for compensating for pathological deficits. Once the relationship of AP parameters and perfusion responses are identified, a method for customizing AP operating parameters may be developed. For example, parameters may be determined that stimulate local myogenic responses to compensate for deficits of neurogenic mechanisms in the microcirculation in the compressed soft tissues in individuals with spinal cord injuries (SCI).

2.1.2 Theoretical basis of modulating blood flow by mechanical stress

Original concept of using AP support surface was to provide periodic relief for compressed tissues (Gardner, 1948; Kosiak, 1961). However, this may not be the sole or even primary benefit associated with AP (Mayrovitz and Smith, 1999). Developments and new knowledge of vascular mechanics has advanced the understanding of relationships between mechanical stress and blood flow regulation (Meininger and Davis, 1992; Mayrovitz et al., 1993; Sumpio, 1993; Nichols and O'Rourke, 1998; Vorp et al., 1998). This new knowledge is important to investigate mechanical and physiological responses of skin to AP. Blood flow induced shear stress acting on the endothelium regulates release of endothelial nitric oxide, a vasodilator (Sumpio, 1993; Arnal et al., 1999; Michiels, 2003). Changes of transmural pressure modulate contraction/relaxation

patterns of vascular smooth muscles (Nichols and O'Rourke, 1998; Levick, 2000). AP results in changes of stress and strain within bulk tissues thereby affecting mechanical stress acting on the vascular smooth muscles and the endothelial cells. Proper loading protocols of AP could elicit vasodilatory responses thereby enhance blood flow. If relationships between loading patterns and blood flow regulation can be identified, AP technology may reduce risk of pressure ulcer by enhancing tissue viability.

2.1.3 Criteria for an indentation system

The essential functions of a system for studying skin perfusion response to AP are the application of a controlled load to the skin and the monitoring of perfusion responses. Two general types of load applicators are found in the literature, controlled-pressure pneumatic systems and dead weight systems. The pneumatic applicators are more common (Mayrovitz et al., 1993; Mayrovitz and Smith, 1999; Mayrovitz et al., 2003) and typically use a commercially available AP support surface to provide the load. Such an approach is limited by its ability to produce reliable interface pressures. Although inflation pressure inside of the air cells is controlled, manipulation of air cell pressure results in unpredictable interface pressures. The interface pressure is dependent on the characteristics of the soft tissue and other factors such as body weight (Krouskop et al., 1986). Interface pressure will therefore vary from subject to subject making comparisons of perfusion responses difficult. The dead weight methods found in the literature use a loading pan and standard weight to manually apply AP loading patterns (Bader, 1990). Such manual operation is more precise than the pneumatic methods, but is tedious and cannot be easily used to study such parameters as loading rate.

Several displacement controlled indentation systems used for the mechanical assessment of properties of bulk tissues (e.g. viscoelastic parameters) have been previously developed and shown to be reliable (Krouskop et al., 1987; Mak et al., 1994; Vannah and Childress, 1996; Zheng and Mak, 1996; Sanders et al., 1997; Pathak et al., 1998; Silver-Thorn, 1999; Wang et al., 2000). Several force or pressure controlled indentation systems have been developed for the perfusion study (Ferguson-Pell et al., 1994; Salcido et al., 1995; Silver-Thorn, 2002). Pneumatic indenters are low cost and easy to develop, but response time is not fast enough to provide complex loading patterns (Ferguson-Pell et al., 1994). An indenter developed by Silver-Thorn is displacement control intended to study effect of bulk tissue properties on the perfusion responses (i.e. study perfusion responses under creep or relaxation test) (Silver-Thorn, 2002). Salcido et al. developed an indenter for use in the animal model (Salcido et al., 1995). The system described here provides force control and easily program ability to simulate various AP protocols.

2.1.4 Necessity of other physiological responses

Heat is an important confounding variable in the investigation of perfusion responses to mechanical stress (Silver-Thorn, 2002). The vasculature of the skin includes nutritional and thermoregulatory capillaries. The thermoregulatory capillaries are larger, horizontal vessels and are located deeper than the nutritional capillaries. They respond to increased temperature by dilating to meet the corresponding increased metabolic demand of the cells as their temperature rises. Accumulation of heat at the body and support surface interface results in an increase in skin temperature thereby increasing metabolic

rate of heated cells (Brown and Brengelmann, 1965). Heat-induced vasodilation is a protective response to remove excessive heat, and is impaired in people with diabetes, peripheral vascular disease, SCI and in the elderly (Belcaro et al., 1990; Timar-Banu et al., 2001; Minson et al., 2002). Maximal skin blood flow can be induced by locally heating skin to 42°C for 20~40 minutes (Kellogg et al., 1999; Minson et al., 2001). We planed to investigate the effects of heat on the microcirculation, hence our system included both temperature control and monitoring capabilities.

Blood pressure is an important factor affecting capillary occlusion pressure as well as perfusion responses to mechanical stress. Blood flow is determined by both blood pressure gradient and flow resistance according to the flow rule (Germann and Stanfield, 2002). Individuals with SCI lose neurogenic control over their cardiovascular system resulting in lower blood pressure. Low blood pressure is also observed in the elderly. Hypotension has been considered a risk factor for developing pressure ulcers (Schubert, 1991). Another potential benefit of measuring blood pressure is to estimate cutaneous flow resistance in pathological populations (Wang et al., 2001). Cutaneous flow resistance is dependent on the skin blood flow and mean arterial pressure. Cutaneous flow resistance is related to vasomotor tone mediated through sympathetic innervation. Greater vasomotor tone implies a higher potential of vasodilatory function higher flow rates (Levick, 2000). Thus, comparing blood pressure between healthy subjects and at-risk population is also an important consideration related to evaluating blood flow responses and pressure ulcer risk.

2.1.5 Edge effect of the indenter head

A final consideration for developing a system to study AP loading effects on the blood flow is the nature of the pressure application. In previous investigations of blood flow responses to external loading, the indenter's edges were curved to minimize rim pressure and provide uniform loading (Newson and Rolfe, 1982; Sacks et al., 1985; Bader and Gant, 1988; Bader, 1990; Ferguson-Pell et al., 1994; Hagisawa et al., 1994). However, no previous attempts were made to justify the selected radius. When the flat end of a rigid cylindrical indenter is pressed into a viscoelastic material like skin or soft tissue, there are considerable stress concentrations at the edge. Stress concentrations would elicit a variable and perhaps unreliable response in the tissue under and surrounding the indenter head. Local blood flow (under the center region of an indenter in our case) is affected by adjacent area (under the rim of an indenter in our case) through transporting of vasodilators up- or down-stream into microcirculatory network (Rivers, 1995; Beach et al., 1998). In order to precisely determine the relationship between loading pressure and blood flow responses, an even pressure distribution is desired.

Timoshenko & Goodier (Timoshenko and Goodier, 1969) and Sacks et al. (Sacks et al., 1985) performed dimensional analysis to describe the pressure distribution under such an indenter. Their analysis was based on the classical elastic theory and did not account for the effect of edge radii. Finite element modeling (FEM) has been used extensively in prosthetic socket design to study interactions between residual limbs and sockets (Silver-Thorn et al., 1996; Zachariah and Sanders, 1996; Zhang et al., 1998). Here FEM is used to predict stress distribution at the interface and within soft tissues and provide insight into of the effects of various socket configurations. Two methods are used

to simulate interface between prosthetic socket and residual limb: the glued method and contact analysis. The advantage of the glued method is that it is easy to construct a FEM. Its disadvantage is that it is less accurate than the contact analysis method. Contact analysis is complex, but provides better accuracy (Zachariah and Sanders, 1996). Simplification of geometric, boundary and material conditions in FEM is useful and needed in preliminary studies such as ours in order to provide insight into mechanical responses (Zhang et al., 1995; Zhang et al., 1998). We used contact analysis to investigate the pressure under a model of our rigid indenter to determine the effect of edge radii. Our development included a non-linear contact finite element model with assumption of linear properties of bulk soft tissues to optimize the edge radius. Adding nonlinear material properties into the contact analysis would have increased the complexity of the model and made convergence to solution difficult (Silver-Thorn et al., 1996; Zhang et al., 1997).

In summary, the approach we have adopted to study blood flow control mechanisms uses a computer-controlled indenter to apply pressure, incorporates laser Doppler flowmetry to monitor perfusion responses including skin blood flow and frequency components, controls temperature in order to simulate clinical environment, and records blood pressure, heart rate and skin temperature. Preliminary results are presented to demonstrate the ability of our system to identify potential parameters associated with beneficial blood flow control mechanisms stimulated by AP. The design and development of our system are described here.

2.2 Design and Development of the System

2.2.1 Computer-controlled indenter design

The completed system is shown in Figure 2-1. The indenter was mounted on a stand that allowed for adjustment with 5 degrees of freedom: translation (A) along and rotation (B) around the vertical axis of the stand, rotation around a horizontal axis extending out along a radius of the vertical axis (C), rotation around a second vertical axis (D) and rotation about the second axis (E). This adjustability is sufficient to allow for the orientation and location of the indenter so that the flat end of the indenter head approaches normal to the skin surface at the desired location on the sacrum.

A stepper-motor (Superior Electric, CT) driven lead screw actuator and two compression springs (Lee Spring, NY; spring constant ~ 385N/m) were used to control the force and, therefore, the interface pressure on the skin. The motor drive control is accomplished using an ISA bus stepper motor control board (Brienza et al., 1996). The springs also served to minimize the adverse effects of any disturbances caused by motion of the subject. A strain gauge, cantilever beam force transducer (Kwiatkowski and Inigo, 1993) was used to monitor force and generate feedback for closed-loop force control. The signal from the strain gage cantilever beam was amplified via force strain gage, and then was sampled by DAP 1204E data acquisition processor (Microstar Laboratories, Bellevue, WA) with 1024K RAM and an 80186 16MHz microprocessor. The force feedback control in the system is aimed to overcome changes of pressure due to participant's unintentional small posture shifts. Feedback control was implemented by comparing differences between desired pattern and force signal every second. The schematic diagram of the system is shown in Figure 2-2. The main control program for

system control and data acquisition is written in LabVIEW 5.0 (National Instrument, TX).

2.2.2 Compound sensor head design

The indenter head contains the laser Doppler blood perfusion probe, thermometer, and heating probe.

Laser Doppler blood perfusion monitor and probe. Laserflo Blood Perfusion Monitor 2 (BPM^2, Vasamedics, MN) and the Softip pencil probe (P-435, Vasamedics, MN) were used to measure capillary blood flow (ml_{LD}/min/100g tissue). BPM^2 provides noninvasive measurement of skin blood flow at a depth of about 1 mm via laser and fiber optics technology (Shepherd and Oberg, 1990). The 0-5 volt analog output of the Laserflo was sampled at 20 Hz using a 16-bit data acquisition card (PCI-MIO-16XE, National Instruments, TX).

Temperature sensor and control device. A five-channel scanning thermistor thermometer (U-08502-14) and two thermistor probes (U-08443-20, Cole-Parmer Instrument Company, IL) were used to measure skin temperature changes both under the indenter and adjacent skin (10 cm from the center of the indenter head). The system provides 0.056°C resolution within a range of -30.0 to 100.0 °C. The analog output (18 mV per °C) was sampled at 20 Hz using the 16-bit data acquisition card (PCI-MIO-16XE, National Instruments, TX). The temperature control device (TCO, Vasamedics, MN) provides 0.5°C precision control of surface temperature. Heat is controlled manually using front panel of the BPM^2. Figure 2-2 shows a schematic diagram showing the locations of the heating element, thermistor, and laser Doppler probe.

Blood pressure monitor. A Critikon Dinamap vital signs monitor (1846SX, Johnson and Johnson, Tampa, FL) was used to monitor systolic blood pressure, diastolic blood pressure and heart rate. The device was connected to the computer via a serial port and was sampled per minute.

2.2.3 Indenter edge radius optimization

Most investigations using rigid soft tissue indenters have utilized a cylindrical indenter with an arbitrary edge radius (Newson and Rolfe, 1982; Sacks et al., 1985; Bader and Gant, 1988; Bader, 1990; Hagisawa et al., 1994). A poor choice of edge radius can result in undesirable high stress concentrations during indentation into soft tissue. This edge effect can be illustrated using classic theory of elasticity. Accordingly, the pressure distribution on an elastic material under the rigid, flat indenter is given by

$$P_x = \frac{F}{2\pi r\sqrt{r^2 - x^2}} \qquad \text{(Equation 2-1)}$$

Where, P_x is the pressure at a distance x from the center of indenter; F is the total applied force; and r is the radius of the indenter (Timoshenko and Goodier, 1969). According to equation (2-1), P_x increases rapidly as x/r approaches 0.8 and, in fact, becomes asymptotic as x approaches 1.0 (Figure 2-3). In other words, the theory predicts a dramatic peak in the contact pressure beginning at a distance approximately $0.8r$ from the center of the indenter.

In our system, the heating probe has a radius of 9.5 mm. The laser Doppler probe is inserted in the center hole of the heating head. The 4.8-mm-diameter thermometer probe is placed adjacent to the heating head. In order to get an even pressure distribution

area under all measuring sites, 80% of the indenter radius should cover all measuring probes. Therefore, an 18-mm-radius rigid indenter head is required to fulfill this goal.

Rounding the edge of the indenter can indeed significantly reduce the edge effect. In this study, finite element modeling was used to determine an optimal edge radius. The ANSYS 5.6 Education version (ANSYS, Pittsburgh, PA) was used to perform a contact pressure analysis. Soft tissue was assumed to be homogeneous, isotropic and bonded to rigid bone (Figure 2-3). For this analysis, a sacral soft tissue thickness of 14 mm was selected based on the average value reported for healthy elderly subjects (Clark et al., 1989). The bulk tissue of the sacrum was assumed to be linearly elastic with a Young's modulus of 100 kPa and a Poisson's ratio of 0.49 (Zhang et al., 1997). A Young's modulus of 70 GPa and a Poisson's ratio of 0.33 were assumed for the aluminum indenter (Spiegel and Limbrunner, 1994). 60 mmHg (7999 Pa) was selected as the indenter's uniform loading pressure. An axisymmetric finite element model (Olukoko et al., 1993; Zhang et al., 1995) was established to perform an indentation test of sacral tissue.

Eight node isoparametric quadrilateral elements (plane82 with axisymmetric behavior, ANSYS, Pittsburgh, PA) were used to represent the indenter and soft tissue. Contact elements were created on either side of the contact surfaces of the indenter (targe169, ANSYS, Pittsburgh, PA) and soft tissue (conta172, ANSYS, Pittsburgh, PA). The penalty function and Lagrange multiplier methods were used to solve the nonlinear contact equations. Due to the contrasting elasticity of the soft tissue compared to the indenter, a low contact stiffness (FKN=0.01) and penetration tolerance ratio (FTOLN=0.1) were used in this model. Due to the nature of alignment of indenter (e.g.

almost vertical alignment of indenter) no slip condition was used between contact elements of the soft tissue and the indenter.

A soft tissue width equivalent to four times the radius of the indenter was considered adequate to represent the infinite boundary for the indentation (Olukoko et al., 1993). The analysis included a flat edge and four additional geometric models with curved edge radii of 0.72, 1.44, 2.16 and 2.88 mm corresponding to 4, 8, 12, and 16 % of radius. The finite element model is shown in Figure 2-4.

2.2.4 Test protocols development

Ten unimpaired subjects (5 male and 5 female) were recruited into the study. The demographic data were as follows: age 30.0 ± 3.1 years, height 162.9 ± 6.8 cm, and weight 58.3 ± 8.6 kg. The following conditions constituted exclusion criteria: the presence of pressure ulcers on the sacrum, diabetes, vascular disease, hypertension, or use of vasoactive medications. An informed consent approved by the University of Pittsburgh Institutional Review Board was obtained from each subject prior to testing. All tests were performed in the Soft Tissue Mechanics Laboratory, University of Pittsburgh. Room temperature was maintained at 24 ± 1°C. For at least 30 minutes prior to testing, all subjects assumed a recumbent, relaxed position in the laboratory to accommodate to the room temperature and achieve a baseline blood flow level.

Test protocols were developed using healthy volunteers to assess the quality of the data obtained and to determine the optimum positioning and loading duration for subsequent studies on AP in pathological populations. The development addressed four protocol issues: 1) comfort and pressure on the exam table for the subject, 2) AP loading

protocol including baseline, loading and post-loading duration, 3) constant and alternating pressure magnitudes and 4) heating protocol parameters.

The sacrum was selected as the site for loading because it is the most common site for pressure ulcers in high-risk populations (Salzberg et al., 1996). Loading the sacrum requires the subjects to lie prone on an examination table, placing pressure on the weight-bearing bony prominences of the dorsum of the foot, knee, anterior pelvis, ribs, elbows, shoulders and face, which must be adequately protected. A positioning protocol was designed to minimize interface pressure and discomfort. The subjects were positioned on a custom-contoured support surface (Versa Form, Sammons Preston Rolyan, IL). A pressure mapping system (FSA, Vista Med, Winnipeg, Canada) was used for interface pressure monitoring.

The specific parameters of the loading regimens proposed for this study (duration of loading, 20 minutes; magnitude of low and high pressure in the AP cycle, 3 and 60 mmHg; and cycle time of AP, 5 minutes) were chosen based either on reports of augmented blood flow in response to either constant (20 minutes to several hours) pressure, low-level (30-70 mmHg) pressure, or AP delivered via existing commercial support surfaces (5 minute inflation-deflation cycle) (Frantz and Xakellis, 1989; Xakellis and Frantz, 1990; Xakellis et al., 1993; Medical Devices Agency, 1995; Medical Devices Agency, 1997; Sanada et al., 1997; Mayrovitz and Smith, 1999; Patel et al., 1999). Since non-zero low pressure after pressure loading has been shown to blunt reactive hyperemia (Mayrovitz and Sims, 2002; Mayrovitz et al., 2003) and the laser Doppler probe requires that it be held in contact with the skin, the low pressure in the AP cycle was chosen to be 3 mmHg. Average pressure was chosen at 30 mmHg; thus constant loading was set at 30

mmHg. The pressure during the high-pressure phase of AP was 60 mmHg to achieve an average of 30 mmHg. The pressure profiles of AP and constant loading are shown in Figure 2-5.

The thermal stress index (TSI) has been used to screen for abnormal microcirculatory functional status in peripheral vascular diseases (Belcaro et al., 1989; Belcaro et al., 1990; Timar-Banu et al., 2001). The procedure for obtaining the TSI consists of measuring the skin blood flow at 35°C for 2 minutes followed by heating the skin at 44°C for 20 minutes, and then recording blood flow for 2 minutes. TSI is defined as blood flow at 44°C / 35°C. A score greater than or equal to five is considered normal. One of our long-term goals is to identify and compensate for deficits to mechanisms responsible for vasodilatory function in people with SCI. However, heating skin at 44°C for 20 minutes (the protocol used in TSI) on individuals with SCI may be unsafe. We suggest the use of a less stressful heating protocol. A modified TSI was designated as skin blood flow at 45°C / 35°Cin which the skin is heated for 15 minutes: three minutes at 35°C, nine minutes of incremental heating (1°C increase per minute) from 35 to 45°C and a final period of three minutes at 45°C.

Parameters measured and analyzed this preliminary investigation included: blood flow (including mean value (ml/min/100g tissue) and frequency content (power)), interface pressure (mmHg), skin temperature (°C) and blood pressure (mmHg). Characteristic frequencies embedded in the blood flow signal are extracted using wavelet analysis (Stefanovska and Bracic, 1999; Stefanovska et al., 1999).

2.3 Results

The contact pressure under the indenter predicted by our FEM is shown in Figure 2-6 for various edge radii. Our goal was to minimize high stress concentrations while accommodating the sensor probes. The specific criteria for selection a curved radius was based on the minimum of the highest pressure under the indenter rim. The best result was achieved with a 1.44 mm (8%) edge radius.

Interface pressure between the participant and the table was monitored throughout the data collection period. One example showing interface pressure during the first and the last minute data collection during the AP protocol is shown in Figure 2-7. Our custom-contoured support surface successfully decreased interface pressure acting on the ventral side of the participants.

Variations in applied pressure caused by respiration and unintentional movements were found to generally be less than 1.5 mmHg (200 Pa). As a result, we established a dead zone of ±1.5 mmHg (200 Pa) around the target pressure in the pressure control program. Figure 2-8 shows a plot of a typical loading pattern with the 3 mmHg (400 Pa) envelope allowed for the adjustment in load. Slow decreases in applied pressure observed in the baseline and low-pressure periods are likely due to stress relaxation in the tissue.

Typical skin blood flow under AP is shown in Figure 2-9. The results show the expected decreased blood flow in the high-pressure phase and increased blood flow in the low-pressure phase of AP. The amplitude of the observed blood flow oscillation pattern was greater in the low-pressure phase of AP. Wavelet analysis was performed on the skin blood flow under AP (data set in Figure 2-9) and is shown in Figure 2-10.

A typical example of skin blood flow during application of the heating protocol is shown in Figure 2-11. A modified TSI score of this case is 8.4. As is evident in Figure 2-11, blood flow is highly correlated with the skin temperature. The temperature on the skin adjacent to the probe head was only slightly increased over baseline measures as the temperature rose under the probe. Diastolic blood pressure, systolic blood pressure and heart rate are relatively constant before and after the experimental procedure.

2.4 Discussion

Underscoring the need for research in AP support surfaces is the wide range of loading parameters used in current commercial products. For example, the cycle times range from 3.6 to 15 minutes in cushions and 7.5 to 15 minutes in mattresses; the setting of inflation/deflation air cell pressure is adjusted based on comfort instead of considering blood flow responses (Medical Devices Agency, 1995; McLeod, 1997; Medical Devices Agency, 1997). This variability in the AP support surface device design parameters is the result of weak scientific evidence (Rithalia, 1995). The system described here is important for the development of systems that are able to simultaneously evaluate tissue biomechanical and physiological characteristics in relation to AP loading methods.

AP appears to stimulate a protective increase in skin blood flow (Bader, 1990; Mayrovitz and Smith, 1999). This mechanism appears to be associated with local autoregulatory microvascular responses that regulate transmural pressure and blood flow within the microvascular system. As autoregulatory responses are thought to be sensitive to a limited range of pressures and loading rates (Schubert and Mulvany, 1999; Butler et al., 2000; Levick, 2000), precise control of the magnitude and rate of loading is essential

for the study skin blood flow responses to AP. Our system is able to provide various settings for both loading rate and pressure that are representative of the inflation and deflation periods of AP devices. Through mechanical stress acting on compressed soft tissue, the microvascular system may be manipulated to elicit protective responses. Autoregulatory control is mediated by changes in the contraction/relaxation state of vascular smooth muscle cells and these changes are reflected in the vasomotion pattern. Thus, the LDF incorporated in our system is the most appropriate method with which to characterize the microvascular responses associated with AP.

Analysis of blood flow oscillations using the Fourier transform has been reported by several investigators (Salerud et al., 1983; Mayrovitz et al., 1993; Schubert et al., 1995). Fourier based analysis does not provide sufficient low frequency resolution to isolate characteristic frequencies associated with blood flow control mechanisms. Wavelet analysis, a relatively new time-frequency (truly time-scale) analysis method, has been proven useful in many fields (Strang and Nguyen, 1997; Mallat, 1999). Our preliminary findings indicated that an increase in skin blood flow during the low-pressure phase of AP is mainly due to an increase in the oscillatory amplitude of blood flow (Figure 2-9). This suggests that the mechanism for increased blood flow in response to mechanical loading is an increase in oscillatory amplitude of vasomotion rather than an increase in oscillatory frequency. This conclusion is supported by the power spectrum in the Figure 2-10, the spectrum shown was calculated using the wavelet transform on the blood flow data shown in Figure 2-9. Comparison of characteristic frequencies of skin blood flow under 60 mmHg and 3 mmHg of contact pressure clearly showed the frequency is relatively constant under two pressure loadings while the power of each

characteristic frequency varied. Our finding suggests that using mean blood flow alone is insufficient for evaluating the status of blood flow control mechanisms.

A limitation of our methodology is that blood flow responses are assessed at the skin level instead of the skeletal muscle level. Although pressure ulcers may develop first in muscle tissue (Daniel et al., 1981; Alterescu and Alterescu, 1988), our method for assessing skin blood flow responses to mechanical stress is still highly relevant in pressure ulcers prevention. Our general hypothesis is that AP elicits protective microvascular responses that modulate vasomotion and enhancing blood flow. The hypothesis is based on the responses of arteriolar vasomotion of the microvascular system both in the skin and the skeletal muscle. Similar research comparing skeletal muscle blood flow responses to rhythmical and static exercise showed that blood flow is lower during static exercise than that during rhythmical exercise (Guyton and Hall, 1996). Rhythmical exercise may create a stimulus similar to AP within the microvasculature of skeletal muscles through intermittent muscle contraction/relaxation. Laser Doppler flowmetry cannot detect blood flow below the skin level. Alternative techniques would be necessary for non-invasive monitoring of microcirculation within skeletal muscles.

Another important factor in the configuration of AP operating parameters concerns ischemia-reperfusion (I/R) injury. I/R injury is defined as cellular injury resulting from the reperfusion process to previously ischemic tissue. I/R has been studied in brain, heart, kidney, liver, lung and muscle tissues (Grace and Mathie, 1999; Collard and Gelman, 2001). The time required to induce I/R injury varies from organ to organ. For example, I/R occurs in the myocardium after 0.25-1 h ischemia, skeletal muscle after 0.5-5 h ischemia, and brain after 10 min to 3h ischemia (Harris and Skalak, 1996). The

time needed to induce I/R injury in the skin is not known. I/R injury has the potential to impact pressure ulcer prevention practices (e.g. repositioning every two hours) (Bouten et al., 2003). If a 2-hour turning routine causes I/R injury, then repositioning a person may harm tissues (Peirce et al., 2000).

I/R injury can be assessed by monitoring microvascular reperfusion after ischemia. In the case where I/R injury has occurred, there will be no reflow into the affected area. The AP protocol used in our study clearly demonstrated an increase in SBF after pressure relief and did not cause the no-reflow phenomenon. We are thus confident that our 5-minute cycle time is safe. The use of a 5-minute cycle is also supported by previous work on ischemic preconditioning, a method to prevent or reduce no-reflow phenomenon associated with I/R injury in skeletal muscles (Jerome et al., 1995). Murry et al. demonstrated four 10-minute-preconditioning cycles (e.g. 5-minute-ischemia and 5-minute-reperfusion) could greatly reduce necrosis of myocardium following prolonged ischemia (Murry et al., 1986).

2.5 Conclusion

A computer-controlled device and test protocols were developed to assess the effect of various loading patterns on skin perfusion. The results indicated that the system can be used to study the relationship between tissue biomechanical and physiological characteristics and loading methods; that it may be useful in the prediction of risk for tissue injury and the design and evaluation of preventive support surfaces.

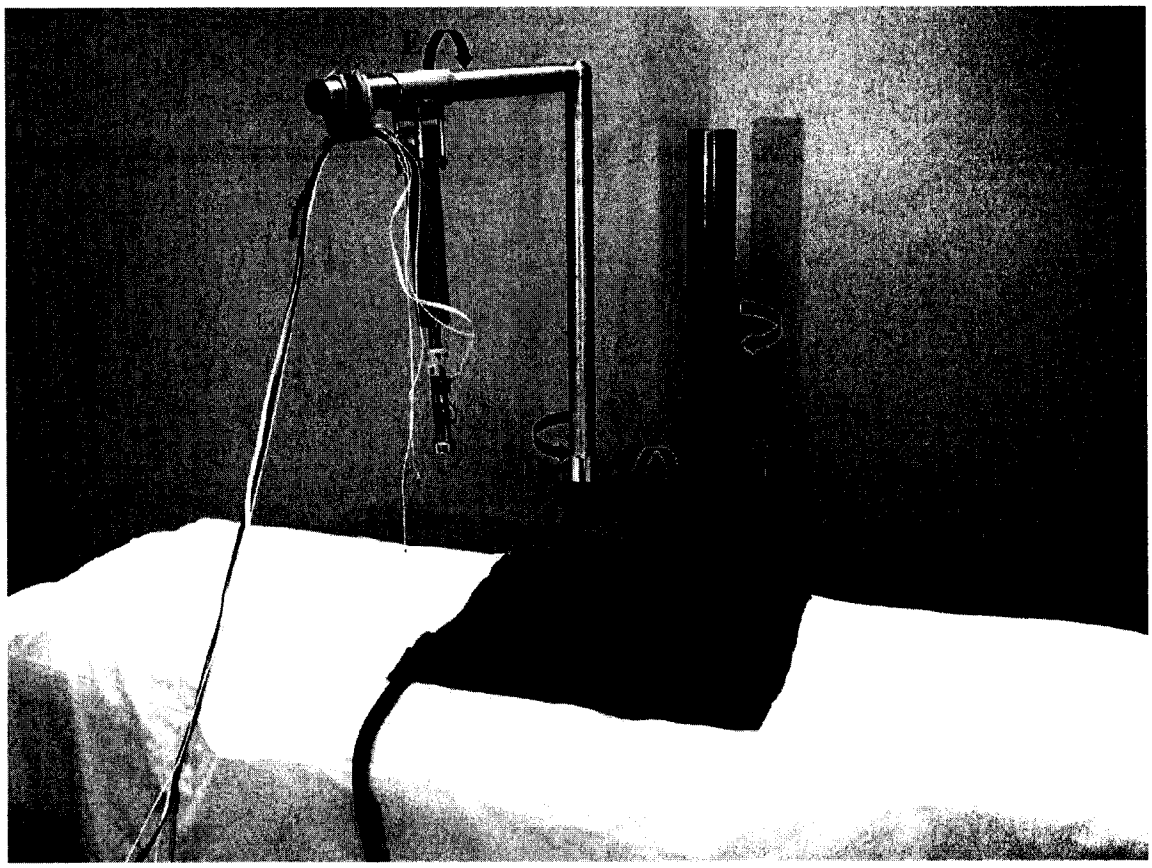

Figure 2-1. Photograph of the indenter system, positioning table and pressure mapping system. (A, B...D indicate degrees of freedom)

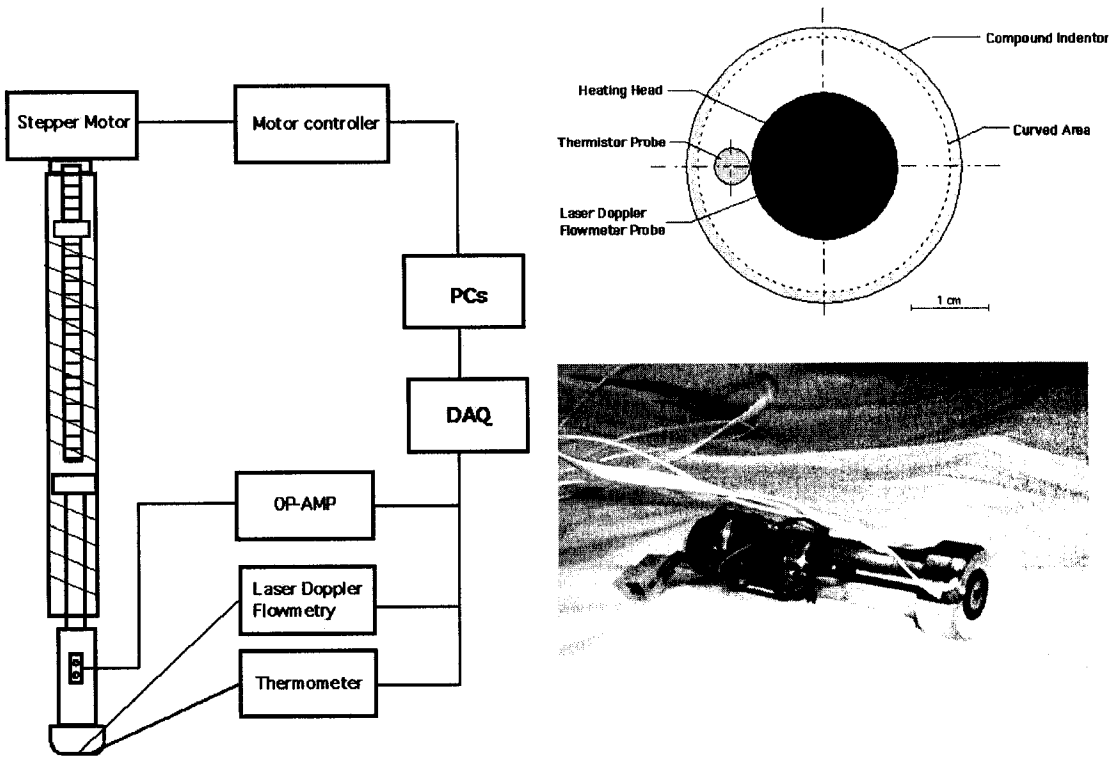

Figure 2-2. Structure and block diagram of the computer-controlled system with detailed locations of probes.

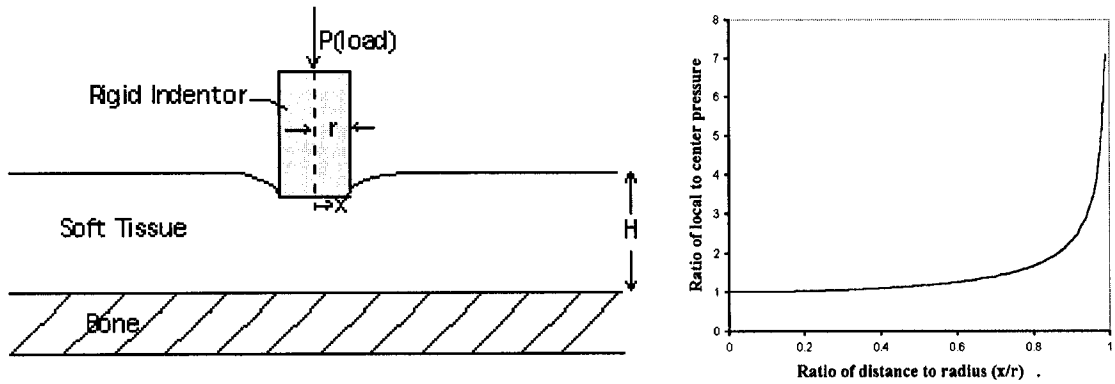

Figure 2-3. A schematic drawing of the simplified relationship of a flat-ended indenter and soft tissue, and relationship of local to central pressure under such indenter

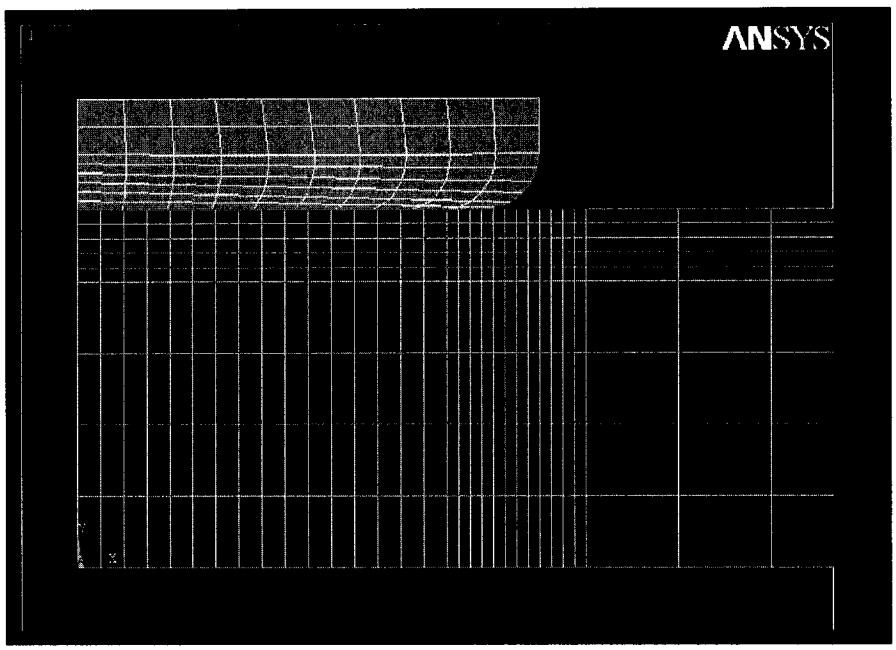

Figure 2-4. An axisymmetric finite element model of indenter and soft tissue.

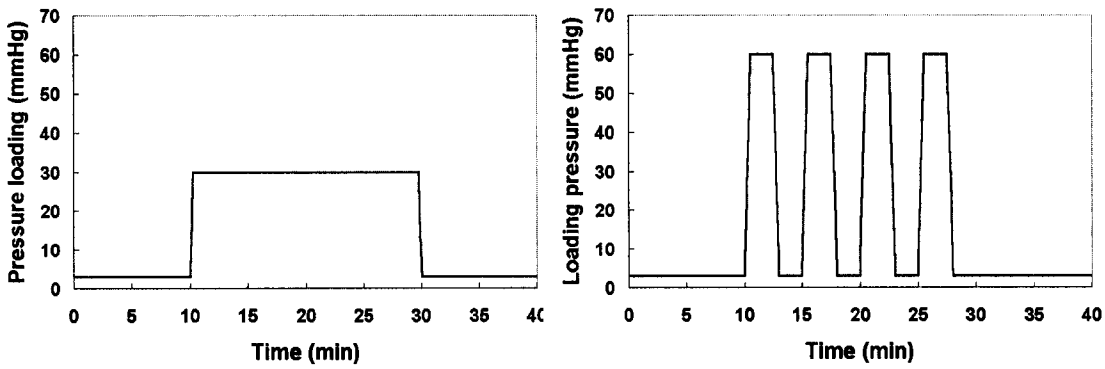

Figure 2-5. Interface pressure profiles of constant loading and alternating pressure protocols.

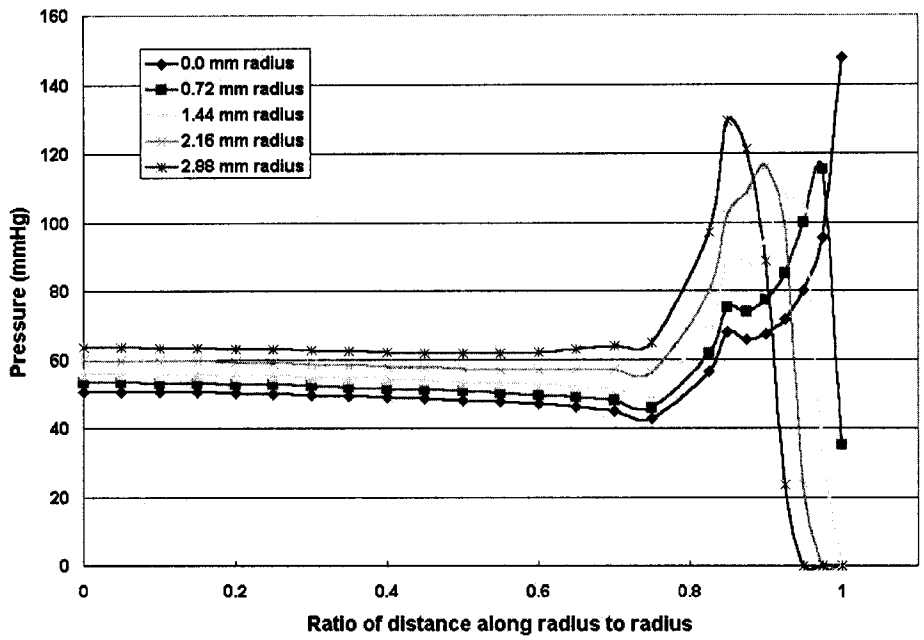

Figure 2-6. Contact pressure under different cutting radius of indenter calculated from non-linear contact finite element model.

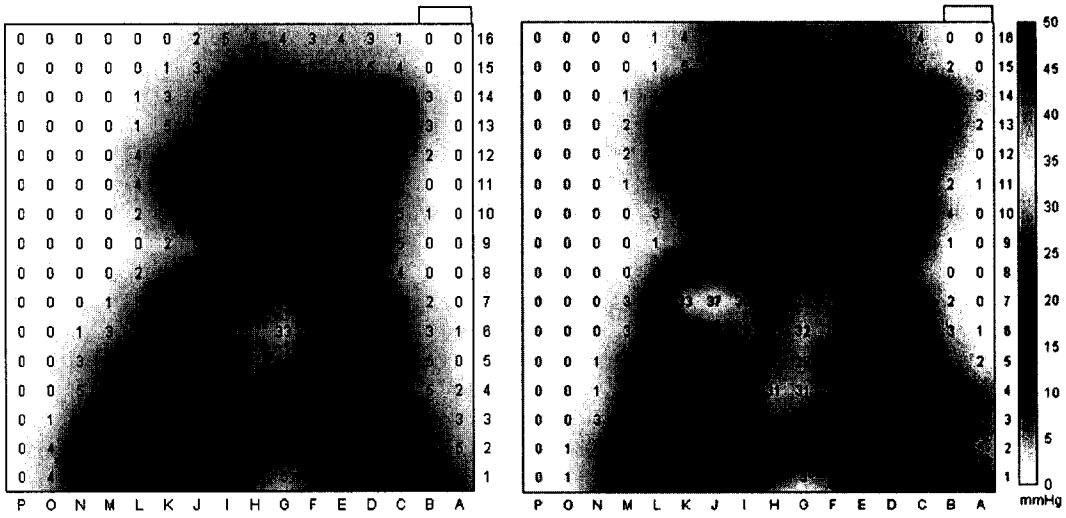

Figure 2-7. An example of the distribution of interface pressure acting on the ventral side of the body during alternating pressure protocol. Right figure is obtained in the initial minute and left figure is obtained in the final minute of the protocol.

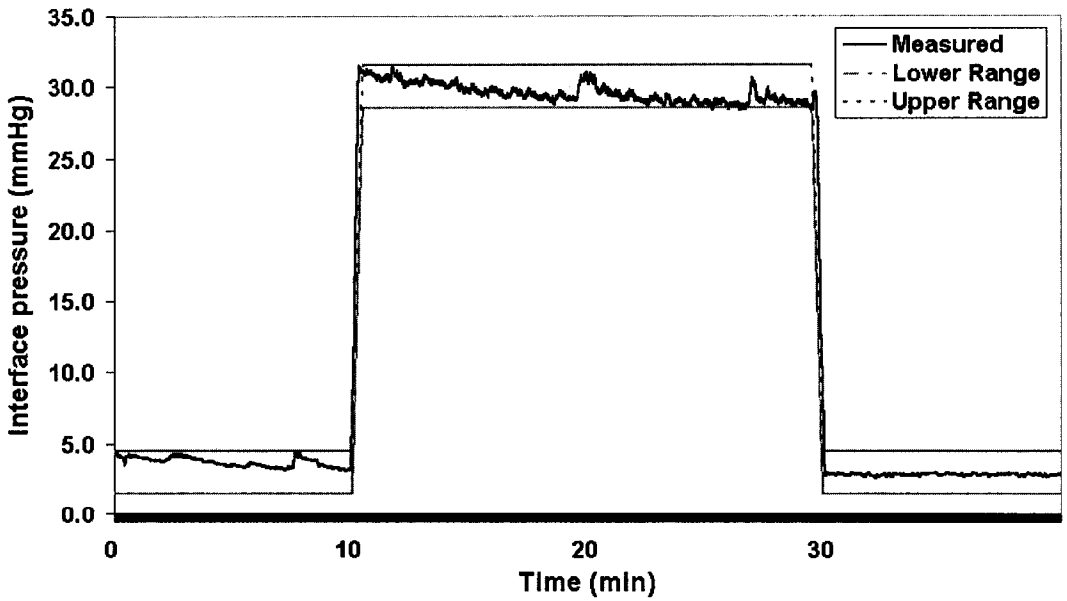

Figure 2-8. A comparison of ideal and measured pressure during constant loading protocol.

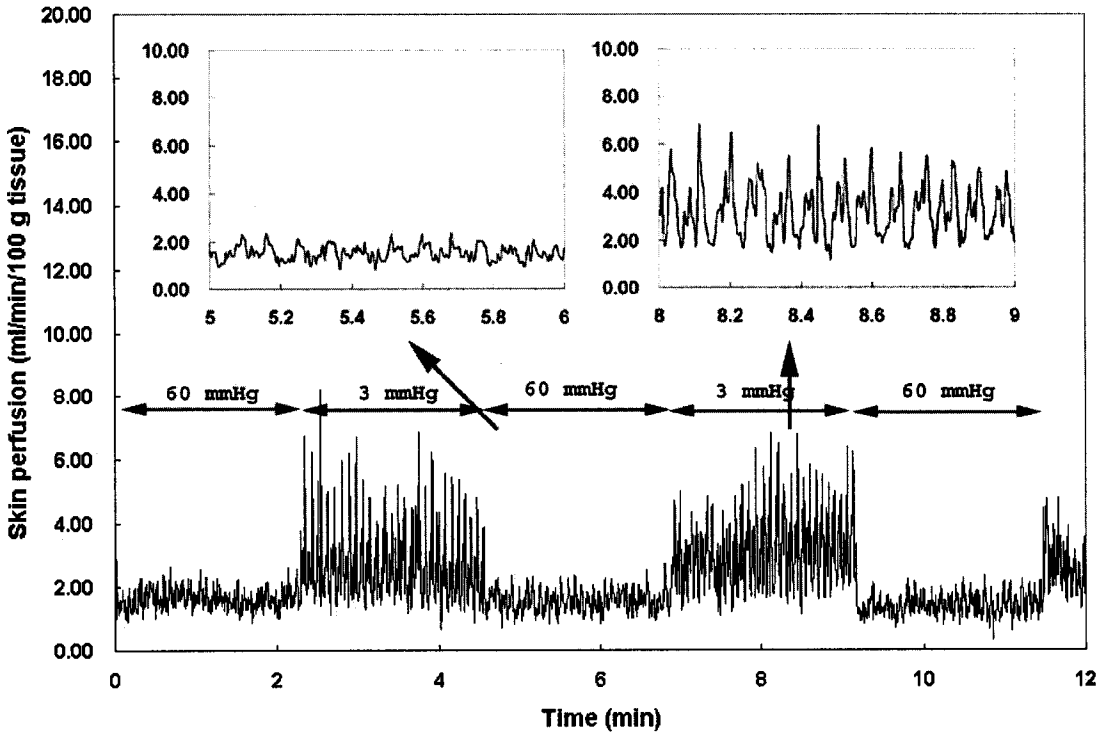

Figure 2-9. A typical example of laser Doppler skin blood flow responses to alternating pressure.

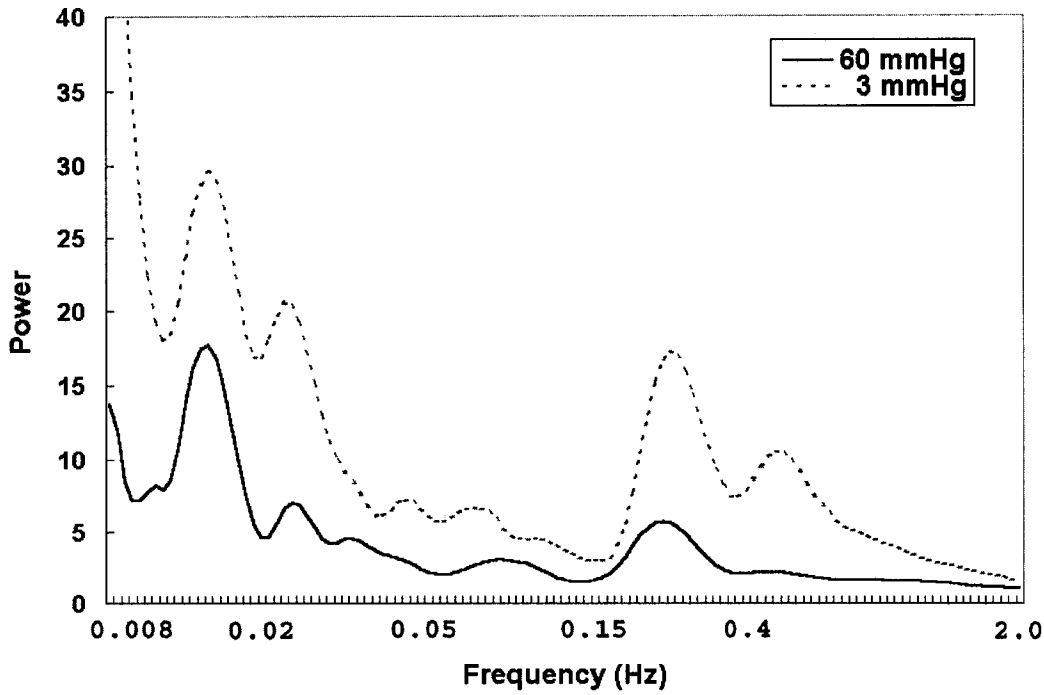

Figure 2-10. Spectral analysis of skin blood flow under alternating pressure (Data set is calculated from data in Figure 2-9).

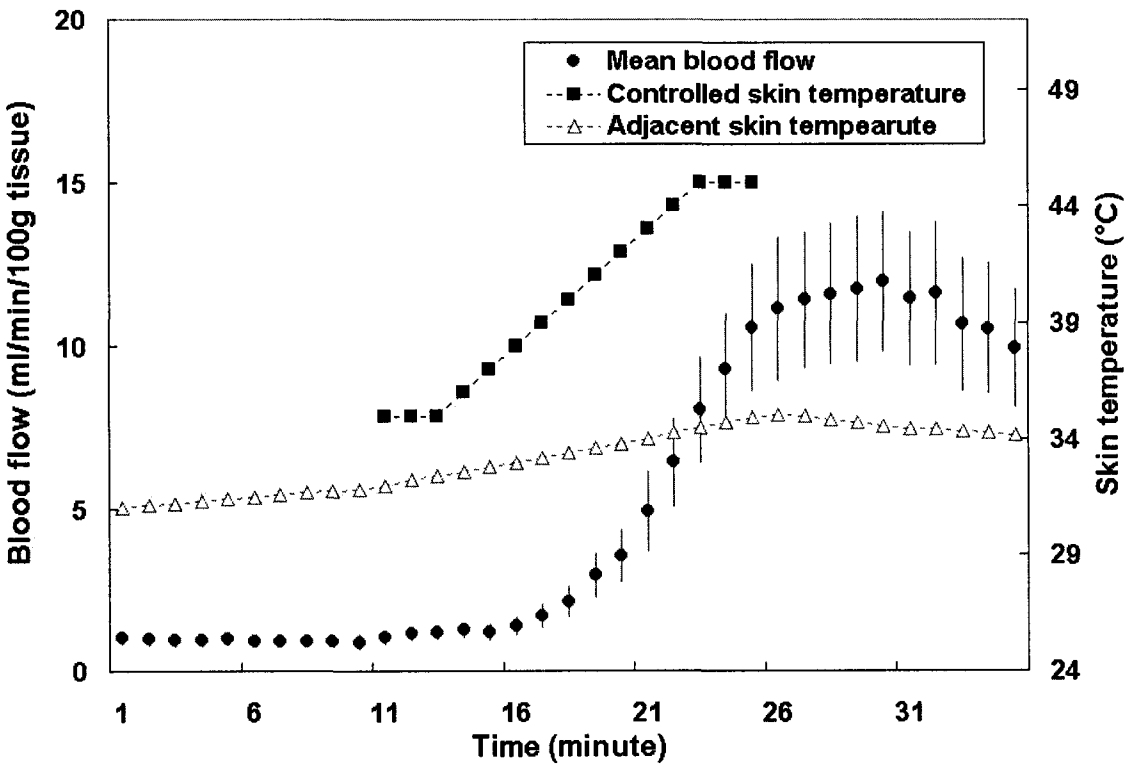

Figure 2-11. An example of skin blood flow under the heating protocol (values are mean ± S.D.).

3.0 USING WAVELET ANALYSIS TO CHARACTERIZE THE THERMOREGULATORY MECHANISMS OF SACRAL SKIN BLOOD FLOW

Abstract – Pressure induced skin blood flow responses measured via laser Doppler flowmetry are commonly reported in the time domain. The usefulness of spectral analysis in examining blood flow control mechanisms has been demonstrated, but traditional Fourier analysis does not provide sufficient resolution to reveal characteristic low frequencies. Time-frequency (wavelet) analysis was performed on ten subjects' sacral skin blood flow responses to heating (45°C) with improved resolution. Five frequency bands were identified (0.008-0.02, 0.02-0.05, 0.05-0.15, 0.15-0.4, 0.4-2.0 Hz) that are thought to be associated with metabolic, neurogenic, myogenic, respiratory or cardiac origins. Significant differences were observed in the mean, normalized power of the metabolic ($p<0.01$) and myogenic frequency bands ($p<0.01$) between pre-heating and maximal heating and pre-heating and post-heating periods. Power increased for the metabolic frequency and decreased for the myogenic frequency. Wavelet analysis successfully characterized thermoregulatory control mechanisms by revealing the contributions of the physiological rhythms imbedded in the blood flow signal.

Key words: heating, laser Doppler flowmetry, skin blood flow, spectral analysis, vasomotion, wavelet transform.

Abbreviations: BPM^2 = Blood Perfusion Monitor 2; LDF = Laser Doppler Flowmetry; TSI = Thermal Stress Index.

3.1 Introduction

Most researchers agree that prolonged exposure to high-pressure gradients causes tissue necrosis via occlusion of capillary blood flow (Levine et al., 1990; Brienza et al., 1993). Therefore, interventions that have been designed to reduce both the magnitude and duration of pressure are an essential component of pressure ulcer prevention and/or treatment regimens; i.e., support surfaces, repositioning, turning and weight shifting protocols. For many years, interface pressure has been used as an indicator of tissue loading tolerance. While interface pressure mapping can identify localized high-pressure areas and evaluate the pressure distribution achieved for a particular individual using a support surface, physiological responses or differences in the mechanical properties of soft tissue either among individuals or at different tissue sites cannot be detected. As these and other systemic factors affect tissue loading tolerance, the range of interface pressures capable of occluding capillary blood flow varies widely (Dinsdale, 1974; Bader, 1990; Xakellis et al., 1993). Therefore, simultaneous measures of multiple tissue responses have recently been adopted as an indication of tissue loading tolerance rather than the solitary use of interface pressure. Future research must seek to quantify and determine the relative significance of the physiological, biochemical and biomechanical tissue responses to mechanical deformation. The monitoring of skin microcirculatory responses via laser Doppler flowmetry (LDF) has potential for use in this manner.

Thermoregulation and nutrition are the two primary functions of the skin microcirculation. Skin microcirculatory changes are controlled by a combination of complex central and local mechanisms. These mechanisms modulate microcirculatory vascular smooth muscle cell activity thereby regulating the periodic constriction/dilation

patterns (oscillations) known as vasomotion (Nicoll and Webb, 1955). While the physiological mechanisms controlling vasomotion are not well known, vascular smooth muscle activity has been shown to be roughly proportional to the tissue's metabolic demand for oxygen (Guyton and Hall, 1996). Vasomotion may also be mediated by metabolic factors such as nitric oxide produced in response to endothelial cell shear stress (Sumpio, 1993). Based on Poiseuille's law, increased amplitude in vasomotion acts to decrease flow resistance with resultant enhancement of local perfusion (Slaaf et al., 1988; Wilkin, 1989; Parthimos et al., 1996; Meyer et al., 2002). Changes in vessel transmural pressure resulting from mechanical deformation have also been shown to decrease smooth muscle cell contractility resulting in vasodilation and enhanced blood flow. This response has been demonstrated in the absence of neural regulation and is mediated by fluctuations in the ion concentrations, mainly Ca^{++}, across the membrane of vascular smooth muscle cells (Osol and Halpern, 1988; Meininger and Davis, 1992; Achakri et al., 1995). Enhanced perfusion appears to increase tissue viability and tolerance to loading (Dinsdale, 1974; Daniel et al., 1981).

LDF has been used extensively to quantify skin perfusion responses to compressive loading (Bennett et al., 1981; Xakellis and Frantz, 1990; Xakellis et al., 1993; Schubert et al., 1994; Colin and Saumet, 1996; Sanada et al., 1997; Mayrovitz and Smith, 1999; Stefanovska et al., 1999). LDF is noninvasive, requires no heating of the skin (as $TcPO_2$ does) and can detect microcirculatory changes at a depth of ~1mm below the surface of the skin. LDF skin capillary blood flow (ml_{LD}/min/100g tissue or au) has been used to monitor responses to loading regimens. Traditionally, LDF blood flow has been reported only in the time domain. Because the physiological rhythms associated

with blood flow control mechanisms are imbedded in the blood flow signal, decomposing the signal via spectral analysis reveals various characteristic frequencies and may contribute to our understanding of these complex mechanisms. Unfortunately, few investigators have used spectral analysis to date and a systematic methodology appears to be lacking (Meyer et al., 1989; Breit and Intaglietta, 1994). To this end, we have designed a series of experiments using this method to investigate skin microcirculatory control mechanisms in response to various stimuli and loading conditions.

Decomposing the LDF blood flow signal into its characteristic frequencies has previously proven useful in studies of physiological control mechanisms. In an early study, Bernardi et al. used autoregressive analysis of the blood flow signal to demonstrate a number of frequency bands. One low frequency band [range 0.017-0.028 Hz] was revealed in 5 of 10 normal subjects. The authors speculated that these fluctuations were due to thermoregulatory influences (Bernardi et al., 1997a). In a second study, Bernardi et al. noted that another frequency band with a peak of ~0.1 Hz had decreased amplitude in diabetics compared to non-diabetics. He theorized that this reduction was due to autonomic neuropathy and; thus, the frequency band reflected a neurogenic origin (Bernardi et al., 1997b). Kastrup et al. also used LDF to study the rhythmical oscillations of blood flow. They classified the observed fluctuations into two categories: 1) α-oscillation [0.0667-0.4333 Hz; median 0.1133 Hz] and 2) β-oscillation [0.0083-0.0467 Hz; median 0.025 Hz]. The amplitude of β-oscillation was two to four times greater than that of α-oscillation. Local administration of lidocaine anesthesia extinguished β-oscillation without affecting α-oscillation; thus, the conclusion was reached that β-oscillation was neurogenic in origin while α-oscillation was non-neurogenic (Kastrup et

al., 1989). This result contradicted Bernardi's previous work in which oscillations in the 0.1 Hz range were attributed to neurogenic origins.

Traditionally, Fourier transform-based power spectrum analysis has been used to study blood flow that is characterized by two peaks. However, Fourier transform analysis does not provide sufficient time resolution for analysis of non-stationary physiological signals, such as heart rate and myoelectric signals (Stefanovska and Bracic, 1999; Karlsson et al., 2000; Lotric et al., 2000). Although windowed Fourier transform method permits time-frequency analysis, obtaining adequate precision in both domains requires selection of a proper window that balances time and frequency resolution. For complex signals with several mixed frequency components (i.e. skin blood flow), this balance is not possible (Stefanovska and Bracic, 1999). To overcome the limitations of the Fourier method, Morlet first conceptualized wavelet analysis in 1983. Later Grossman and Morlet laid the mathematical foundation for the wavelet transform permitting multi-resolution, time-frequency analysis (Grossmann and Morlet, 1984) of the blood flow signal.

Stefanovska and Bracic used the wavelet transform to analyze respiration, electrocardiogram, blood pressure, and LDF signals. They recorded responses at rest and during exercise in subjects with normal, well-conditioned and pathological cardiovascular systems. According to their eloquently reported results, wavelet analysis of blood flow reveals various peaks in the power spectrum corresponding to specific origins: 1) heart rate (0.4-2.0 Hz), 2) respiratory activity (0.15-0.4 Hz), 3) vascular myogenic responses (0.06-0.15 Hz), 4) neurogenic responses (0.02-0.06 Hz) and 5) metabolic responses (0.0095-0.02 Hz) (Bracic and Stefanovska, 1998; Stefanovska and Bracic, 1999;

Stefanovska et al., 1999). Since Stefanovska et al. demonstrated that wavelet analysis permits examination of the contributions of the myogenic, neurogenic and metabolic components of vasomotion relative to an exercise stimulus, the authors theorized that this method might also be useful in differentiating the effects of thermal stress from tissue loading. In addition to being used in the study of exercise effect, wavelet analysis has also been used in the fields: 1) study of cutaneous microcirculation of free flap tissue (Soderstrom et al., 2003) and 2) study of SBF responses to acetylcholine (endothelium-dependent vasodilator) and sodium nitroprusside (endothelium-independent vasodilator) (Kvernmo et al., 1999).

This report describes the first in a series of studies investigating the skin's microcirculatory response to various stimuli and loading conditions as measured by LDF. This study aimed to: 1) thermally-induce maximal sacral skin blood flow responses, 2) identify the characteristic frequency bands in the blood flow signal using the wavelet transform and 3) determine the relative power or contribution of the various bands to the total blood flow. In this manner, the response to thermally induced maximal blood flow could be characterized. Thus, in subsequent studies, the control mechanisms associated with the effects of heating could be differentiated from those associated with tissue loading.

3.2 Methods

Ten unimpaired subjects (5 male and 5 female) were recruited into the study. The demographic data were as follows: age 30.0 ± 3.1 years, height 162.9 ± 6.8 cm, and weight 58.3 ± 8.6 kg. The following conditions constituted exclusion criteria: the

presence of pressure ulcers on the sacrum, diabetes, vascular disease, hypertension, or use of vasoactive medications. An informed consent approved by the University of Pittsburgh Institutional Review Board was obtained from each subject prior to testing. All tests were performed in the Soft Tissue Mechanics Laboratory, University of Pittsburgh. Room temperature was maintained at 24 ± 1°C. For at least 30 minutes prior to testing, all subjects assumed a recumbent, relaxed position in the laboratory to accommodate to the room temperature and achieve a baseline blood flow level.

A Laserflo Blood Perfusion Monitor 2 (BPM^2, Vasamedics, MN) and Softip pencil probe (P-435, Vasamedics, MN) were used to measure capillary blood perfusion (ml_{LD}/min/100g tissue). A temperature control module (TCO, Vasamedics, MN) with heater probe (P-422, Vasamedics, MN) was used to heat the skin to 45°C to obtain a maximal skin blood flow response. Laser Doppler skin blood flow was sampled at 20 Hz. A computer-controlled indenter system and other system components were designed and developed for use in this study and are described elsewhere (see Chapter 2).

With subjects lying prone on a customized, conforming support surface, blood flow over the sacrum was recorded at rest for 10 minutes to establish baseline flow (pre-heating period). Fifteen minutes of heating followed consisting of three minutes at 35°C, nine minutes of incremental heating (1°C increase per minute) from 35 to 45°C and a final period of three minutes at 45°C. Skin blood flow monitoring continued throughout a 10-minute post-heating period.

Wavelet analysis provides a multi-resolution, time-frequency analysis of sacral skin blood flow. Wavelet transform decomposes a signal over dilated and translated

wavelets (Strang and Nguyen, 1997). Continuous wavelet transform of a signal *f(u)* was defined as:

$$\hat{f}(s,t) = \int_{-\infty}^{\infty} \psi_{s,t}(u) f(u) du \qquad \text{(Equation 3-1)}$$

where $\hat{f}(s,t)$ is a wavelet coefficient and $\psi_{s,t}(u)$ is a wavelet function and was defined as

$$\psi_{s,t}(u) = \frac{1}{\sqrt{s}} \psi\left(\frac{u-t}{s}\right) \qquad \text{(Equation 3-2)}$$

A family of time-frequency wavelets is obtained by scaling function ψ by parameter s (scale factor) and translating it by t (time factor). Continuous wavelet transform is easier to interpret data or recognize patterns because its complete scales tend to reinforce the traits and make all information more visible than using discrete wavelet transform (Hubbard, 1996). Thus continuous wavelet transform was used in this study to perform time-frequency analysis of skin blood flow.

The Morlet wavelet model was used to perform wavelet transform analysis. Morlet wavelet is a Gaussian function defined as:

$$\psi_{s,t}(u) = \frac{1}{\sqrt[4]{\pi}} \cdot \left(e^{-i\omega_0 u} - e^{-\omega_0^2/2}\right) \cdot e^{-u^2/2} \qquad \text{(Equation 3-3)}$$

whereas ω_0 was chosen as 2π. Therefore, a simple relationship between frequency and scale could be expressed as $frequency = \frac{1}{scale}$ (Bracic and Stefanovska, 1998). Morlet wavelet, a Gaussian function, allows the best time-frequency localization according to Heisenberg uncertainty principle (Meste et al., 1994; Bracic and Stefanovska, 1998).

In order to quantify the amplitude of power within the characteristic frequency bands, the average amplitude of each frequency band was calculated using the following equation:

$$A_i(f_{i1}, f_{i2}) = \frac{1}{t}\int_0^t \frac{1}{f_{i2} - f_{i1}} \int_{1/2\pi f_{i2}}^{1/2\pi f_{i1}} \frac{1}{s^2} \hat{f}(s,t)\,ds\,dt \qquad \text{(Equation 3-4)}$$

where f_{i1} and f_{i2} are the limits of a given frequency band; e.g., myogenic characteristic frequency band.

In order to permit comparisons of the subjects' power distributions, the relative contribution of each frequency band (myogenic, neurogenic, etc.) was used in this study and was defined as ratio of the average amplitude of total frequency range (0.008-2.0 Hz) for each condition; i.e., pre-heating, heating, post-heating:

$$a_i(f_{i1}, f_{i2}) = \frac{A_i(f_{i1}, f_{i2})}{A_{total}} \qquad \text{(Equation 3-5)}$$

Since physiological signals in the skin blood flow rarely have frequencies higher than 2 Hz (Stefanovska et al., 1999), the specified range of frequencies for the wavelet analysis was established at 0.007 to 2.5 Hz. The limits of each frequency band were chosen based on the ranges previously reported by Stefanovska and Bracic and our research data. Selection was also contingent upon the peak frequency for each heating condition and each subject falling within each band.

The thermal stress index (TSI) has been used to screen for abnormal microcirculatory functional status in peripheral vascular diseases and diabetes (Belcaro et al., 1989; Belcaro et al., 1990; Timar-Banu et al., 2001). The procedure consists of measuring the skin blood flow at 35°C for 2 minutes followed by heating the skin at 44°C for 20 minutes, and then recording blood flow for 2 minutes. TSI is defined as blood flow

at 44°C / 35°C and scores $\geq$ 5 are considered normal. A modified TSI was designated as skin blood flow at 45°C / 35°C.

All mathematical functions were developed using Matlab 5.2 and wavelet toolbox (MathWorks, MA). A skewness test was used to test the normality of the data. A Mauchly's test of sphericity was used to test the equal variances of the repeated measures data. A one-way ANOVA with repeated measures was used to compare differences among pre-heating, heating, and post-heating periods. Where skewed distributions existed, Kruskal-Wallis one-way analysis of variance was used to compare differences among frequency bands during pre-heating, heating and post-heating periods. The level of significance for post-hoc multiple comparisons of repeated measures was adjusted to 0.017 based on the Bonferroni correction (Portney and Watkins, 2000).

3.3 Results

The mean skin blood flow for each temperature was plotted and is shown in Figure 3-1. Skin blood flow shows step increases for skin temperature beyond 42°C. The mean blood flow during post-heating period was higher than that at 45°C (maximum thermal stress). The increase in skin blood flow during post-heating compared to maximal heating was observed in 7 subjects.

A modified TSI was calculated for each subject. Based on our definition, all subjects demonstrated normal microcirculatory function with modified TSI scores $\geq$ 5 and a group mean $\pm$ S.D. = 8.44 $\pm$ 2.43.

The wavelet transforms are expressed in the time-frequency domain (Scalogram, the squared magnitude of the wavelet transform) (Figure 3-2). In this form, the data

cannot be easily compared or quantified. However, by averaging the data points in the time domain during a specific time event, two-dimensional time and frequency data may be reduced to one-dimensional frequency domain data. All participants' time-averaged wavelet transforms of skin blood flow at 45°C is shown in Figure 3-3.

Based on the distribution of power, five frequency modes were identified (Figure 3-3). [Since inter-subject peak frequency location varied slightly for each degree of thermal stress, the authors determined that a frequency band that captured the peaks for all of the subjects would more accurately characterize the blood flow frequency elements. All subjects' power spectra during maximal heating were plotted for comparisons.] Five frequency bands were chosen to represent the frequency elements for all subjects. The five frequency bands were designated as 1) metabolic (0.008-0.02 Hz), 2) neurogenic (0.02-0.05 Hz), 3) myogenic (0.05-0.15 Hz), 4) respiratory (0.15-0.4 Hz) and 5) cardiac (0.4- 2.0 Hz) (Figure 3-3).

In order to compensate for variability in total flow, the power for each frequency band was normalized to the total power for each condition (Figure 3-4) (data set in Figure 3-4 was calculated from the same subject in Figure 3-2). A skewness test for each frequency band showed significant differences in respiratory frequency band during pre-heating and heating periods ($p<0.05$). A Mauchly's test of sphericity for each frequency band demonstrated no statistically significantly differences ($p>0.05$). Analysis using one-way ANOVA with repeated measures demonstrated a significant difference between the mean metabolic and myogenic frequency bands for all conditions ($p<0.001$). Kruskal-Wallis one-way analysis of variance was used to test respiratory frequency band, and showed no significant differences among pre-heating, heating and post-heating periods.

Following post-hoc analysis; however, statistically significant differences were demonstrated only between 1) the mean pre-heating and 45°C heating period and 2) the pre-heating and post-heating period for the metabolic and myogenic frequency bands. The relative contribution in the metabolic frequency band was increased ($p<0.01$) while the myogenic frequency band decreased ($p<0.01$) (Figure 3-5).

3.4 Discussion

The vasodilation induced by local heating is primarily mediated by neurogenic reflexes and locally released substances. However, the interactions between these mechanisms are complex and poorly understood (Pergola et al., 1993; Magerl and Treede, 1996; Kellogg et al., 1999). To further clarify and objectively characterize these mechanisms, we used wavelet analysis to reveal five different frequency bands associated with the various control mechanisms of thermally induced vasomotion imbedded in the maximal blood flow signal.

Skin blood flow and control mechanisms of vasomotion in response to local heating depend on the temperature, the duration of exposure and the rate in which the heat is applied. Varying these factors stimulates different control mechanisms resulting in different perfusion responses (Minson et al., 2001). For example, increasing the temperature quickly or beyond 45°C could stimulate nociceptive receptors and increase the neurogenic component of the signal. In other studies of thermally induced maximal blood flow (including TSI), the skin is heated rapidly (over 2-3 minutes) to 42~44°C and the temperature is held constant for 20-40 minutes (Taylor et al., 1984; Johnson et al., 1986). This rapid heating of the skin produces two signal peaks: the first due to local

sensory nerve activity (axon reflex) and the second due to release of endothelial nitric oxide (Kellogg et al., 1999; Minson et al., 2001). In this study, a stepwise heating from 35 to 45°C was applied to the skin over the sacrum to avoid nociceptive stimulation and to permit analysis of the blood flow response at each temperature.

As our rate of heating was much slower than the rapid increase in heat conventionally used to achieve maximal vasodilation, the typical biphasic blood flow response to heating was not observed. In our study, only the second peak was observed during maximal heating. This peak extended into the post-heating period. The nitric oxide phenomenon may account for the increased flow observed during the post-heating recovery period. We believe that the slower, incremental heating rate in this study may account for the absence of the first peak (local sensory nerve activity).

Direct and indirect evidence supports the designation of the frequency bands' origins. While direct evidence supports the designation of the higher frequencies to cardiac and respiratory origins (Muck-Weymann et al., 1996; Stefanovska and Bracic, 1999), the evidence supporting assignment of the lower frequencies to metabolic, neurogenic or myogenic origins is primarily indirect (Kastrup et al., 1989; Kvernmo et al., 1999; Stefanovska and Bracic, 1999; Soderstrom et al., 2003).

Several reports provide indirect evidence to support the designation of the ~ 0.1 Hz peak frequency to local control mechanisms (Kastrup et al., 1989). The smooth muscle cells respond continually to changes in transmural pressure (Osol and Halpern, 1988; Meininger and Davis, 1992; Achakri et al., 1995). This ion-mediated response has been demonstrated in isolated animal vessels either by dynamically measuring the change in vessel diameter (Bertuglia et al., 1994), or ion concentration (Gustafsson et al., 1994).

This myogenic theory appears to be the most accepted explanation for local control of vasomotion (Ursino and Fabbri, 1992; Achakri et al., 1994); therefore, the 0.05-0.15 Hz frequency band was attributed to myogenic control mechanisms.

Stefanovska and Bracic demonstrated an isolated peak at ~0.01Hz in all cardiovascular signals with a variance in its peak location characteristic of local phenomena. They cited indirect evidence for attributing this frequency to endothelial cell metabolic processes (Kvernmo et al., 1998a; Stefanovska et al., 1999). These processes may be mediated by oxygen demand or nitric oxide release. Kvernmo et al. demonstrated 0.008-0.02 Hz frequency is a endothelial dependent response by comparisons of skin blood flow responses to acetylcholine (endothelium-dependent vasodilator) and sodium nitroprusside (endothelium-independent vasodilator) (Kvernmo et al., 1999). We also hypothesize that the 0.008-0.02 Hz frequency band is metabolic in origin; i.e., mediated by any locally released vasodilators.

Kastrup's blood flow recordings under both anesthesia and denervation clearly identified the neurogenic origin of the 0.0083-0.0467 Hz (β-oscillation) frequency range. However, due to the superior resolution provided by wavelet transform at low frequencies, our data revealed two peaks within this range. We chose to associate the higher frequency band, 0.02-0.05Hz, as having a neurogenic origin and, as previously stated, the lower frequency band, 0.008-0.02 Hz, with metabolic effects (Guyton and Hall, 1996). Although designation of the frequency bands in this study is based solely on the data obtained from 10 subjects, we believe this method will prove useful in determining the relative significance of the various control mechanisms of skin blood flow in response to loading or other stimuli.

The increased metabolic influence was anticipated and may be explained by the increase in metabolic activity associated with tissue heating. Higher ambient temperatures have been shown to cause an increase in tissue metabolism and oxygen consumption on the order of 10% for every 1°C (Brown and Brengelmann, 1965).

The decreased myogenic activity was also anticipated as heat produces a mild inflammatory reaction in which local chemical mediators act to decrease smooth muscle tone and increase capillary and post-capillary venule permeability resulting in vasodilation of resistance vessels (Michlovitz, 1990). Local myogenic control is considered to be independent of any neural or humoral influences and has been associated with the application of mechanical force to the vascular smooth muscle cell (Osol and Halpern, 1988; Meininger and Davis, 1992; Achakri et al., 1995). In this study, thermal stress was applied with minimal mechanical force and deformation to the soft tissue. Therefore, compared to pre-heating blood flow, heat stress accounts for the observed decreased power in the myogenic frequency band shown in the heating and post-heating periods.

Due to the increase in total blood flow resulting in increased total power for the maximal heating period, significant differences between the pre-heating and maximal heating period were demonstrated for each frequency band. In order to permit comparisons of the energy contributions from each frequency band for all subjects during all periods, the power of each frequency band (myogenic, neurogenic, etc.) was normalized to the total power for each period; i.e., pre-heating, heating, post-heating. Subsequently, statistically significant differences between the pre-heating and heating period and the pre-heating and post-heating period were demonstrated for only the

frequency bands at 0.008-0.02 Hz (metabolic) and 0.05-0.15 Hz (myogenic). Wavelet transforms must be normalized to the total power for a specific period in order to reveal the relative contribution of the physiological rhythms imbedded in the blood flow.

Skin blood flow response to local heat appears to increase the amplitude of vasomotion, without affecting the characteristic frequencies . This finding contradicts the traditional view that the frequency of the flow signal shifts in response to stimulation (Thoresen and Walloe, 1980; Salerud et al., 1983). Our analysis supports the evidence reported by Stefanovska and Bracic demonstrating that the peaks of the average wavelet transforms from all cardiovascular signals (ECG, blood pressure and LDF) appear at similar or, in some instances, at exactly the same frequencies (Stefanovska and Bracic, 1999). Thus, thermal stress enhances and maintains vasomotion in the microcirculatory flow. The increased amplitude of vasomotion results in decreased effective vascular resistance resulting in increased blood flow based on Poiseuille's law (Funk et al., 1983).

3.5 Conclusion

Our long-term goal is to improve our understanding of the control mechanisms of blood flow associated with tissue loading in order to optimize critical parameters that enhance tissue viability. Through wavelet analysis of the LDF skin blood flow signal, our study identified five characteristic frequency bands. Our findings indicate that 1) local heating increases skin blood flow at the sacrum and 2) that the relative contribution in the metabolic frequency band was increased while the myogenic frequency band decreased. Thus, our study has established a means of evaluating the relative influence of specific control mechanisms in response to local heating. Using this method it will be possible to

differentiate heating effects from loading effects in future studies. Our results encourage the use of this method for differentiating the control mechanisms of blood flow in response to a variety of stimuli. For example, since the degree of vasodilation induced by local heating has been used as a clinical diagnostic tool for evaluation of sympathetic function (Sandeman et al., 1991; Carberry et al., 1992), this method could further aid in the diagnosis of specific pathologies affecting the vasodilatory response.

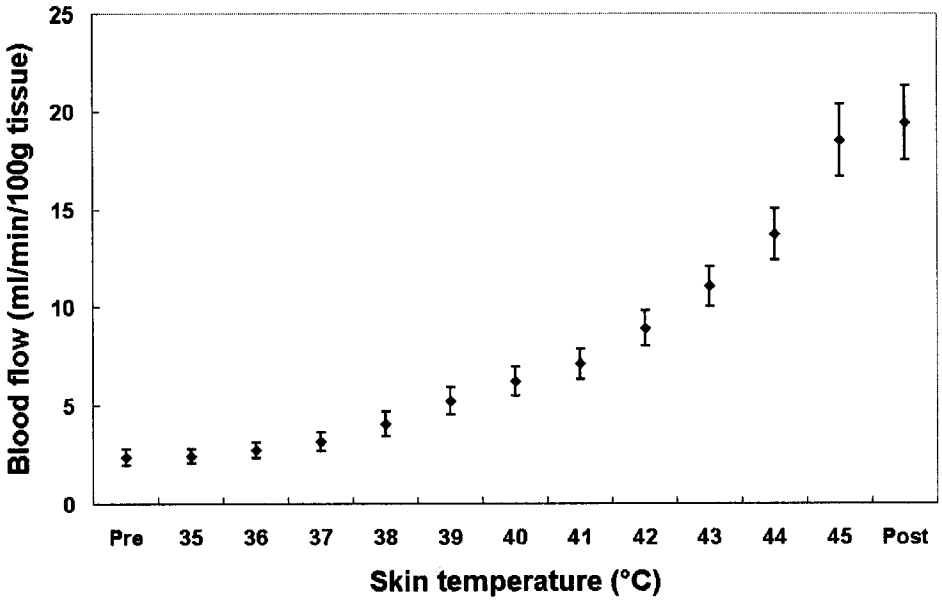

Figure 3-1. Mean skin blood flow at different skin temperature (values are mean ± S.E.). (Time period is 10 minutes for pre-heating and post heating, 3 minutes for 35°C & 45°C, and 1 minute for other temperature.)

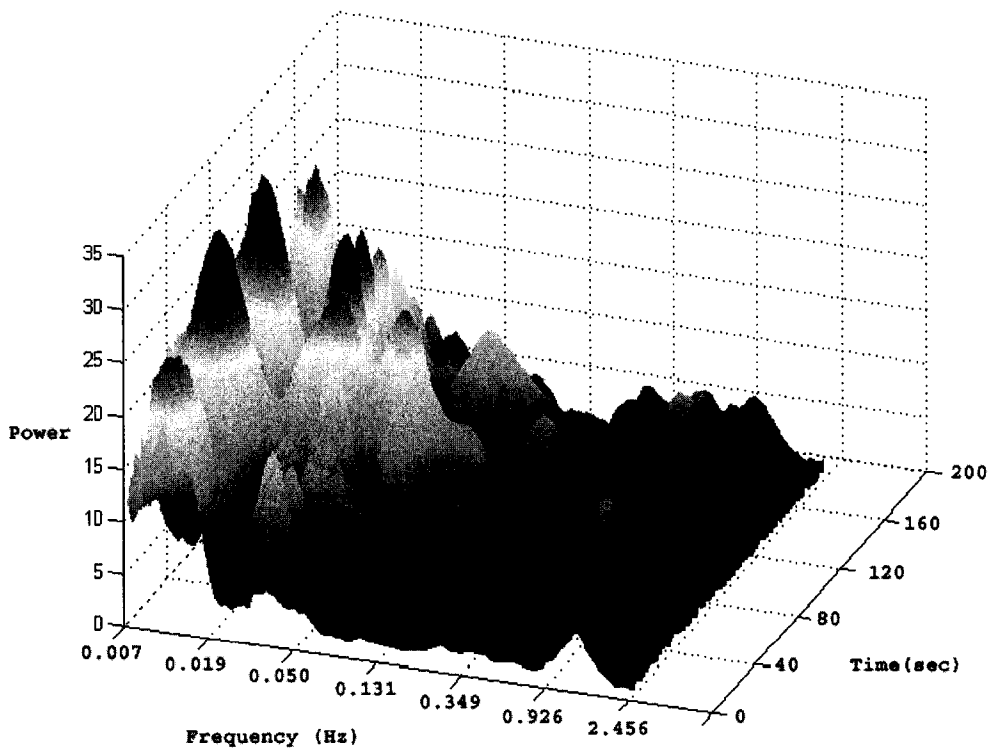

Figure 3-2. A typical example of Scalogram of skin blood flow at 45°C.

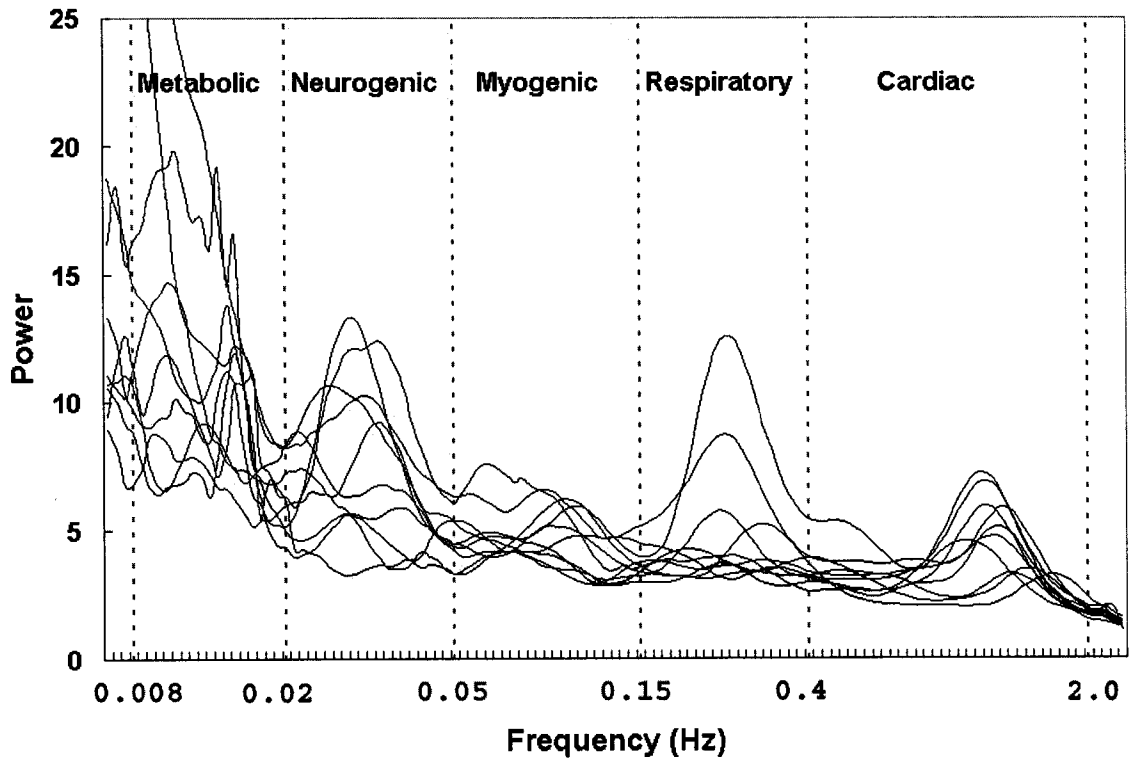

Figure 3-3. Time-period-averaged wavelet transforms of skin blood flow at 45°C for all participants' spectra.

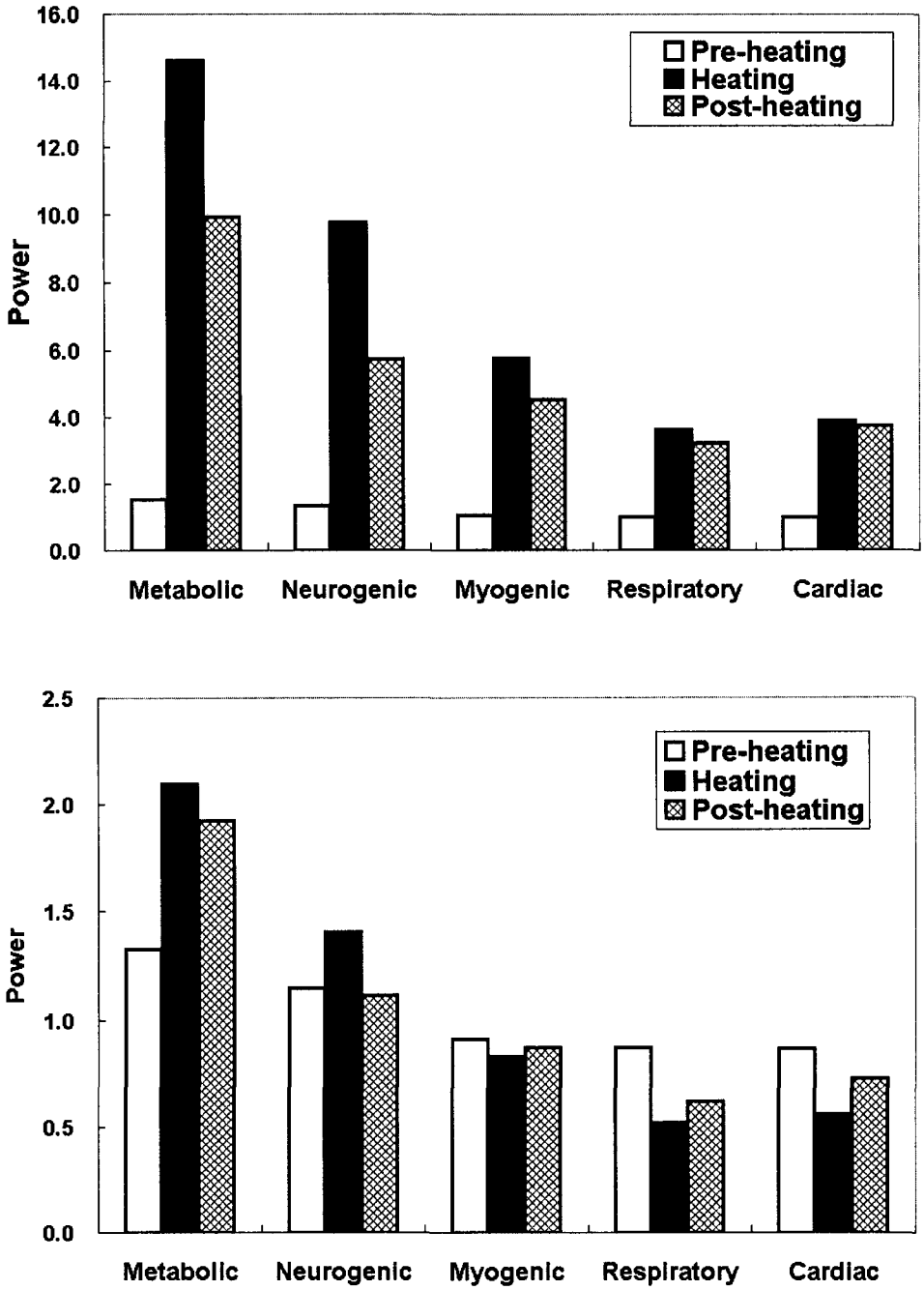

Figure 3-4. A typical example of non-normalized (upper figure) and normalized (lower figure) power of five characteristic frequency bands during different periods. Data set in Figure 3-4 are calculated from the same subject in Figures 3-2. Normalization method of upper figure is described in equation 3-5.

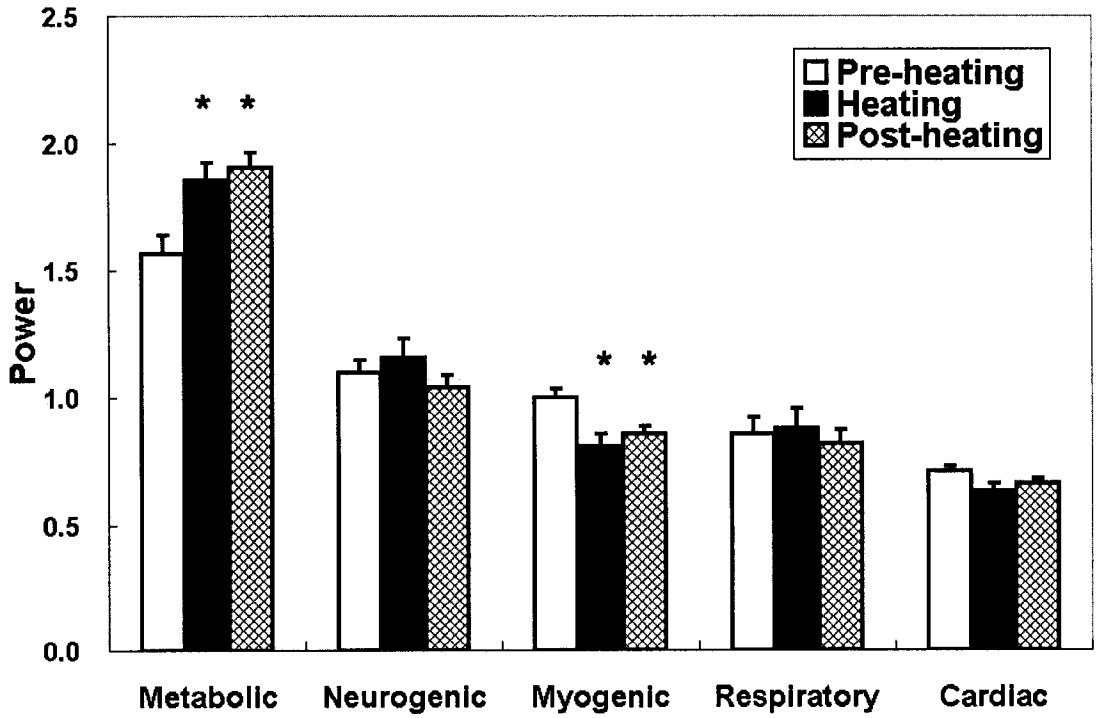

Figure 3-5. Comparisons of each characteristic frequency band during different periods for all participants (values are mean ± S.E.) (* indicates $p<0.01$).

4.0 A COMPARISON OF CHANGES IN RHYTHMS OF SACRAL SKIN BLOOD FLOW IN RESPONSE TO HEATING AND INDENTATION

Abstract – Studies on alternating pressure (AP) and skin blood flow (SBF) are confounded by the interrelationships between its control mechanisms (metabolic, neurogenic and myogenic) and associated stimuli. Our objective is to differentiate blood flow control mechanisms associated with indentation from those associated with heating. Ten healthy, young adults participated in this study. Incremental heat (35 to 45°C, 1° step/minute) and pressure (0 to 60 mmHg, 5 mmHg step/3 min) were applied to the skin overlying the sacrum using a computer-controlled indenter. Sessions for heat and pressure protocols were separated by 7±2 days. Wavelet analysis was used to decompose the blood flow signal into frequency bands reported to be associated with metabolic, neurogenic, myogenic, respiratory and cardiac control mechanisms. Power in the myogenic frequency range was higher after incremental pressure and lower after incremental heating while power in the metabolic frequency range was lower after incremental pressure and higher after incremental heating ($p<0.01$). Mean blood flow decreased as pressure increased from 0 to 15 mmHg; mean blood flow increased as pressure increased from 15 to 60 mmHg. Skin blood flow appears to be mediated by myogenic control after application of incremental external pressure and appear to be mediated by metabolic control after incremental local heat exposure.

Key Words: heating, indentation, laser Doppler flowmetry, skin blood flow, wavelet transform.

Abbreviations: AP = Alternating Pressure; SBF = Skin Blood Flow.

4.1 Introduction

Pressure ulcer prevention strategies attempt to minimize the effects of interrelated factors known to be associated with their etiology. Specific strategies to prevent sitting-acquired pressure ulcers have been focused on periodic un-weighting of the ischial area and the provision of support surfaces designed to maintain tissue integrity (Brienza et al., 1991; Brienza et al., 1993; Brienza and Karg, 1998; Brienza et al., 2001). The support surface's role in maintaining tissue integrity is to distribute weight as evenly as possible over the surface, thus reducing pressure gradients, preventing extreme heat transfer rates, controlling wetness, and minimizing shear forces. Product developers have focused their efforts on reducing pressure and pressure gradients. However, pressure is but an indirect measure of the detrimental effects of a person's body weight pressing through soft tissue to the underlying support surface. Blood flow measurements provide a more direct measure of risk because it is the blood that provides the tissue with the critical nutrients necessary for its survival.

The interaction between interface pressure and microvascular blood flow appears to be complex. Different pathologies result in various deficits of normal physiological responses to mechanical stresses. For one example, loss of neurogenic control over the cardiovascular system following spinal cord injury impairs blood flow regulation (Byrne and Salzberg, 1996; Teasell et al., 2000). For another, loss of elastin and/or degradation of the collagen matrix in aging skin reduces the body's ability to withstand external pressure without excessive deformation of the blood vessels (Bader, 1990; Ballas and Davidson, 2001). In some circumstances, it may be impossible to reduce and hold interface pressure below critical levels. A rapid reduction in or elimination of ischemia-

inducing external pressure results in reactive hyperemia, a protective increase in skin blood flow to levels above baseline levels. The magnitude and duration of this reactive hyperemia appears to be related to the magnitude and duration of the applied pressure (Lewis and Grant, 1926; Guyton and Hall, 1996). The application of external pressures below the level for which blood vessels become occluded also induces a protective increase in flow (Xakellis et al., 1993; Patel et al., 1999; Abraham et al., 2001). Alternating pressure support surfaces - surfaces that provide cyclic changes in interface pressure - appear to reduce risk of tissue damage by increasing microvascular blood flow. However, the underlying physiological mechanisms responsible for the increases are not understood (McLeod, 1997; Mayrovitz and Smith, 1999; Rithalia and Gonsalkorale, 2000).

Blood flow regulation is attributed to vasomotion, the rhythmic constriction and dilation of blood vessels (i.e. arterioles and venules) (Intaglietta, 1991; Colantuoni et al., 1994; Lossius and Eriksen, 1995; Bertuglia et al., 1996). Vasomotion is controlled by central neurogenic, local myogenic, and metabolic mechanisms (Ursino and Fabbri, 1992; Achakri et al., 1994; Bracic and Stefanovska, 1998; Stergiopulos et al., 1998; Schubert and Mulvany, 1999; Butler et al., 2000). Blood flow changes are necessary to meet various thermoregulatory and local nutrient demands (Salerud et al., 1983; Wilkin, 1986; Wilkin, 1988; Intaglietta, 1991). Increased amplitude of time-varying changes to the radius of a blood vessel result in decreased flow resistance, thereby enhancing blood flow according to Poiseuille's law (Funk et al., 1983; Slaaf et al., 1988; Wilkin, 1989; Parthimos et al., 1996; Meyer et al., 2002).

In previous work, we successfully identified five distinct frequency bands embedded in the laser Doppler blood flow signal thought to correspond to metabolic, neurogenic, myogenic, respiratory and cardiac control mechanisms (see Chapter 3). Our results suggest increased contribution of metabolic mechanisms and decreased contribution of myogenic mechanisms in the maximal skin blood flow induced by local heat (45°C) compared to baseline blood flow. We postulated that the dominant role of metabolic mechanisms are associated with the release of nitric oxide (Kellogg et al., 1999; Minson et al., 2001), thus supporting the designation of the metabolic frequency band. The designation of each characteristic frequency band was based on the wavelet-transformed power spectrum and previously reported research (Bracic and Stefanovska, 1998; Stefanovska and Bracic, 1999; Stefanovska et al., 1999; Kvandal et al., 2003; Soderstrom et al., 2003). Our primary objective here is to investigate the effect of externally applied stress on SBF by comparing it to the response to externally applied heat.

Increases in skin blood flow in response to mechanical stress have been considered a protective response by other investigators (Sacks et al., 1988; Frantz and Xakellis, 1989; Xakellis and Frantz, 1990; Xakellis et al., 1993; Sanada et al., 1997; Mayrovitz and Smith, 1999; Patel et al., 1999), and may be impaired in at-risk populations. Physiological mechanisms responsible for increases in skin blood flow are not well understood; however, the autoregulation of local microcirculation may be associated with this protective response. Autoregulation is the tendency of the local microcirculatory system to maintain constant blood flow. It is considered a local protective mechanism independent of neurogenic control. We postulate that

autoregulation is the underlying physiological mechanism responsible for the stress dependent protective increase in skin blood flow.

Although autoregulation aims to hold flow constant in the steady state, the value of baseline blood flow is determined and is frequently altered by changes in sympathetic control and local metabolic rate (Levick, 2000). Vascular smooth muscle cells receive information from interrelated blood flow control mechanisms thereby setting the contraction rhythm to provide sufficient blood flow (Johnson, 1989). These interrelated systemic neurological and local metabolic and myogenic mechanisms work together to respond to different stimuli. By using wavelet analysis, the understanding of interactions of metabolic, myogenic and neurogenic responses to different stimuli (e.g. heating and various loading patterns) may be advanced. Improved knowledge of blood flow control mechanisms may allow assessment of the effectiveness of alternating pressure therapy and thereby contribute to the determination of optimal alternating pressure parameters.

Both metabolic and myogenic factors have been considered to be associated with autoregulation of the local microcirculatory system. Both factors are mechanical stress dependent (Lewis and Grant, 1926; Duff and Shepherd, 1953; Aulick et al., 1977; Freund et al., 1981; Osol and Halpern, 1988; Meininger and Davis, 1992; Achakri et al., 1995; Butler et al., 2000). The myogenic response was first reported by Bayliss in 1902 (Bayliss, 1902), and has been considered a local control mechanism responsible for control of transmural pressure. Transmural pressure is the pressure difference across a blood vessel wall, that is, the intra-blood vessel pressure minus external blood vessel pressure. Myogenic responses are triggered for pressures within a limited range (Schubert and Mulvany, 1999; Levick, 2000). The strength of the myogenic response is determined

by blood vessel wall tension and depends on transmural pressure and vessel radius according to LaPlace's law (Nichols and O'Rourke, 1998). The changes in arteriolar radius may take up to 60 seconds to fully develop (Levick, 2000). Mechanical stress exerted by body weight results in stress and strain within soft tissues that affect transmural pressure and therefore may enhance the myogenic response.

The role of metabolic control mechanisms in autoregulation must also be considered. Under stress (i.e. hypoxia or tissue loading), the tissue's metabolic wastes and oxygen demand stimulate release of vasodilators to modulate blood flow. One important vasodilator in cutaneous microcirculation is endothelial nitric oxide. Release of nitric oxide is determined by the magnitude of shear stress acting on endothelium (Arnal et al., 1999; Michiels, 2003). Shear stress is dependent on the magnitude of blood flow according to Poiseuille's law (Sumpio, 1993). Endothelial nitric oxide is also sensitive to changes of flow rate (Butler et al., 2000).

Prevention of pressure ulcers and enhanced tissue integrity may be possible using alternating pressure support surfaces. Alternating pressure may stimulate a protective increase in skin blood flow, but the physiological mechanism by which this occurs needs closer examination. The investigation of alternating pressure and skin blood flow is confounded by the interrelated control mechanisms (i.e. metabolic, neurogenic, myogenic, respiratory and cardiac) and stimuli (i.e. heating and indentation) effecting SBF. In this study, we compared blood flow responses to heating and indentation, and aimed to differentiate their associated control mechanisms. We were particularly interested in isolating the metabolic and myogenic responses. This is the second in a series of studies investigating skin's microvascular response to various stimuli and

loading conditions as measured by laser Doppler flowmetry. Findings from this study may enhance understanding of pressure ulcer etiology and could be used to design and assess prophylactic support surfaces.

4.2 Methods

Ten unimpaired subjects (5 male and 5 female) were recruited for this study. Their demographic data were as follows (values are mean ± S.D.): age 30.0 ± 3.1 years, height 162.9 ± 6.8 cm, and weight 58.3 ± 8.6 kg. The following conditions constituted exclusion criteria: the presence of pressure ulcers on the sacrum, diabetes, vascular disease, hypertension, or use of vasoactive medications. Informed consent approved by the University of Pittsburgh Institutional Review Board was obtained from each subject prior to testing. All tests were performed in the Soft Tissue Mechanics Laboratory at the University of Pittsburgh. Room temperature was maintained at 24 ± 1°C. Test subjects assumed a recumbent, relaxed position in the laboratory for at least 30 minutes prior to testing in order to become acclimated to the room temperature and achieve a steady baseline blood flow level.

A Laserflo Blood Perfusion Monitor 2 (BPM^2, Vasamedics, MN) and Softip pencil probe (P-435, Vasamedics, MN) were used to measure capillary blood perfusion (ml_{LD}/min/100g tissue). A temperature control module (TCO, Vasamedics, MN) with heater probe (P-422, Vasamedics, MN) was used to heat the skin to 45°C to obtain a maximal skin blood flow response. A computer-controlled indenter system and other system components were designed and developed for use in this study (see Chapter 2).

Heating and indentation were applied over the sacrum, the most common site for pressure ulcers (Salzberg et al., 1996). The sacrum was heated from 35 to 45°C. The details of the heating protocol are described in Chapter 3. A computer-controlled device was used to apply incremental loading from 0 to 60 mmHg in 5-mmHg steps. Pressure was held constant for 3 minutes at each level to allow enough time to observe microvascular responses. Subjects underwent the heating protocol in the first session and incremental loading in the second session. Test sessions were separated by 7±2 days to avoid carryover effects on baseline blood flow. The baseline (i.e. pre-heating or pre-loading) and recovery (i.e. post-heating or post-loading) periods for both heating and loading protocols were 10 minutes.

Each characteristic frequency band was extracted from LDF blood flow data using wavelet transforms. The details of the wavelet analysis and normalization methods used are described in Chapter 3. The rationale for designation of frequency range for each characteristic frequency band's control mechanism is also described in Chapter 3. The characteristic frequency bands associated with the individual control mechanisms are as follows: metabolic (0.008-0.02 Hz), neurogenic (0.02-0.05 Hz), myogenic (0.05-0.15 Hz), respiratory (0.15-0.4 Hz), and cardiac (0.4-2.0 Hz). The power in each characteristic frequency band during and/or after stimuli (e.g. heating or indentation) was normalized to the power in the same frequency band during the baseline blood flow measurement. All mathematical functions were developed using Matlab 5.2 (MathWorks, MA).

A skewness test was used to test the normality of the data. Paired t-tests were used to compare power in the corresponding frequency bands for the following conditions: pre-loading and pre-heating, post-loading and post-heating, and pre-loading and post-

loading. Had skewed distributions existed, Wilcoxon matched-pairs signed-ranks test would have been used. A one-way ANOVA with repeated measures was used to compare differences of power in each characteristic frequency band during incremental loading. Where skewed distributions existed, Kruskal-Wallis one-way analysis of variance was used to compare differences of power in each characteristic frequency band during. Linear and quadratic trend analysis were used to describe responses in each characteristic frequency band under incremental loading (Portney and Watkins, 2000). The significance level was set at 0.05. All data were analyzed by using SPSS 10.1.

4.3 Results

Mean skin blood flow under incremental loading is shown in Figure 4-1. The results indicate that blood flow decreases under initial pressure loading (0-15 mmHg), is relatively constant between 15 and 30 mmHg, and then increases as pressure increases above 35 mmHg.

Comparisons in each characteristic frequency band obtained from pre-loading and post-loading blood flow are shown in Figure 4-2. Results show that only blood flow response in the myogenic frequency band is significantly different ($p<0.05$).

Skewness tests showed no statistically significantly differences ($p>0.05$) in each characteristic frequency band during post-heating, incremental loading and post-heating except in the power in the metabolic frequency band during 55 mmHg loading pressure.

Kruskal-Wallis one-way analysis of variance was used to test metabolic frequency band, and showed significant differences during incremental loading ($p<0.05$). A quadratic relationship between power in the metabolic frequency band and incremental

pressure was detected ($p<0.05$). For incremental pressure, trend analysis shows that power in the metabolic frequency band decreased as pressure increased from 5-20 mmHg and increased above 20 mmHg (Figure 4-3).

Analysis using one-way ANOVA with repeated measures showed no significant differences in power in the neurogenic frequency band during incremental loading ($p>0.05$). No trends were found in the neurogenic frequency band (Figure 4-4).

Analysis using one-way ANOVA with repeated measures demonstrated a significant difference in power in the myogenic frequency band during incremental loading ($p<0.05$). A quadratic trend relating power in the myogenic frequency band to incremental pressure was detected ($p<0.05$). The power in the myogenic frequency band increases for increasing pressure under 25 mmHg and decreases for increasing pressure above 25 mmHg (Figure 4-5).

When comparing normalized power in each characteristic frequency band post-loading to post-heating, the results show that SBF response in the metabolic frequency band is more dominant during post-heating ($p<0.01$), while SBF response in the myogenic frequency band is more dominant during post-loading ($p<0.01$) (Figure 4-6).

4.4 Discussion

The primary finding of our study is that SBF control mechanisms associated with indentation (nonnoxious incremental pressure stimulus) are different from those associated with heating. We showed that the myogenic response causes an increase in SBF during loading (pressure induced vasodilation) and after loading (reactive hyperemia). Pressure induced vasodilation and reactive hyperemic responses are well

known, but not well understood. Both phenomena have been considered to be associated with metabolic and myogenic factors and/or sensory nerves (Larkin and Williams, 1993; Guyton and Hall, 1996; Joyner and Dietz, 1997). Reactive hyperemia occurs in denervated tissues, and is usually considered a local response to tissue ischemia (Lewis and Grant, 1926; Duff and Shepherd, 1953; Guyton and Hall, 1996). Engelke et al. demonstrated that the blocking of endothelial nitric oxide release does not eliminate the maximal vasodilation observed during reactive hyperemia and postulated that a myogenic response is responsible for the hyperemia (Engelke et al., 1996). Our data supports Engelke's theory because it suggests that there is an increased contribution of myogenic control after incremental loading. Although mechanical stress acting on the sacrum may also modulate shear stress acting on the endothelium that initiates nitric oxide release, our results suggest that metabolic control has a minor role in SBF's response to mechanical stress. Shear stress is the primary stimulus for the regulation of nitric oxide release (Arnal et al., 1999; Michiels, 2003), and is dependent on the blood flow and inverse third power of vessel radius according to Poiseuille's law (Sumpio, 1993). When external pressures approach the higher levels in our experiment, the flow is reduced to levels near biological zero (Shepherd and Oberg, 1990) thus nearly eliminating flow induced shear stress altogether.

In contrast to the response to indentation, our data shows post-heating increases in SBF occurring in the 0.008-0.02 Hz frequency band. We postulate that this increase is primarily due to nitric oxide release and, as such, supports our designation of this frequency range as being related to metabolic control mechanisms, endothelial nitric oxide in this case. Kellogg et al. and Minson et al. determined that maximal blood flow

response to local heating is a biphasic response in which an initial peak is related to local sensory nerve activity and a second is due to release of endothelial nitric oxide (Kellogg et al., 1999; Minson et al., 2001). This conclusion is also supported by the results of Stefanovska and colleagues' research (Kvernmo et al., 1999; Stefanovska and Bracic, 1999; Stefanovska et al., 1999; Soderstrom et al., 2003). These investigators compared SBF responses to acetylcholine (endothelium-dependent vasodilator) and sodium nitroprusside (endothelium-independent vasodilator), and demonstrated that the 0.01 Hz frequency component of the SBF signal is endothelium dependent.

As blood flow increases, the blood vessel dilates due to the release of nitric oxide. The resulting low, but sustained, output of endothelial nitric oxide reduces the vasomotor tone of adjacent myocytes and thus reduces blood pressure and shear stress (Levick, 2000). Since we observed that a decrease in SBF is accompanied by a decrease in metabolic response for external pressures in the range of 5-20 mmHg loading and an increase in SBF is accompanied by an increase in metabolic response under 25-55 mmHg loading, our finding supports the theory that metabolic control is a flow-mediated response. However, the overall metabolic response under loading was less than that of baseline SBF, which suggests a decreased contribution of metabolic control under loading.

An inverse relationship was observed between the metabolic and myogenic responses under incremental pressure loading. That is, the metabolic response increased and myogenic response decreased for pressure in the range of 25-55 mmHg. This is evidence of one aspect of the interrelationships among vasodilators, sensory nerves, and myogenic responses for the regulation of SBF (Ping and Johnson, 1992; Larkin and

Williams, 1993; Engelke et al., 1996). Given the complexity of the control, the simultaneous monitoring of blood flow control mechanisms using the wavelet analysis technique developed for this study may advance understanding of patho-physiological changes in the microvascular system (Ping and Johnson, 1992).

Understanding the responses to both heat and pressure is advantageous when designing support surfaces intended to reduce risk of pressure ulcer development. Both heat and pressure are interface parameters that are affected by support surface design. For alternating pressure support surfaces, it may be possible to adjust and/or optimize loading parameters to enhance the beneficial myogenic mediated increase in SBF (Meininger and Davis, 1992; McLeod, 1997; Mayrovitz and Smith, 1999). Increase in SBF during loading may lead to improved pressure ulcer prevention. There may a particularly important application for the population of people with spinal cord injuries where neurogenic control over the cardiovascular system is impaired, thereby causing a high risk of developing pressure ulcers. For individuals with impaired or diminished endothelial function (i.e. elderly or smokers) (Barua et al., 2001; Minson et al., 2002), the protective vasodilation induced by heat for removing excessive heat may not function well. Thus heat dissipation can be an important factor in choosing support surfaces for individuals with endothelial dysfunction. Improved understanding by clinicians and manufactures of the risk factors and associated physiological mechanisms has the potential to improve pressure ulcer prevention outcomes (Bergstrom et al., 1996).

The optimization of alternating pressure parameters will need to consider the complex relationship between applied pressure and the response of the microcirculatory system. In our study, mean blood flow reached minimum levels for applied pressures in

the range of 15-20 mmHg. As pressure exceeds 35 mmHg, the response showed a trend of increasing blood flow. This response is consistent with the findings of other researchers (Xakellis et al., 1993; Patel et al., 1999). Patel et al. has also suggested that it may be due to a myogenic response (Patel et al., 1999). The myogenic response is thought to be sensitive to just a limited range of applied pressures (Schubert and Mulvany, 1999; Butler et al., 2000; Levick, 2000). In our study, we found the myogenic response was enhanced under pressure loading. We found that the myogenic response is most dominant between 15-25 mmHg loading. This finding has potentially important implications for choosing low and high pressure settings for AP support surfaces. However, this protective mechanism may be due to stress relaxation resulting from viscoelastic properties of soft tissue including blood vessels and surrounding skin components.

In comparing our results with the results of others who have used spectral analysis to characterize control mechanisms of the cardiovascular system, we find both similarities and differences (Keselbrener and Akselrod, 1998; Krongold et al., 1999; Stefanovska and Bracic, 1999; Mitsis et al., 2002). Several investigators have used laser Doppler flowmetry combined with spectral analysis (Fourier-based analysis) to analyze the physiological role of vasomotion in the microvascular system (Intaglietta, 1991; Colantuoni et al., 1994; Lossius and Eriksen, 1995; Bertuglia et al., 1996). Using fast Fourier transform to analyze laser Doppler blood flow signal, Bernardi et al. concluded that 0.03-0.15 Hz is associated with sympathetic control over skin blood flow (Bernardi et al., 1989). In a later study, they observed reductions near 0.1 Hz in patients with diabetes and concluded that the reduction is due to neuropathy (Bernardi et al., 1997b).

However, 0.1 Hz falls within our designation of the myogenic control mechanism. A possible explanation for this apparent inconsistency between our result and theirs is that the frequency resolution provided by the Fourier transform used by Bernardi et al. was not high enough. Using wavelet transform, a multi-resolution time-frequency analysis with improved frequency resolution, we were able to differentiate between the 0.02-0.05 Hz and 0.05-0.15 Hz ranges corresponding to the neurogenic and myogenic origins respectively. Although autonomic neuropathy in diabetic patients may cause an abnormal pattern of vasomotion (Benbow et al., 1995; Stansberry et al., 1996), structural changes of vascular smooth muscle associated with chronic denervation have also been observed (Bevan and Tsuru, 1979). Thus, impaired myogenic response may also exist in diabetic patients which in turn would cause reduction in 0.1 Hz frequency range.

4.5 Conclusion

The purpose of this study was to differentiate blood flow control mechanisms associated with indentation from those associated with heating. We found that the response to heat tended to be in the frequency range corresponding to metabolic activity, 0.008-0.02 Hz, and that the response to pressure tended to be in the frequency range corresponding to myogenic control, 0.05-0.15 Hz. We validated our novel method for monitoring microvascular system responses using heating and indentation stimuli. The method is potentially important in advancing pressure ulcer prevention research as well as in improving understanding of the beneficial protective mechanisms associated with alternating pressure support surfaces.

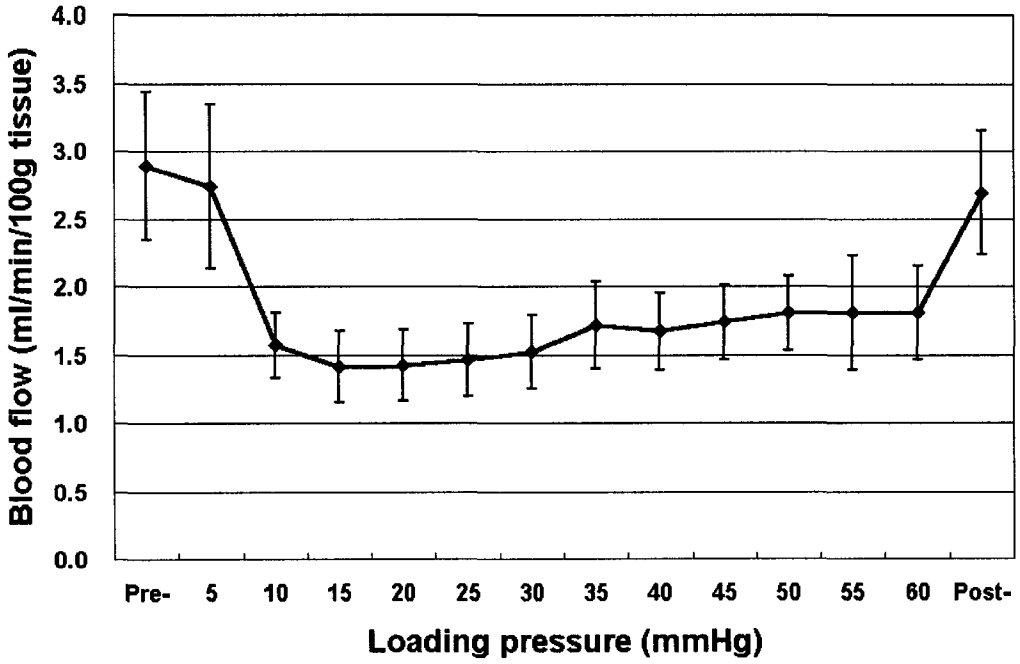

Figure 4-1. Mean blood flow under incremental loading (0 to 60 mmHg at 5 mmHg step/ 3 min) (values are means ± S.E.).

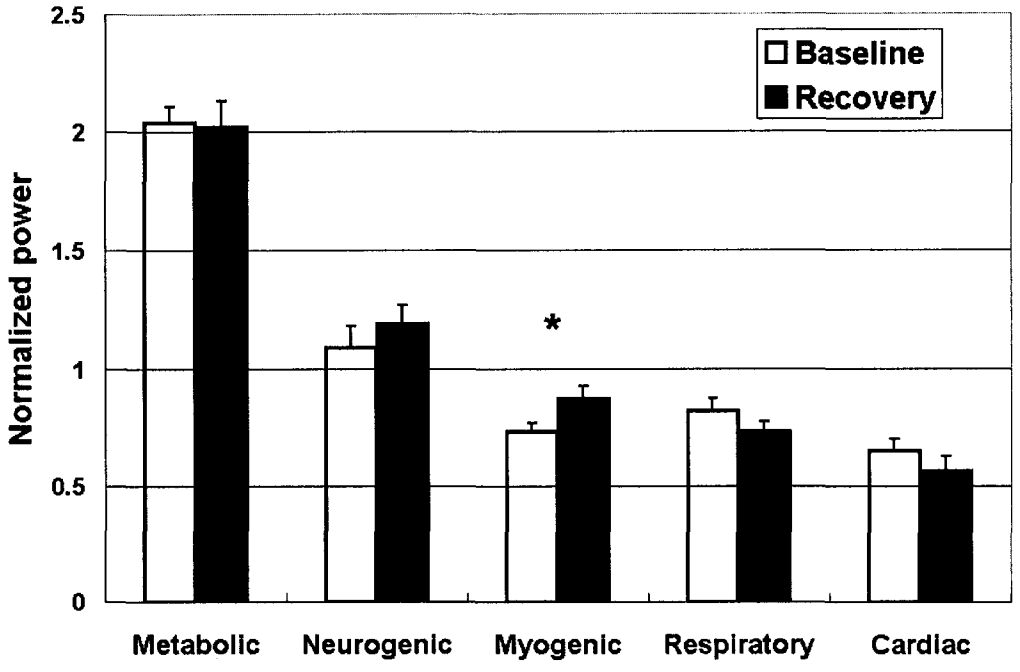

Figure 4-2. Comparisons of power of five characteristic frequencies of baseline and recovery periods (* indicates $p<0.05$) (values are means ± S.E.).

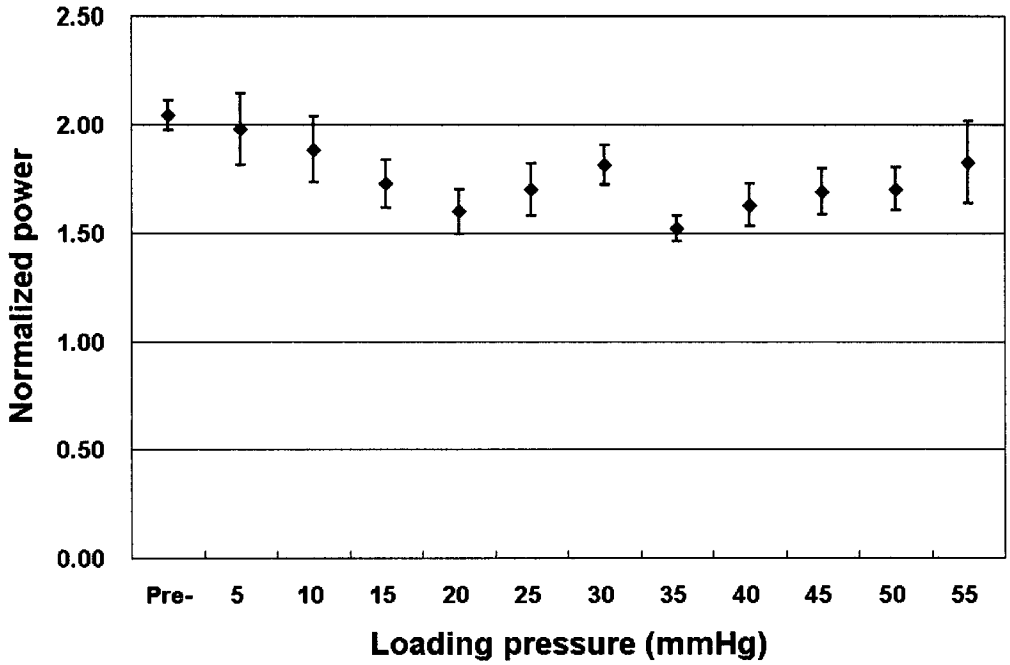

Figure 4-3. Power of metabolic frequency under incremental pressure (values are means ± S.E.).

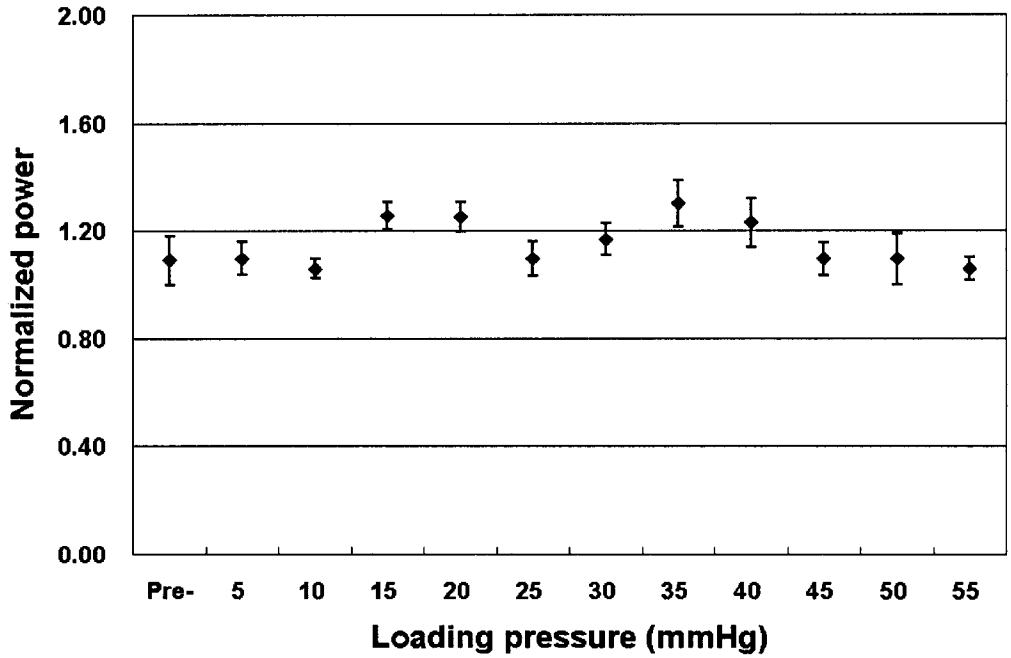

Figure 4-4. Power of neurogenic frequency under incremental pressure (values are means ± S.E.).

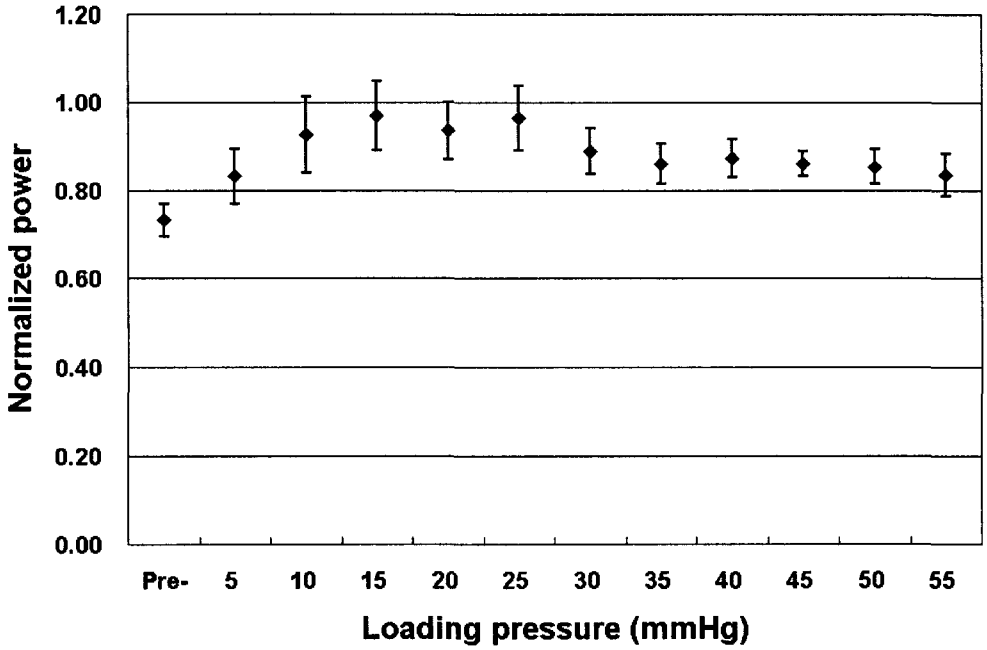

Figure 4-5. Power of myogenic frequency under incremental pressure (values are means ± S.E.).

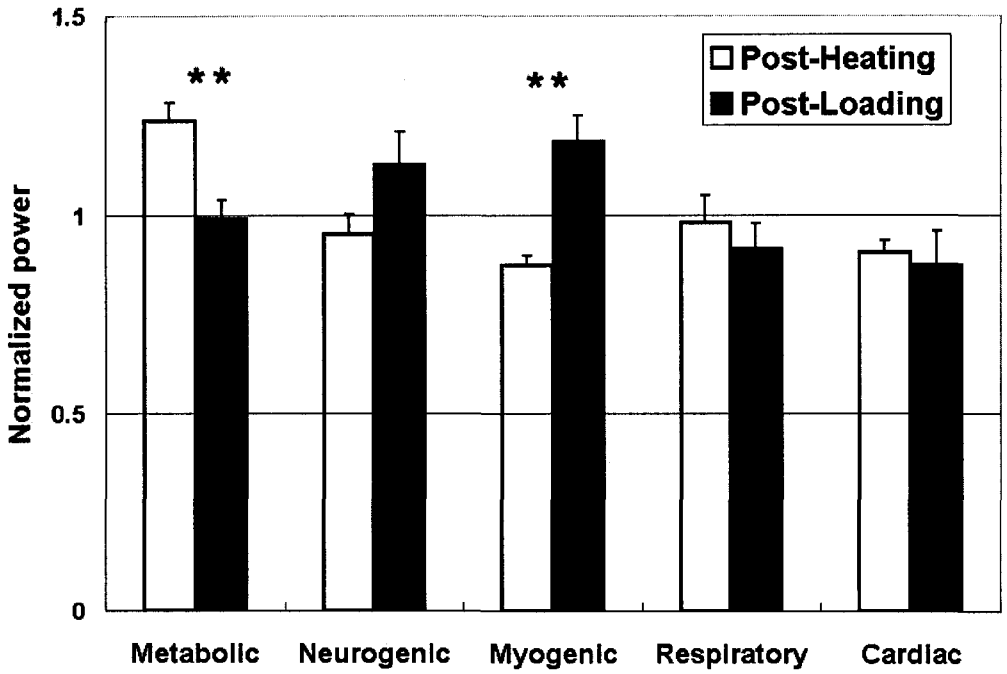

Figure 4-6. Comparisons of power of five characteristic frequency bands between post-heating and post-loading (** indicates $p<0.01$).

5.0 A TIME-FREQUENCY APPROACH USING WAVELETS TO STUDY WEEK-TO-WEEK VARIABILITY IN BLOOD FLOW OSCILLATIONS

Abstract – Skin blood flow (SBF) is regulated by interrelated control mechanisms and responds to various stimuli. The study of blood flow responses is confounded by temporal variability in blood flow measurement. Spectral analysis has been shown to be useful in isolating the effects of distinct control mechanisms to various stimuli in the microcirculatory system. However, the sensitivity of spectral analysis to temporal blood blow variability has not been reported. This study investigates the effectiveness of a wavelet analysis technique in reducing week-to-week variability in blood flow measurements. Baseline blood flow and maximal blood flow response of 10 healthy young subjects were recorded once per week for three consecutive weeks. Wavelet analysis was used to decompose the laser Doppler blood flow signal into frequency bands determined to be associated with metabolic, neurogenic, myogenic, respiratory and cardiac control mechanisms. The results show that coefficients of variation for the power in each frequency band at baseline are smaller than the coefficients of variation of blood flow at baseline or maximal blood flow ratio ($p<0.05$). Myogenic and respiratory frequencies showed the highest coefficients of variation among the five frequency bands. Our study suggests that wavelet analysis is effective in reducing temporal blood flow variability.

Key words: laser Doppler flowmetry, skin blood flow, variability, wavelet transform.

Abbreviations: ANOVA = Analysis of Variance; BPM = Blood Perfusion Monitor; CoV = Coefficient of Variation; LDF = Laser Doppler Flowmetry; SBF = Skin Blood Flow.

5.1 Introduction

Skin blood flow is regulated by interrelated control mechanisms and responds to various stimuli. The study of blood flow responses is confounded by temporal variability in blood flow measurement (Hertzman and Randall, 1948; Tenland et al., 1983; Gaehtgens, 1992; Hoffmann et al., 1993; Mayrovitz et al., 1997; Gardner-Medwin et al., 2001). Standardization Group of the European Society of Contact Dermatitis warned that research using laser Doppler flowmetry needs to consider the effect of temporal variability on baseline blood flow measurements on results (Bircher et al., 1994). Two methods have been reported to compensate for temporal variability in baseline blood flow measurements: 1) expressing baseline blood flow as a percentage of thermally induced maximal blood flow obtained at the time of testing (Mayrovitz et al., 1999) and 2) measuring baseline blood flow at the controlled skin temperature (e.g. 37°C) (Creutzig et al., 1987). A third method used to overcome temporal variability in blood flow response measurements is expressing blood flow response as a percentage of baseline blood flow (Olsson and Bende, 1986; Mayrovitz et al., 1993; Sanada et al., 1997; Mayrovitz and Sims, 2002). However, the success of these methods in reducing temporal blood flow variability has been mixed.

Analysis of skin blood flow oscillations has been shown to be useful in isolating the effects of distinct control mechanisms to various stimuli in the microcirculatory system (Salerud et al., 1983; Kastrup et al., 1989; Bernardi et al., 1997b; Stefanovska et al., 1999; Soderstrom et al., 2003). However, the sensitivity of spectral analysis to temporal blood blow variability has not been reported. The potential advantage of using spectral analysis in reducing temporal variability is related to the basic principle of blood

flow regulation. Blood flow is distributed within the cardiovascular system to meet the current demands of individual organs and tissues. At any given time, the blood flow to a particular body organ or system is dependent on its need and the current demands of all other organs and systems. In this way, the body is able to prioritize its distribution and manage the entire body with only a limited capacity to circulate blood. Blood flow in the skin serves several purposes including thermoregulation and as a supply of nutrients to the skin tissues. The flow is also affected by changes in sympathetic control and local metabolic rate (Levick, 2000). The magnitude of skin blood flow will therefore be dependent on these needs plus the demands of other organs and systems. However, regulation of flow in the microvascular system of the skin includes responses to these various needs and associated stimuli with oscillatory rhythms in distinct frequency ranges (Salerud et al., 1983; Kastrup et al., 1989; Bernardi et al., 1997b; Stefanovska et al., 1999; Soderstrom et al., 2003). We are therefore able to filter out portions of the SBF variability that correspond to control mechanisms of flow regulation not associated with our targeted responses. For example, in our studies we are interested in examining the metabolic, neurogenic and myogenic control mechanisms in response to externally applied pressure. Using spectral analysis allows us to filter out the variability associated with temporal changes in respiratory and cardiac function.

Several spectral analysis methods have been used to analyze blood flow oscillations. These techniques include the Fourier transform (Salerud et al., 1983; Kastrup et al., 1989; Stauss et al., 1998), autoregressive analysis (Colantuoni et al., 1994; Bernardi et al., 1997a) and wavelet analysis (Stefanovska et al., 1999). The disadvantages of using the Fourier transform are related to its limited frequency resolution and inability

to analyze non-stationary signals such as those resulting from blood flow measurements (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Aubert et al., 1999). Thus a time-frequency approach to characterize these characteristic frequencies is needed. The simplest time-frequency analysis is the short time Fourier transform. The short time Fourier transform uses a fixed time window to calculate frequency components embedded in a signal. However, the fixed window method limits its applicability in the analysis of signal containing several characteristic frequencies (Stefanovska and Bracic, 1999). The autoregressive analysis techniques are also not idea for blood flow signals. The need to select the order in the parametric model to provide the best fit of the data is limiting (Aubert et al., 2003). Using a Wigner-Ville distribution, a more complicated time-frequency analysis based on the Cohen's class distribution (Cohen, 1989; Novak and Novak, 1993), to study a signal containing multiple characteristic frequencies needs to consider cross-terms problem (Keselbrener and Akselrod, 1996).

Wavelet analysis is a relatively new time-frequency (actually time-scale) analysis method and is used in numerous biomedical applications (Hubbard, 1996; Mallat, 1999). Wavelet analysis provides good time resolution at high frequency and good frequency resolution at low frequency. This multi-resolution, time-frequency analysis has been shown to be a good method for analyzing blood flow oscillations (Bracic and Stefanovska, 1998). A limitation of using wavelet analysis is the need to select an appropriate wavelet function. There is little general guidance or well-defined rules available for selecting an wavelet function (Kumar et al., 2003). Some of the popular wavelet functions are Coiflet, Daubechies, Haar, Mexican Hat, Morlet and Symmlet

(Strang and Nguyen, 1997). Several wavelet functions have been compared to in characterizing reactive hyperemia (Humeau et al., 2000). The Morlet wavelet is a Gaussian function that gives the best time-frequency localization within the limits according to the uncertainty principle (Meste et al., 1994; Bracic and Stefanovska, 1998). (The time resolution Δt and frequency resolution Δf are mutually exclusive and are interdependent according to the equation $\Delta t \times \Delta f \geq c$, where c is a constant. Equality in this equation is only achieved by a Gaussian function.) Thus we have selected the Morlet wavelet for this study in order to maximize both time and frequency resolution (Stefanovska et al., 1999; Humeau et al., 2004)(see Chapter 3).

Skin blood flow oscillations are attributed to vasomotion, that is, the rhythmical constriction and dilation of arterioles and venules (Nicoll and Webb, 1955; Intaglietta, 1991; Colantuoni et al., 1994). In previous work, we successfully identified five distinct frequency bands embedded in the laser Doppler blood flow signal reported to correspond to metabolic, neurogenic, myogenic, respiratory and cardiac origins (Stefanovska et al., 1999; Kvandal et al., 2003; Soderstrom et al., 2003)(see Chapter 3 and 4). Analysis methods were proposed to study relative contributions in each characteristic frequency to total blood flow (see Chapter 3). Here we hypothesize that this method may be useful to identify and filter out the inconsequential variability associate with the cardiac, respiratory control mechanisms and higher frequency components, thus allowing us to make better assessments of the metabolic, neurogenic and myogenic control mechanisms.

The specific aims of this study were to: 1) compare the effectiveness of wavelet analysis to traditional methods used by other researchers for reducing blood flow variability (e.g. maximal blood flow ratio (Mayrovitz et al., 1999)), 2) identify the control

mechanism responsible for temporal variability of baseline blood flow, 3) assess temporal variability of blood flow during pre-heating, heating and post-heating periods and 4) examine repeatability of maximal blood flow responses once per week for three consecutive weeks.

5.2 Methods

Ten unimpaired subjects (5 male and 5 female) were recruited for this study. Their demographic data were as follows (values are mean ± S.D.): age 30.0 ± 3.1 years, height 162.9 ± 6.8 cm, and weight 58.3 ± 8.6 kg. The following conditions constituted exclusion criteria: the presence of pressure ulcers on the sacrum, diabetes, vascular disease, hypertension, or use of vasoactive medications. Informed consent approved by the University of Pittsburgh Institutional Review Board was obtained from each subject prior to testing. All tests were performed in the Soft Tissue Mechanics Laboratory at the University of Pittsburgh. Room temperature was maintained at 24 ± 1°C. Subjects were brought into the laboratory on three occasions separated by 7 ± 2 days for measurement of baseline and thermally induced maximal sacral blood flow for three consecutive weeks. Blood flow over the sacrum was recorded during 10 minutes of rest to establish baseline flow followed by 15 minutes of incremental heating from 35 to 45°C. Measurements continued during a 10-minute recovery (post-heating) period following the removal of the heat source. Test subjects laid in recumbent, relaxed position in the laboratory for at least 30 minutes prior to testing in order to become acclimated to the room temperature and achieve a steady baseline blood flow level.

Laserflo Blood Perfusion Monitor 2 (BPM^2, Vasamedics, MN) and Softip pencil probe (P-435, Vasamedics, MN) were used to measure capillary blood perfusion (ml_{LD}/min/100g tissue). A temperature control module (TCO, Vasamedics, MN) with heater probe (P-422, Vasamedics, MN) was used to heat the skin to 45°C to obtain a maximal skin blood flow response. A computer-controlled indenter system and other system components were designed and developed for use in this study (see Chapter 2).

Wavelet analysis was used to decompose blood flow signals into frequency components determined to be associated with metabolic, neurogenic, myogenic, respiratory and cardiac control mechanisms. Analysis methods were used to study relative contributions in each characteristic frequency band to total blood flow (see Chapter 3). The rationale for designation of frequency range for each characteristic frequency band's control mechanism is described in Chapter 3. The characteristic frequency bands associated with the individual control mechanisms are as follows: metabolic (0.008-0.02 Hz), neurogenic (0.02-0.05 Hz), myogenic (0.05-0.15 Hz), respiratory (0.15-0.4 Hz), and cardiac (0.4-2.0 Hz). Maximal blood flow ratio was defined as the ratio of baseline blood flow to thermally induced maximal blood flow at 45°C. A Mauchly's test of sphericity was used to test for equal variances of the repeated measures data. One-way analysis of variance (ANOVA) with repeated measures was used to test for differences among baseline blood flow between measurements taken once per week for three consecutive weeks. Mean baseline blood flow was calculated based on 1, 2, and 5 minute-period to study short-term variation. SBF variability related to changing physiological needs and conditions over long time scales confounds research that relies on repeated measures. To minimize the impact of this inherent variability,

researchers have developed various techniques to normalize their measurements (Creutzig et al., 1987; Sanada et al., 1997; Mayrovitz et al., 1999). A common method for assessing such variability in SBF is the coefficient of variation (Hertzman and Randall, 1948; Tenland et al., 1983; Gaehtgens, 1992; Hoffmann et al., 1993; Mayrovitz et al., 1997; Gardner-Medwin et al., 2001). The coefficient of variation (CoV) is independent of units of measurement. Here the CoV is used to analyze the impact of inherent SBF variability on various normalization methods by comparing its magnitude between total blood flow at baseline, power within each characteristic frequency band at baseline and baseline SBF normalized to the maximal blood flow according to the ratio method

5.3 Results

The subjects' mean blood flow and standard deviation for baseline blood flow and blood flow at temperatures between 35°C and 45°C for measurements taken once per week for three consecutive weeks is shown in Table 5-1. Mauchly's tests of sphericity of baseline blood flow and characteristic frequencies show no statistically significant difference (Table 5-2). One-way ANOVA with repeated measures do not show a significant difference between corresponding measurements taken on different weeks ($p>0.05$) (Table 5-3).

The CoV for the blood flow signal power in each individual frequency band at baseline (CoV range from 0.08 to 0.15) are smaller than that of blood flow at baseline (0.28) or maximal blood flow ratio (0.41) ($p<0.05$) (Figure 5-1). Myogenic and respiratory frequencies have higher CoV among five characteristic frequencies bands in baseline blood flow (Figure 5-1).

CoV of blood flow signals during pre-heating, heating and post-heating periods in three consecutive weeks show that there is more variability in the blood flow signal during heating compared to pre-heating (baseline) and post-heating (recovery) except in the myogenic frequency band where there is more variability during the preheating (baseline) time period (Figure 5-2).

Heating produced consistent increases of blood flow signal power in the metabolic frequency range and decreases in signal power in the myogenic frequency band for each of the three measurement sessions ($p<0.01$).

In the short-term variation study, mean baseline blood flow calculated over a 2-minute-period is shown in Table 5-4. All three time periods (1, 2, 5-minute) show no significant differences in mean baseline blood flow ($p>0.05$).

A contour plot of wavelet coefficients showing time information across the spectrum of the five characteristic frequency bands under 45°C heating is shown in Figure 5-3.

5.4 Discussion

To best of our knowledge, this is the first study published using wavelet analysis to investigate temporal variability of laser Doppler blood flow measurements. However, time-frequency approaches have been used widely in the study of heart rate variability (Aubert et al., 2003). Heart rate variability is well accepted as one of the most promising physiological markers for identifying cardiovascular mortality (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). We postulated that the study of skin blood flow variability has

great potential to advance understanding of blood flow control mechanisms and to provide early detection of pathological changes in the skin (i.e. foot ulcers in diabetes mellitus, survival of free flap or stage I pressure ulcer).

Time-frequency data was reduced to frequency only information by averaging wavelet coefficients during specific time events. This method allows us to quantify time-frequency data for statistical testing. Time-averaged wavelet coefficients also serve as a basis for studying relative contributions in each characteristic frequency band to the total blood flow signal. Using this approach we successfully determined that metabolic control mechanisms are responsible for thermally-induced blood flow regulation (see Chapter 3) and myogenic control mechanisms are responsible for pressure-induced vasodilation and reactive hyperemia (see Chapter 4). In this study we further showed that frequency components extracted by wavelet analysis are effective in overcoming temporal variability. However, averaging wavelet coefficients during specific time events eliminates the time information in that characteristic frequency band. This approach is necessary to interpret features of blood flow oscillations since physiological meanings associated with these characteristic frequency bands are as of yet unknown. Direct interpretation of time-frequency data such as the data shown in Figure 5-3 is very difficult. Thus, a wavelet technique with quantification methods was proposed to study blood flow variability in this study. Our results suggest that this method is effective in reducing blood flow measurement variability. We intend to use the technique originally developed to study heart rate variability to study these time-varying features of SBF in characteristic frequency bands (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Blood flow signal power in the myogenic frequency band (0.05-0.15 Hz) showed the highest week-to-week variability in baseline blood flow. This phenomenon may be caused by a local spontaneous contractions and relaxations of vascular smooth muscles in the microvascular system. Spontaneous contraction/relaxation of vascular smooth muscles has been observed *in vitro* in experiments on vessels without innervation. Thus myogenic response detected by LDF probe is sensitive to the position of the underlying microvasculature featured for spatial inhomogeneity (Braverman, 2000). Myogenic response accordingly is the most variable control mechanism in the cutaneous microcirculation. However, externally applied stimuli may enhance the degree of synchronism of myogenic oscillations in adjacent areas thereby increasing unit blood flow (Silverman and Stout, 2002).

Signal power in the respiratory frequency band (0.15-0.4 Hz) showed the second highest week-to-week variability among five characteristic frequencies. Hoffmann et al. used the frequency histogram method to compare baseline blood flow at two different days, and found that variability of baseline blood flow was mainly due to an inconsistency of 0.2867Hz frequency component (Hoffmann et al., 1993). Also, the effect of breathing rhythm on LDF blood flow is well documented (Bernardi et al., 1989; Muck-Weymann et al., 1996).

Four of five characteristic frequencies (e.g. metabolic, neurogenic, respiratory and cardiac) showed higher variability during heating compared to CoV during pre-heating (baseline) and post-heating (recovery) periods. This result is not consistent with the methodologies used by others in which local heat is used to reduce temporal variability (Creutzig et al., 1987; Mayrovitz et al., 1999). Mayrovitz et al. suggested that heating the

skin to 45°C for 5 minutes and expressing baseline blood flow as a percentage of blood flow at 45°C reduces variability. Mayrovitz did not provide a rationale was for choosing 5 minutes. This relatively short time period is likely not of sufficient duration to induce maximal blood flow response (Kellogg et al., 1999; Minson et al., 2001), and may only provoke axon reflex mediating vasodilation, the first of the two mechanisms mediating thermally-induced maximal blood flow response. In this study we use 15 minutes of heating for inducing maximal blood flow response. However, 15 minutes may not be long enough to reach peak blood flow mediated by endothelial nitric oxide (Minson et al., 2001).

Laser Doppler technology has proven a reliable tool to study cutaneous microcirculation; however, some limitations are noted (Shepherd and Oberg, 1990; Braverman et al., 1992). Measurement values are derived from frequency shifts and power levels of reflected laser light compared to that of emitted laser light. A scaling factor is necessary to convert optical signal parameters to physiological blood perfusion. In the absence of a well-accepted universal scaling factor, LDF blood flow is often expressed in arbitrary units or relative changes (Bircher et al., 1994). There are also poor standards for calibration of laser Doppler flowmetry (Oberg, 1990). Analysis of relative LDF blood flow measurements may contribute to the temporal variability seen in many studies. However, studying relative contribution of each characteristic frequency to the total LDF blood flow is independent of the scaling factor and is robust to overcome the limitation of laser Doppler technology. Thus, a decrease in blood flow variability using wavelet analysis is expected in this study.

Variations in laser Doppler blood flow measurements were investigated for relatively short 1, 2, and 5 minute periods. We were interested in assessing the time varying characteristics of the signals so that non-stationary components could be ruled out as a potential factor in any variability we recorded in our longer-term (week-to-week) variability study. We found no significant difference in mean skin blood flow over these short time periods. Thus our results indicated that mean blood flow does not have much variation when averaged over these short time spans. Since blood flow is a non-stationary signal especially in long-term measurements, the calculation of mean blood flow is theoretically affected by the time-period used. The time period used for calculating mean blood flow in other studies ranges from 10 seconds to 6 minutes (Tenland et al., 1983; Breit et al., 1993; Mayrovitz and Larsen, 1994a; Mayrovitz and Larsen, 1994b; Strucl et al., 1994; Sanada et al., 1997). Although our data showed no difference of mean blood flow averaged over 1, 2 and 5 minute periods, a single sample measurement would likely have a high degree of variability caused by the 0 to 2.5 Hz oscillatory patterns in the blood flow signal.

The sampling frequency used for laser Doppler measurement can have significant impact on the results and their variability. The sampling frequency should be at least twice as high as the highest frequency component in the blood flow signal. Therefore, in order to accurately assess the cardiac related rhythms, a minimum 5-Hz sampling frequency is required (i.e. heart rate may be up to 150 beats/min at rest). Most commercial laser Doppler instruments provide an averaging function over time period to smooth blood flow data. Longer averaging time periods are preferred in clinical applications where measurement higher frequency oscillations are undesirable. In

research where the high frequency components are of interest, shorter averaging time periods are necessary. The length of the sampling time period should be long enough to cover at least one cycle of lowest frequency signal component (i.e. 100 seconds for 0.01 Hz corresponding to metabolic frequency).

Probe placement and regional variations in the cutaneous microvascular system is a potential confounding factor in research using laser Doppler flowmetry. Laser Doppler flowmetry provides a single point measurement (~1 mm^2) of cutaneous microcirculation. Consistent positioning of the laser Doppler probe over the sacrum is difficult and therefore unlikely. We attempted to minimize variability caused by positioning variations by using a single evaluator throughout the study. As an alternative to the single point measurement technique employed here other researchers have used scanning Laser Doppler system that samples blood flow on a grid. However, such instruments do not sample fast enough and do not permit the simultaneous application of pressure to the measurement site, a requirement for our studies.

5.5 Conclusion

The major finding of this study is that wavelet analysis was shown to be an effective method for managing temporal variability in skin blood flow measurements. Using the wavelet analysis techniques described here, our results from three consecutive weeks of blood flow measurements suggest that using individual frequency components embedded in baseline blood flow separately provides a more reliable basis on which to make quantitative comparisons and other analyses for various experimental conditions. Lower variability will increase statistical power. The wavelet technique produced data

with significantly lower variability compared to the baseline normalization and maximal blood flow normalization technique found in the literature.

Higher week-to-week variability was observed in myogenic and respiratory related frequency bands when the skin was heated. The maximal blood flow ratio method failed to reduce variability in baseline blood flow. An increase in power in the metabolic frequency bands and a decrease in power in the myogenic frequency band were observed for the maximal blood flow response (local heat at 45°C).

Table 5-1. The subjects' mean blood flow and standard deviation for baseline and recovery blood flow and blood flow at temperatures between 35°C and 45°C for measurements taken once per week for three consecutive weeks.

	Baseline	Skin Temperature (^{0}C)											Recovery
		35	36	37	38	39	40	41	42	43	44	45	
S01	2.88±1.57	2.97±1.24	3.01±0.81	3.34±1.09	3.88±1.30	4.84±2.19	5.76±2.51	6.84±2.64	8.45±2.68	10.04±2.95	11.74±3.89	14.40±5.00	12.96±2.88
S02	1.19±0.36	1.30±0.13	1.55±0.20	1.87±0.26	2.53±0.09	3.86±0.28	4.97±0.93	6.27±0.98	8.63±1.08	11.94±1.59	15.07±2.51	19.18±3.99	21.14±4.40
S03	2.53±0.40	2.61±0.38	2.88±0.41	3.73±0.49	4.81±0.65	6.85±1.23	8.79±2.04	10.20±2.55	12.23±2.32	14.00±2.31	16.81±3.60	20.96±6.07	20.87±5.15
S04	3.60±0.51	4.03±0.53	3.68±0.18	3.90±0.24	4.70±0.94	5.51±1.75	5.56±1.50	5.95±1.55	6.21±2.01	7.33±3.54	10.02±4.36	15.06±9.80	15.79±9.05
S05	1.82±0.67	1.72±0.68	2.04±0.67	2.61±1.02	3.43±1.19	4.64±1.91	5.58±1.88	7.18±2.26	8.34±2.90	9.22±2.42	11.51±3.56	14.08±5.44	17.64±7.08
S06	2.94±1.55	3.86±2.36	3.96±1.42	4.62±1.96	5.25±0.57	6.61±1.16	7.51±3.01	9.08±1.75	11.45±1.35	13.72±2.15	17.45±2.66	21.99±0.76	24.36±2.29
S07	1.68±0.51	2.22±0.63	2.87±0.74	3.31±0.86	4.22±0.91	5.73±1.09	7.45±0.57	8.85±0.70	11.42±0.73	15.00±3.29	17.44±3.28	22.67±2.40	22.42±7.59
S08	6.04±0.48	4.62±1.57	5.09±1.09	5.65±1.49	7.20±2.07	8.48±3.35	8.85±2.23	9.40±1.81	10.57±2.14	11.03±2.23	12.93±1.29	17.94±2.52	19.46±5.29
S09	1.05±0.19	1.35±0.34	2.17±0.94	2.23±0.97	2.73±1.18	3.47±1.56	4.56±2.26	5.87±2.55	7.22±3.22	10.61±5.18	13.52±6.14	18.36±8.10	17.81±6.35
S10	1.28±0.29	1.31±0.19	1.36±0.16	1.64±0.39	2.04±0.29	2.89±0.96	4.83±0.86	5.98±1.42	8.47±2.46	9.79±3.26	11.80±3.79	16.26±6.24	17.29±6.38

Skin blood flow is expressed in the format, mean ± standard deviation

Table 5-2. Mauchly's test of sphericity of skin blood flow and five characteristic frequency bands for measurements taken once per week for three consecutive weeks.

	Baseline	Heating	Ratio	Metabolic	Neurogenic	Myogenic	Respiratory	Cardiac
Mauchly's W	0.653	0.828	0.653	0.935	0.964	0.865	0.849	0.792
Significance	0.181	0.470	0.182	0.764	0.863	0.560	0.521	0.393

Table 5-3. One-way ANOVA with repeated measures of baseline blood flow and five characteristic frequency bands in skin blood flow measurements taken once per week for three consecutive weeks.

	Baseline	Heating	Ratio	Metabolic	Neurogenic	Myogenic	Respiratory	Cardiac
Sum of Squares	0.269	77.011	113.424	0.054	0.040	0.002	0.089	0.004
Mean Square	0.135	38.505	56.712	0.027	0.020	0.001	0.045	0.002
Significance	0.828	0.316	0.456	0.432	0.198	0.949	0.268	0.588

Table 5-4. Comparisons of short-term variation of mean baseline blood flow measurements taken once per week for three consecutive weeks: two-minute averaging period.

	First Week					Second Week					Third Week				
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
S01	4.81	4.73	4.78	4.59	4.41	1.76	1.72	1.65	1.73	1.77	2.45	2.32	2.29	2.18	1.97
S02	1.08	1.11	1.13	1.05	1.00	0.96	0.95	0.89	0.85	0.85	1.44	1.55	1.62	1.71	1.67
S03	2.55	2.87	2.92	3.00	2.83	2.78	2.57	2.73	2.78	2.55	2.09	2.09	2.09	1.98	2.12
S04	2.92	2.88	3.02	2.77	3.50	3.91	3.73	3.28	4.52	4.43	3.94	3.87	3.88	3.69	3.71
S05	1.62	1.85	1.75	1.70	1.79	1.65	1.80	1.94	2.83	4.39	1.15	1.15	1.12	1.36	1.11
S06	1.68	1.65	1.70	1.71	1.71	3.97	3.65	3.74	6.13	5.90	2.46	2.38	2.34	2.49	2.57
S07	1.20	1.25	1.14	1.14	1.12	1.76	1.63	1.70	1.64	1.58	2.39	2.15	2.17	2.13	2.13
S08	5.47	5.89	6.59	7.14	6.57	6.18	7.36	6.57	4.08	3.27	5.93	6.09	7.11	7.02	5.41
S09	0.99	0.93	0.94	0.90	0.89	1.31	1.30	1.30	1.23	1.23	0.72	0.87	1.16	1.06	0.96
S10	1.81	1.53	1.61	1.52	1.48	1.34	1.43	1.21	1.08	1.11	1.13	1.00	1.06	0.89	0.96

"First" indicates mean skin blood flow of the first 2-minute in the 10-minute skin blood flow signal.

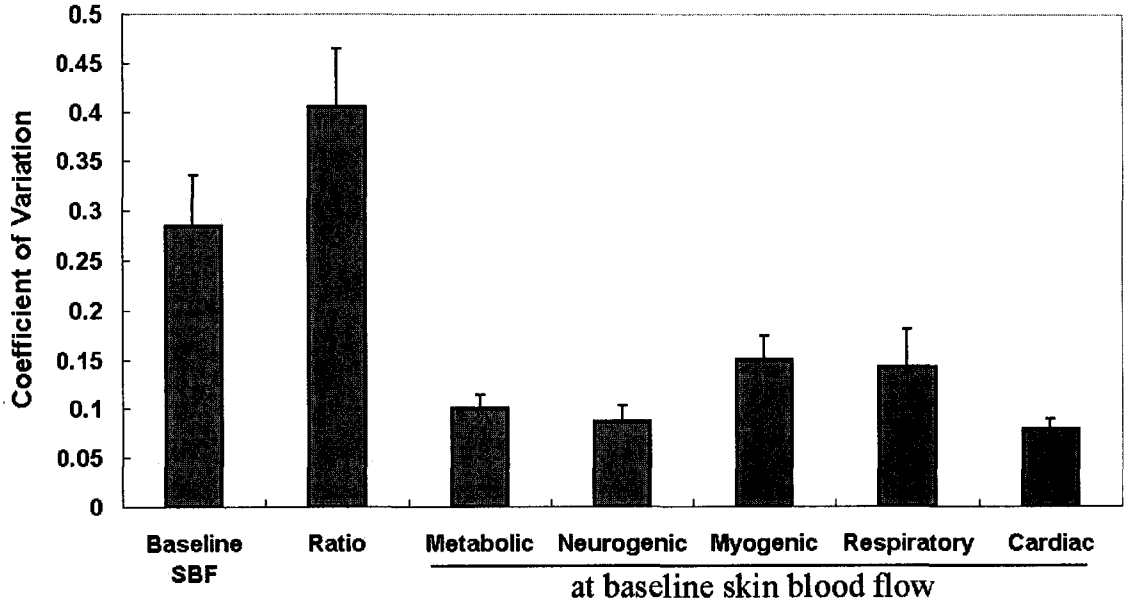

Figure 5-1. Comparisons of coefficients of variation of skin blood flow at baseline, maximal blood flow ratio method (blood flow at baseline/at 45°C) and five characteristic frequency bands isolated from baseline blood flow in three consecutive weeks (values are mean ± S.E.).

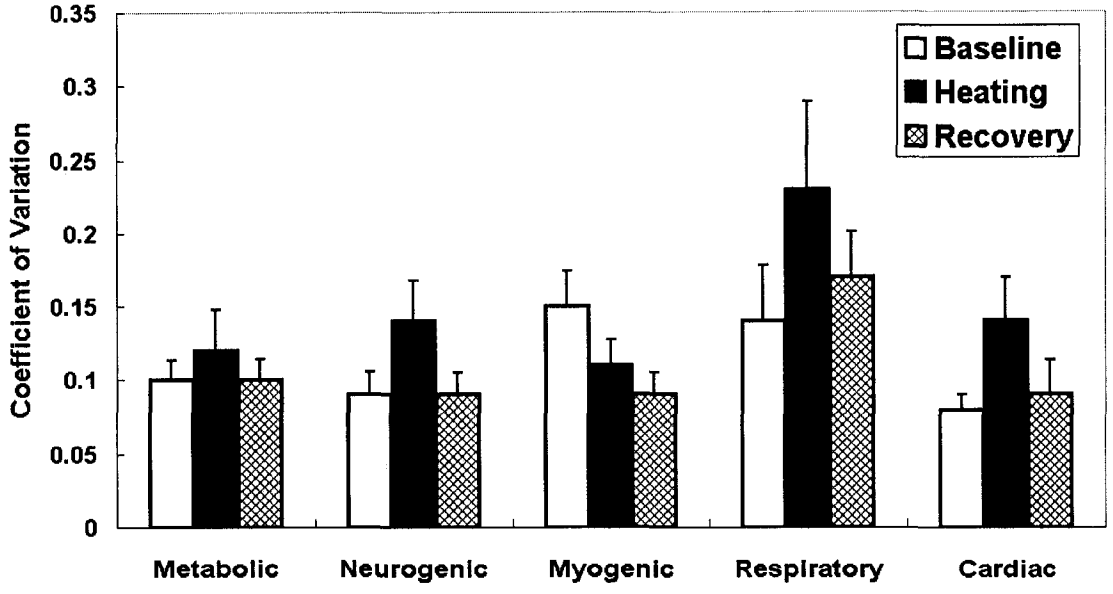

Figure 5-2. Comparisons of coefficients of variation of five characteristic frequency bands during pre-heating, heating and post-heating periods in three consecutive weeks (values are mean ± S.E.).

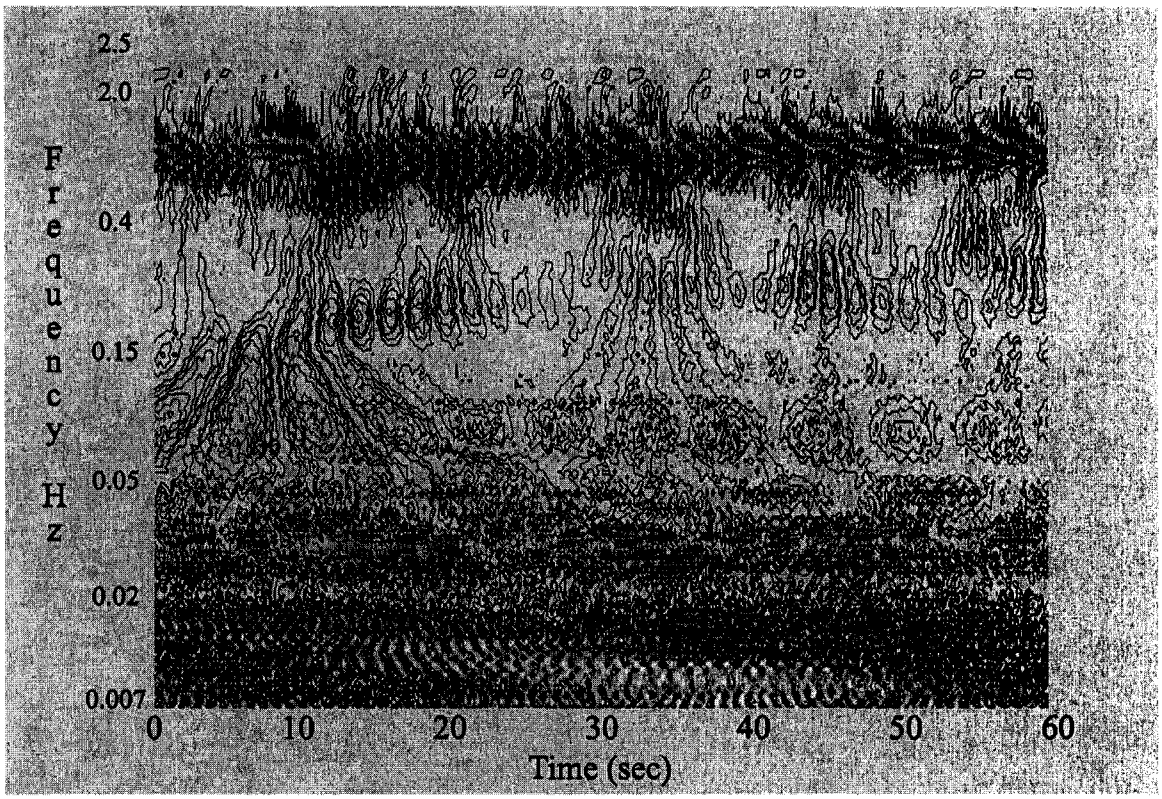

Figure 5-3. A contour plot of wavelet coefficients showing time information of five characteristic frequencies under 45°C heating.

6.0 EFFECTS OF ALTERNATING PRESSURE AND CONSTANT LOADING ON THE OSCILLATORY COMPONENTS OF SKIN BLOOD FLOW

Abstract – Prevention of pressure ulcers and enhanced tissue integrity may be possible using alternating pressure (AP) support surfaces. AP appears to stimulate a protective increase in skin blood flow (SBF), but the mechanism by which this occurs is not well understood. Ten healthy participants were subjected to both constant loading for 20 min at 30 mmHg and alternating pressure for 20 min (5 min cycle × 4) at either 60 mmHg or 3 mmHg on their sacrum. Wavelet analysis was used to decompose the blood flow signal. The power spectrum was divided into five ranges associated with metabolic, neurogenic, myogenic, respiratory and cardiac control mechanisms. The results indicate AP stimulates an increase in SBF compared to constant loading ($p<0.01$). SBF during the high-pressure phase of four AP cycles shows an increasing trend. An increase in power in metabolic frequency range and a decrease in power in the myogenic frequency range during AP was observed compared to SBF prior to loading. Power increased in the myogenic frequency range during the low-pressure phase of AP and decreased during the high-pressure phase. Our study suggests that optimization of AP amplitude, frequency and offset parameters to compensate for impaired control mechanisms in pathological populations may be possible using wavelet analysis of blood flow oscillations.

Key Words: alternating pressure, decubitus ulcer, laser Doppler flowmetry, skin blood flow, wavelet transform.

Abbreviations: AP = Alternating Pressure; BPM = Blood Perfusion Monitor; LDF = Laser Doppler Flowmetry; SBF = Skin Blood Flow; RCT = Randomized Control Trial.

6.1 Introduction

When pressure exceeds capillary blood pressure for a sufficient time, pressure ulcers develop due to insufficient blood supply and removal of metabolites. Because even support surfaces with the best pressure distribution characteristics do not necessarily maintain interface pressure below capillary blood pressure, a support surface providing periodic pressure relief has been suggested as the best strategy to prevent pressure ulcers (Gardner, 1948; Gardner and Anderson, 1948; Kosiak et al., 1958). The first commercial alternating pressure (AP) device (a small cell ripple bed) was introduced in the 1960s in the United Kingdom (Bliss et al., 1967). Since that time, there have been numerous efforts to develop AP support surfaces. Recent clinical trials on AP devices have had inconsistent results regarding their ability to prevent pressure ulcers (Andersen et al., 1982; Exton-Smith et al., 1982; Stapleton, 1986; Conine et al., 1990; Bliss, 1995; Aronovitch et al., 1999; Russell and Lichtenstein, 2000). Better evidence to support their use as a preventive measure against developing pressure ulcers is needed before third party payers such as the Centers for Medicare and Medicaid Services (CMS) will reimburse their clients for the use of AP devices in this way. In current practice, AP devices are only used in the treatment of stage III or IV pressure ulcers (Baeke, 2000). The inconsistent results seen in recent randomized control trials (RCT) on AP devices could be due to variability in the configuration (e.g. air cell size) and operating parameters (e.g. frequency, amplitude and offset) used in current technology (McLeod, 1997; Rithalia and Gonsalkorale, 2000). A clearer understanding of the effects of configuration and operating parameters on the microcirculation in at-risk tissues is needed before significant progress can be made toward providing improved AP devices.

Previous research has not clearly established the efficacy of intermittent relief or provided explanations of relevant physiological compensatory mechanisms (Bader, 1990; Mayrovitz et al., 1993; Mayrovitz and Smith, 1999; Rithalia and Gonsalkorale, 2000). Bader stated that AP stimulates an increase in oxygen levels in healthy subjects, but not in individuals with MS or SCI (Bader, 1990). Mayrovitz concluded that AP stimulates an increase in SBF in at-risk subjects (e.g. elderly women), but not in healthy subjects (Mayrovitz et al., 1993; Mayrovitz and Smith, 1999). The contradiction between these findings regarding the effect in healthy individuals may be due in part to differences in loading methods. Bader used a rigid indenter and Mayrovitz employed a pneumatic pressure applicator. There is some evidence that pneumatic devices result in unpredictable loading pressures acting on the soft tissues (Krouskop et al., 1986).

Loading pressure is the primary factor determining SBF responses (Sumpio, 1993; Guyton and Hall, 1996; Schubert and Mulvany, 1999; Levick, 2000). The application of mechanical stress on the skin (pressure) alters the stress and strain within the microvascular system, and causes changes in SBF. Endothelial cells and vascular smooth muscle cells sense mechanical changes in their environment, and respond by modulating contractions of the blood vessels to counteract the effect of pressure stimulus. Endothelial cells sense changes in shear stress and regulate the release of nitric oxide to ultimately minimize the shear stress. Vascular smooth muscle cells respond to changes in wall tension, which depends on transmural pressure, and change contraction and relaxation rhythms (Nichols and O'Rourke, 1998; Arnal et al., 1999; Levick, 2000; Michiels, 2003). The application of particular patterns of externally applied pressure may

modulate the local tissue perfusion (Johnson, 1981; Meininger and Davis, 1992; McLeod, 1997; Mayrovitz and Smith, 1999).

Much of the research discussed above points to the potential of externally applied mechanical stress for enhancing SBF under weight-bearing soft tissue. However, more information is required concerning the interrelated control mechanisms before this theory can be fully utilized in practice. Mechanical responses of skin to externally applied pressure are mainly mediated by local control mechanisms such as the myogenic control mechanism. Neurogenic control mechanisms are responsible for whole body regulation (Guyton and Hall, 1996). Modulating myogenic responses may overcome impaired vasodilation in response to mechanical stress in individuals with endothelial dysfunction (i.e. elderly or smokers) (Barua et al., 2001; Minson et al., 2002). Prevention of pressure ulcers in individuals with SCI may be possible by stimulating metabolic or myogenic control mechanisms.

In our previous research we demonstrated increase in SBF under loading and post-loading, and postulated that a myogenic response is the underlying mechanism responsible for these increases (see Chapter 4). This finding was made possible by the use of a relatively new methodology for simultaneously monitoring metabolic and myogenic responses to mechanical stress during external loading. This method allows studying interactions of different SBF control mechanisms, and may greatly advance the understanding of the etiology of pressure ulcers.

This is the third in a series of studies aiming to study mechanisms associated with AP (see Chapter 3 and 4). Our long-term goal is to identify optimal operating parameters and configurations for AP devices to enhance SBF in individuals with SCI and other

conditions that increase their risk of developing pressure ulcers. This series of studies compares SBF and frequency band separated responses to various loading patterns in healthy subjects. Improved understanding of blood flow control mechanisms' responses to mechanical stress should provide insight into the possible methods for optimizing AP parameters to enhance SBF for various pathological patients. The specific hypothesis of this study is that the applications of AP stimulate myogenic responses as measured from wavelet analysis of blood flow oscillation, thereby enhancing SBF.

6.2 Theoretical Basis of the Hypothesis

Blood flow oscillations are attributed to metabolic, neurogenic, myogenic, respiratory and cardiac factors (Salerud et al., 1983; Kastrup et al., 1989; Kvernmo et al., 1999; Stefanovska and Bracic, 1999; Soderstrom et al., 2003). Control mechanisms responding to stimuli alter the blood flow oscillation patterns, thereby modulating blood flow to meet organs' needs. We hypothesize that the application of AP stimulates myogenic responses and that, in turn, enhances SBF. Our experimental test of this hypothesis involves analyzing changes in the power spectrum of the laser Doppler SBF signal.

Regulation of blood flow can be achieved by modulating either pressure gradient or flow resistance. The relationship between these factors is

$$\text{Blood flow} = \frac{\text{Pressure gradient along a blood vessel}}{\text{Flow resistance}} = \frac{\Delta P}{R} \quad \text{(Equation 6-1)}$$

In most cases, the cardiovascular system regulates blood flow through widening and narrowing of arterioles (e.g. vasomotion), thus modulating flow resistance, R, and altering blood flow.

A typical mean arterial pressure is about 90 mmHg. Central venous pressure is close to 0 mmHg; thus a typical pressure gradient across the entire cardiovascular system is about 90 mmHg. The total cardiac output of cardiovascular system can be expressed as

$$\text{Cardiac Output} = \frac{\text{Mean arterial pressure}}{\text{Total peripheral resistance}} \qquad \text{(Equation 6-2)}$$

For the skin, blood flow = Mean local arteriolar pressure/local flow resistance. Local flow resistance is dependent on the vessel radius, vessel length, blood viscosity, and manner of flow according to Poiseuille's law (Nichols and O'Rourke, 1998; Germann and Stanfield, 2002):

$$R = \frac{8L\mu}{\pi r^4} \qquad \text{(Equation 6-3)}$$

where L is the length of the vessel, μ is the viscosity of the blood flow, and r is the internal radius of the vessel.

To illustrate the effect of oscillations in the radius on the magnitude of blood flow, consider two blood vessels with the same average radius (r_0), one with constant radius, r_1, and the other time varying, $r_2 = r(t) = r_0(1 + \lambda \sin \omega t)$ where $0.3 < \lambda < 0.8$ (Bertuglia et al., 1996; Ursino et al., 1996). Assume that the length of the vessel and viscosity of the fluid are the same in the two blood vessels. Blood flow in the vessel with constant radius, F_1, can be expressed as

$$F_1 = \frac{\Delta P}{R} = \frac{\Delta P}{\frac{8\mu L}{\pi r_1^4}} = \frac{\Delta P \cdot \pi r_1^4}{8\mu L} \qquad \text{(Equation 6-4)}$$

where ΔP is pressure drop over the length L of a cylindrical blood vessel with radius r_1 (where $r_1 = r_0$.), and μ is the viscosity of the fluid.

Blood flow in the vessel with the time oscillating radius, F_2, can be expressed as

$$F_2 = \frac{\int_0^T F(t)dt}{T} = \frac{\omega}{2\pi}\int_0^{2\pi/\omega} F(t)dt$$

$$= \frac{\omega}{2\pi}\int_0^{2\pi/\omega} \frac{\Delta P \cdot \pi \cdot r_0^4 \cdot (1+\lambda \sin \omega t)^4}{8\mu L} dt$$

$$= \frac{\omega \cdot \Delta P \cdot \pi \cdot r_0^4}{16\pi \cdot \mu \cdot L}\int_0^{\frac{2\pi}{\omega}} (1+\lambda \sin \omega t)^4 \, dt$$

$$= \frac{\pi \cdot \Delta P}{8\mu \cdot L}(1+3\lambda^2+\frac{3}{8}\lambda^4)\cdot r_0^4 \qquad \text{(Equation 6-5)}$$

To compare F_1 and F_2, consider the ratio:

$$\frac{F_2}{F_1} = \frac{(\frac{\pi \cdot \Delta P}{8\mu \cdot L}(1+3\lambda^2+\frac{3}{8}\lambda^4)\cdot r_0^4)}{(\frac{\Delta P \cdot \pi r_1^4}{8\mu L})}$$

$$= 1+3\lambda^2+\frac{3}{8}\lambda^4 \qquad \text{(Equation 6-6)}$$

The relative increase in flow, F_2/F_1, with respect to oscillation amplitude is plotted in Figure 6-1.

6.3 Methods

6.3.1 Subjects

Ten unimpaired subjects (5 male and 5 female) were recruited into the study. The demographic data were as follows: age 30.0 ± 3.1 years, height 162.9 ± 6.8 cm, and weight 58.3 ± 8.6 kg. The following conditions constituted exclusion criteria: the presence of pressure ulcers on the sacrum, diabetes, vascular disease, hypertension, or use

of vasoactive medications. An informed consent approved by the University of Pittsburgh Institutional Review Board was obtained from each subject prior to testing.

6.3.2 Instrumentation

A computer-controlled indenter was developed to provide constant loading and alternating pressure on the sacrum of each subject. The indenter was mounted on a stand that allowed for adjustment with 5 degrees of freedom for the orientation and location of the indenter so that the flat end of the indenter head approaches normal to the skin surface at the desired location on the sacrum. A stepper-motor (Superior Electric, CT) driven lead screw actuator and two compression springs (Lee Spring, NY; spring constant ~ 385N/m) were used to control the force and, therefore, the interface pressure on the skin. The motor drive control is accomplished using an ISA bus stepper motor control board (Brienza et al., 1996). The springs served to minimize the adverse effects of any disturbances caused by motion of the subject. A strain gauge, cantilever beam force transducer (Kwiatkowski and Inigo, 1993) was used to monitor force and generate feedback for closed-loop force control. The main control program is written in LabVIEW 5.0 (National Instrument, TX) (see Chapter 2).

The indenter head contains the laser Doppler blood perfusion monitor, thermometer, and heating probes. The radius on the edge of the cylindrical head was optimized to reduce stress concentrations based on a finite element model (see Chapter 2).

Laserflo Blood Perfusion Monitor 2 (BPM^2) and the Softip pencil probe (P-435) (Vasamedics, MN) were used to measure capillary blood flow (ml_{LD}/min/100g tissue).

BPM^2 provides noninvasive measurement of skin blood flow at a depth of about 1 mm via laser and fiber optics technology. A low power beam (2 mW) of Helium-Neon laser light (780 nm wavelength) is delivered to the sample tissue. The 0-5 volt analog output of the Laserflo was sampled at 20 Hz using a 16-bit data acquisition card (PCI-MIO-16XE, National Instruments, TX).

A five-channel scanning thermistor thermometer (U-08502-14) and two thermistor probes (U-08443-20, Cole-Parmer Instrument Company, IL) were used to measure skin temperature changes under the indenter. The system provided 0.056°C temperature measurement resolution within a range of -30.0 to 100.0 °C. The analog output (18 mV per °C) was sampled at 20 Hz using the 16-bit data acquisition card.

6.3.3 Protocols

All tests were performed in the Soft Tissue Mechanics Laboratory at University of Pittsburgh in the School of Health and Rehabilitation Sciences. Room temperature was maintained at 24 ± 1 °C. Subjects were kept relaxed for at least 30 minutes prior to testing in order to achieve a stable SBF and to allow them to acclimate to the room temperature. During the resting period, subjects were asked to empty their bladder. A custom contoured support surface (Versa Form, Sammons Preston, IL) was provided to minimize interface pressure during all tests to protect compressed tissue from damage and to prevent discomfort (see Chapter 2).

A crossover design was used in which the order of treatment, constant or alternating pressure on the sacrum, was randomly assigned (Portney and Watkins, 2000). The specific parameters of the loading regimens proposed for this study (duration of

loading, 20 minutes; magnitude of low and high pressure in the AP cycle, 3 and 60 mmHg; and cycle time of AP, 5 minutes) were chosen based either on reports of augmented blood flow in response to either constant (20 minutes to several hours) pressure, low-level (30-70 mmHg) pressure, or AP delivered via existing commercial support surfaces (5 minute inflation-deflation cycle) (Frantz and Xakellis, 1989; Xakellis and Frantz, 1990; Xakellis et al., 1993; Medical Devices Agency, 1995; Medical Devices Agency, 1997; Sanada et al., 1997; Mayrovitz and Smith, 1999; Patel et al., 1999). Since non-zero low pressure after pressure loading has been shown to blunt reactive hyperemia (Mayrovitz and Sims, 2002; Mayrovitz et al., 2003) and the laser Doppler probe requires that it be held in contact with the skin, the low pressure in the AP cycle was chosen to be 3 mmHg. Average pressure was chosen at 30 mmHg; thus constant loading was set at 30 mmHg. The pressure during the high-pressure phase of AP was 60 mmHg to achieve an average of 30 mmHg.

Subjects underwent the following loading regimens in randomized order: 1) an AP regimen consisting of 20 minutes (5 minute cycle x 4) with the loading pressure applied alternately at either 60 mmHg or 3 mmHg and 2) a constant pressure regimen consisting of a 20 minute loading period at 30 mmHg. SBF was also recorded for at least 10 minutes before and after loading in order to monitor baseline (pre-loading) and recovery (post-loading) responses (Figure 6-2). A washout period of 40 minutes between regimens was used.

6.3.4 Data analysis

Wavelet analysis provides a multi-resolution, time-frequency analysis of sacral skin blood flow. Wavelet transform decomposes a signal over dilated and translated wavelets (Strang and Nguyen, 1997). Continuous wavelet transform of a signal *f(u)* was defined as (Grossmann and Morlet, 1984):

$$\hat{f}(s,t) = \int_{-\infty}^{\infty} \psi_{s,t}(u) f(u) du \qquad \text{(Equation 6-7)}$$

where $\hat{f}(s,t)$ is a wavelet coefficient and $\psi_{s,t}(u)$ is a wavelet function and was defined as

$$\psi_{s,t}(u) = \frac{1}{\sqrt{s}} \psi\left(\frac{u-t}{s}\right) \qquad \text{(Equation 6-8)}$$

A family of time-frequency wavelets is obtained by scaling function ψ by parameter s (scale factor) and translating it by t (time factor). Continuous wavelet transform is easier to interpret data or recognize patterns because its complete scales tend to reinforce the traits and make all information more visible than using discrete wavelet transform (Hubbard, 1996). Thus continuous wavelet transform was used in this study to perform time-frequency analysis of skin blood flow.

The Morlet wavelet model was used to perform wavelet transform analysis. Morlet wavelet is a Gaussian function defined as:

$$\psi_{s,t}(u) = \frac{1}{\sqrt[4]{\pi}} \cdot \left(e^{-i\omega_0 u} - e^{-\omega_0^2/2}\right) \cdot e^{-u^2/2} \qquad \text{(Equation 6-9)}$$

whereas ω_0 was chosen as 2π. Therefore, a simple relationship between frequency and scale could be expressed as $frequency = \frac{1}{scale}$ (Bracic and Stefanovska, 1998). Morlet

wavelet, a Gaussian function, allows the best time-frequency localization according to Heisenberg uncertainty principle (Meste et al., 1994; Bracic and Stefanovska, 1998)(see Chapter 5).

In order to quantify the amplitude of power within the characteristic frequency bands, the average amplitude of each frequency band was calculated using the following equation:

$$A_i(f_{i1}, f_{i2}) = \frac{1}{t}\int_0^t \frac{1}{f_{i2} - f_{i1}} \int_{1/2\pi f_{i2}}^{1/2\pi f_{i1}} \frac{1}{s^2} \hat{f}(s,t)\,ds\,dt \quad \text{(Equation 6-10)}$$

where f_{i1} and f_{i2} are the limits of a given frequency band; e.g., myogenic characteristic frequency band.

In order to permit comparisons of the subjects' power distributions, the relative contribution of each frequency band (myogenic, neurogenic, etc.) was used in this study and was defined as ratio of the average amplitude of total frequency range (0.008-2.0 Hz) for each condition; i.e., pre-loading, loading, post-loading periods:

$$a_i(f_{i1}, f_{i2}) = \frac{A_i(f_{i1}, f_{i2})}{A_{total}} \quad \text{(Equation 6-11)}$$

Since physiological signals in the skin blood flow rarely have frequencies higher than 2 Hz (Stefanovska et al., 1999), the specified range of frequencies for the wavelet analysis was established at 0.007 to 2.5 Hz. The limits of each frequency band were chosen base on the ranges previously reported by Stefanovska and Bracic (Stefanovska and Bracic, 1999) and our research data (see Chapter 3 and 4).

Paired t-tests were used to compare mean blood flow and power for constant loading and AP in each characteristic frequency band and during pre-loading, loading and post-loading periods. Linear and quadratic trend analysis were used to study changes

within characteristic frequency bands with time during loading (Portney and Watkins, 2000). All statistical tests will be performed at an alpha level of 0.05. All data were analyzed by using SPSS 10.1.

6.4 Results

Three responses to constant loading and alternating pressure were analyzed: skin temperature, mean skin blood flow and power within three characteristic frequency bands.

6.4.1 Skin temperature

Mean skin temperature during alternating pressure and constant loading is shown in Figure 6-3. Relative changes of mean skin temperature during alternating pressure and constant loading is shown in Figure 6-4. Skin temperature was not shown to be significantly different during pre-loading, constant loading, alternating pressure and post-loading periods ($p>0.05$) (Figure 6-5). Mean skin temperature for all subjects increased by 1.04°C from constant loading to post-loading and 1.35 °C from AP to post-loading.

6.4.2 Mean skin blood flow

Mean skin blood flow was greater during alternating pressure compared to constant pressure ($p<0.01$) (Figure 6-6). During the high-pressure phase of AP (60 mmHg), skin blood flow was similar in value to the SBF recorded during constant pressure (30 mmHg) ($p>0.05$) (Figure 6-7). The ratio of SBF during loading to the mean value during pre-loading increased for AP loading and remained relatively flat for

constant loading. SBF during high- and low-pressure phases of the AP cycles were generally higher than the value during constant pressure ($p<0.05$) (Figure 6-8). The SBF was higher during post-loading for AP ($p=0.01$) (Figure 6-8).

6.4.3 Characteristic frequency bands

No statistically significant differences were found between constant loading and AP when the SBF data was analyzed in the individual metabolic, neurogenic and myogenic frequency bands for the post-loading period ($p>0.05$). However, SBF in the myogenic frequency band was higher after AP compared to constant loading (Figure 6-9).

Trend analysis demonstrated a quadratic relationship between power in the metabolic frequency band and time during constant loading ($p<0.05$). During constant loading, SBF signal power in the metabolic frequency band decreases for the first 10 minutes then remains relatively flat for the final 10 minutes (Figure 6-10).

A linear relationship between power in the neurogenic frequency band and time during constant loading was observed ($p<0.05$). SBF signal power in the neurogenic frequency band tends to increase with time during constant pressure (Figure 6-11).

Trend analysis demonstrated a quadratic relationship between power in the myogenic frequency band and time during constant loading ($p<0.05$). In the myogenic frequency band, SBF signal power increases after 5 minutes of constant loading, peaks at the 10 minutes, then decreases during constant loading (Figure 6-12).

Trend analysis demonstrated a quadratic relationship between power in the metabolic frequency band and time during AP ($p<0.05$). During AP, the SBF signal

power in the myogenic frequency band is higher than during the pre-loading period (Figure 6-10). The more dominant role of metabolic control was shown under AP rather than under constant loading.

No trends were found in the neurogenic frequency band during AP ($p<0.05$). A generally increasing trend in SBF signal power in the neurogenic frequency band was observed during AP (Figure 6-11). Neurogenic control appears to be more dominant during AP compared to constant pressure.

Trend analysis demonstrated a quadratic relationship between power in the myogenic frequency band and time during AP ($p<0.05$). The power of the SBF signal in the myogenic frequency band is lower compared to the pre-loading period (Figure 6-12). There was a decrease in SBF signal power in the myogenic frequency band during AP compared to constant pressure.

6.5 Discussion

Constant loading

During constant pressure, SBF signal power in the metabolic frequency band decreases for the first 10 minutes then remains relatively flat for the final 10 minutes. Although the metabolic frequency band (0.008-0.02 Hz) in the SBF has been shown to be endothelium dependent, the exact vasodilator responsible for the response is not known (Kvernmo et al., 1998b). We speculated that nitric oxide is potentially related to responses in this frequency band. Release of nitric oxide is mediated by shear stress on the vessel wall that is dependent on the blood flow according to Poiseuille's law (Sumpio, 1993; Arnal et al., 1999; Michiels, 2003). Our data showed that SBF during 30-

mmHg of constant pressure is decreased thereby theoretically decreasing the release of nitric oxide. This is consistent with changes of power in the metabolic frequency band shown in our data. We also showed increases in power in the metabolic frequency band during after removal of constant pressure in the post-loading period, which further supported the assertion that responses in this frequency band are flow dependent. Accordingly, the flow dependent vasodilator in the microcirculation is considered as endothelial nitric oxide. Thus, supporting this frequency band as a metabolic related control mechanism (i.e. endothelial nitric oxide). Further evidence is that the power in the 0.008-0.02 Hz frequency band decreases in the first 10 minutes of constant pressure then remains constant in the following 10 minutes. This is consistent with metabolism of nitric oxide by local cells (El-Farra et al., 2003).

The observation that the power in the metabolic frequency band reaches minimum and then stays constant is consistent with the SBF reaching the no blood flow status (biological zero) completely inhibiting the release of nitric oxide (see Chapter 4). This finding suggests the loading time of AP support surface should be kept less than 10 minutes in order to maintain the beneficial vasodilation effect of nitric oxide on the compressed skin.

In our previous research, the increase in SBF occurring within 35-60 mmHg of applied pressure under incremental loading (0 to 60 mmHg at a 5 mmHg step/3 min) is due to a myogenic response (see Chapter 4). In this study, constant loading at 30 mmHg for 20 minutes does not elicit an increase in SBF, although an increasing trend of a higher myogenic response was observed. Increase in SBF within 35-60 mmHg under incremental pressure loading (0-60 mmHg) while decrease under 30-mmHg constant

loading. This finding suggests protective vasodilation is more related to the loading pattern rather then on the absolute value of loading pressure. Myogenic response is a rate dependent response (Schubert and Mulvany, 1999), thus myogenic response may not be triggered under constant loading to overcome tissue ischemia. This finding suggests constant loading is more harmful than dynamic loading pattern in the aspect of SBF.

Alternating pressure

Reactive hyperemia occurs after the release of applied pressure (Lewis and Grant, 1926). Increases in SBF during low-level pressure have also been observed (Abraham et al., 2001)(see Chapter 4). However, the benefits associated with AP cannot be explained by either phenomenon. For example, our data showed after several cycles of AP the minimum level of SBF increased even under the same high pressure (60 mmHg). Similar findings were reported by Bader and Mayrovitz (Bader, 1990; Mayrovitz and Smith, 1999). Comparing mean SBF under 20-minute constant pressure and AP clearly indicates that AP permits more SBF of the compressed tissue. Comparing SBF of 4 cycles of AP shows an increasing trend during the high pressure phase of AP, especially starting from the second cycle. The specific mechanism associated with the increase in SBF associated with AP is still not clear (Bader, 1990; Mayrovitz and Smith, 1999). However, periodic changes in transmural pressure may stretch vascular smooth muscles leading to a more synchronous oscillation pattern of adjacent vascular smooth muscles (Wilkin, 1986). An increased degree of synchronization of vasomotion may enhance blood flow output. In our previous work, incremental pressure (0 to 60 mmHg at 5 mmHg step/3 min) elicits vasodilation after 35 mmHg. This was attributed to the increased myogenic response.

While this vasodilation occurs, the amount of increased blood flow is far less than the increased SBF induced by AP reported by Mayrovitz (Mayrovitz and Smith, 1999). Our findings suggest that interactions of pressure-induced vasodilation and reactive hyperemia or other protective mechanisms may be involved.

Increased power in the metabolic frequency band and decreased power in the myogenic frequency band for AP compared to the pre-loading period were observed. Our result showing increased power in the metabolic frequency band during AP does not support our hypothesis. This unexpected result may be due to the high-pressure phase (60 mmHg) of the AP cycle completely inhibiting the myogenic responses of the sacral cutaneous microcirculation. This finding is supported by several research studies showing decreased myogenic response when perfusion pressure was decreased (e.g. increased externally applied pressure) (Osol and Halpern, 1985; Gustafsson et al., 1994; Gros et al., 2002). Although we observed a reduced contribution of myogenic control mechanisms under AP compared to pre-loading SBF, an increase in myogenic control activity is observed during the low-pressure phase of the AP cycle. This is consistent with our assertion that myogenic control is the primary factor responsible for SBF increases after removal of pressure (Engelke et al., 1996)(see Chapter 4).

Studies on isolated blood vessels have shown that myogenic responses are pressure sensitive (Sumpio, 1993; Guyton and Hall, 1996; Schubert and Mulvany, 1999; Levick, 2000). Our previous study showed that this holds true *in vivo,* on a macroscopic scale as increased myogenic control was observed in the post-loading after incremental pressure loading ($p<0.05$). In this study, myogenic responses increase after alternating pressure but not after constant loading ($p>0.05$). This finding supports Johnson's

conclusion that myogenic response is dependent on loading patterns rather than solely on the magnitude of loading pressure (Johnson, 1989). An increase in power in the myogenic frequency band in the 10 minutes post-loading suggests that the myogenic response induced by externally applied mechanical stress is not a short transient response. The lasting effect of an enhanced myogenic response could be a potential solution for modulating SBF to compensate for pathological changes.

SBF signal power in the neurogenic frequency band is relatively flat during both alternating and constant pressure when compared to the magnitude of changes seen in the metabolic and myogenic frequency bands. The purpose of neurogenic control in the cardiovascular system is to regulate whole body blood flow in response to system-wide changes. Local stimuli (i.e. local heat or locally applied pressure) elicit responses mediated by metabolic or myogenic control mechanisms (Guyton and Hall, 1996)(see Chapter 3 and 4). The flat response in the neurogenic frequency band and the larger changes seen in the metabolic and myogenic frequency bands in response to the local stimuli of heat and pressure are consistent with this understanding. Sympathetic vasoconstrictor noradrenergic fibers in the skin adjust vasomotor tone to stabilize blood pressure (Levick, 2000). The changing nature of AP results in an unstable transmural pressure of the arterioles which may, in turn, stimulate neurogenic regulation. Although these experiments do not permit the testing of this hypothesis, repeating the experiments on subjects with SCI may help to identify the source of the regulation in the neurogenic frequency band related to mechanical stress.

Our results give us some beginning guidance on the optimal operating parameters for AP support surfaces. The pressure during the low-pressure phase of the AP cycle

should be zero. The pressure level used here during the high-pressure phase (60 mmHg) is within the range of pressure induced vasodilation (Abraham et al., 2001). But, comparison of metabolic frequency under constant loading and AP in the first 2.5 minutes indicates that 60 mmHg is probably too high for eliciting myogenic responses. Thus, a pressure range between 30 to 60 mmHg should be used for the high pressure of AP. However, if the pressure is too low during the high-pressure phase it may not stimulate a significant reactive hyperemic response. The use of 3-cell or 4-cell geometric configurations for AP support surface would decrease the pressure necessary to support body weight during the high-pressure phase and yet allow for lower peak pressures in the range where myogenic responses are stimulated.

Several researchers have shown that at-risk subjects have lower occlusion pressure thresholds, impaired reactive hyperemia, and diminished maximal vasodilatory function (i.e. smokers, elderly, SCI, cardiovascular dieses) (Schubert and Fagrell, 1991; Schubert and Heraud, 1994; Barua et al., 2001; Minson et al., 2002). These impaired microcirculatory functions result in diminished responses to different stimuli. Although different pathology exists in different patients, the final common pathway is insufficient blood flow supply (Dinsdale, 1974; Daniel et al., 1981). Myogenic responses and metabolic control are inherent properties of cutaneous microcirculation and are spared in people with SCI population. Thus, using AP to stimulate an increase in SBF in SCI to enhance tissue viability and prevent pressure ulcers may be possible. For individuals with endothelial dysfunction (i.e. smokers, elderly population) the configuration of AP should focus on enhancing myogenic responses which could be monitored from wavelet analysis of blood flow oscillations.

Regulation of skin blood flow is a process involving interrelated control mechanisms and stimuli. Although manipulation of responses of metabolic and myogenic control mechanisms could be used to enhance blood flow in individuals with SCI, several factors may limit its effectiveness. Myogenic response is dependent on the transmural pressure of the blood vessel. Individuals with SCI level at T6 and above lose sympathetic control over blood vessels that result in a lower vasomotor tone (Byrne and Salzberg, 1996; Teasell et al., 2000). The consequences of a lower vasomotor tone associated with SCI are a higher baseline skin blood flow and an impaired vasodilatory function (Schubert and Fagrell, 1991; Schubert et al., 1995). Insufficient vasomotor tone may reduce the potential for significant myogenic responses to AP. Endothelial nitric oxide is dependent on shear stress determined by blood flow volume (Davies, 1995; Arnal et al., 1999; Michiels, 2003). Impaired vasodilatory function may also result in a decreased response of this flow-mediated vasodilation.

Skin blood flow is highly dependent on the skin temperature, thus monitoring of skin temperature is necessary (see Chapter 2 and 3). The temperature of the skin surface is affected by heat transfer with the environment (Barnett and Ablarde, 1995). Our data shows a steady increase in skin temperature during constant pressure ($\triangle t$=1.04°C) and AP ($\triangle t$=1.35°C). This phenomenon may be due to the heat accumulation between indenter and the sacrum. While the range of skin temperature in this study does not result in large heat-mediated vasodilation (see Chapter 3). A higher skin temperature after application of AP compared to constant loading is related to a higher skin blood flow after AP.

6.6 Conclusion

A significant finding of this study was that SBF during AP was higher compared to constant pressure over 20 minutes. Since increased SBF may enhance tissue viability, our study suggests that AP may enhance tissue viability. Our analysis of the SBF signal power in individual frequency bands thought to be associated with corresponding metabolic, neurogenic, and myogenic control mechanisms showed that these control mechanisms respond differently to constant and alternating pressure. We found the increase in SBF associated with AP cannot be explained by reactive hyperemia or pressure induced vasodilation. Our study clearly shows SBF of tissue under loading is dependent on the loading patterns of the externally applied pressure. Using this analysis may allow for the optimization of AP loading patterns for enhanced tissue integrity in individuals with impairments to specific control mechanisms.

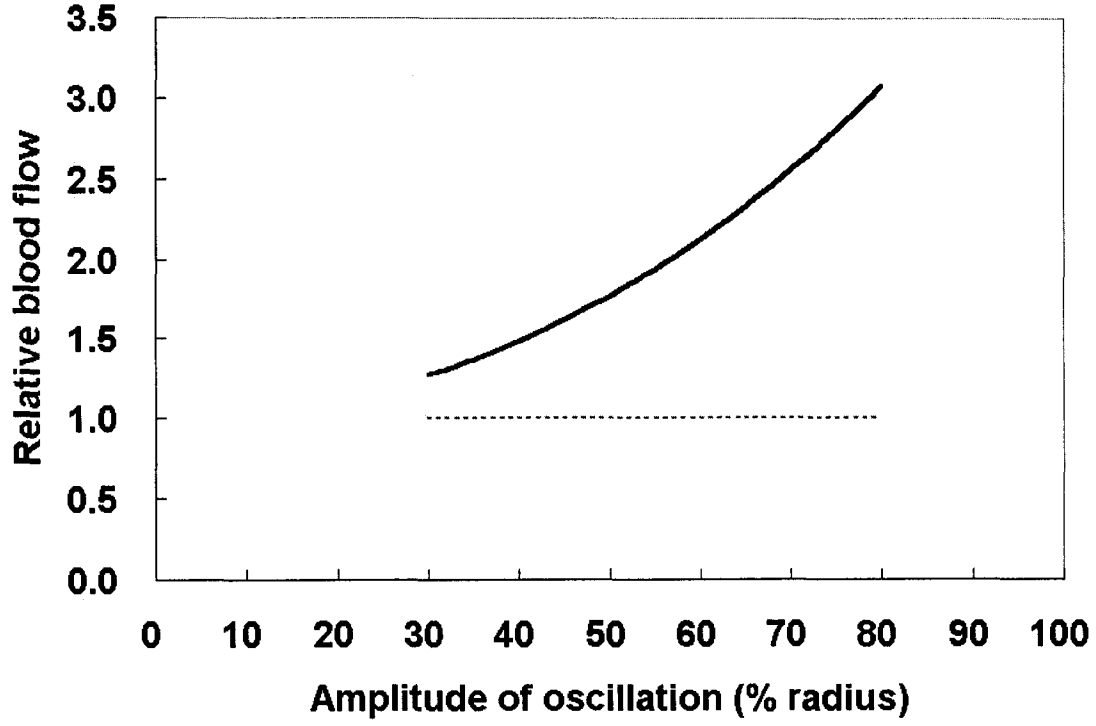

Figure 6-1. Effect of time-varying radius of a blood vessel on skin blood flow was compared to a blood vessel with constant radius at the same average radius. (amplitude of oscillation = amplitude of oscillation/average radius; relative skin blood flow = blood flow of a vessel with a time-varying radius /blood flow of a vessel with a constant radius)

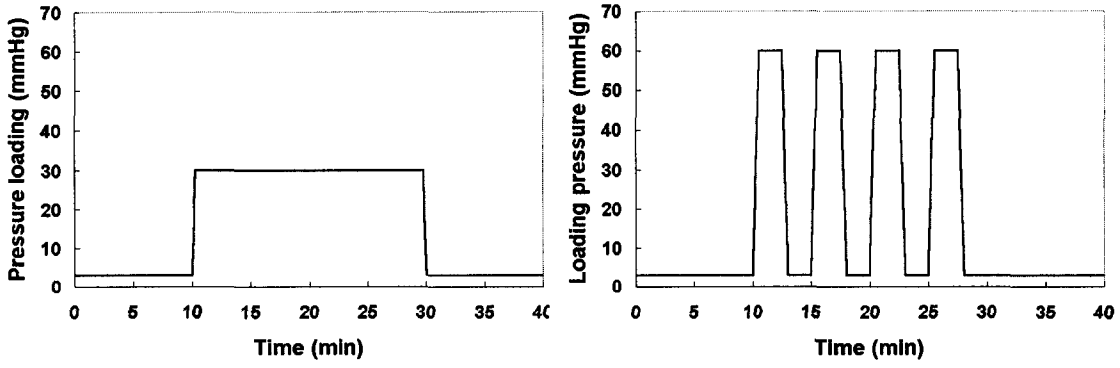

Figure 6-2. Interface pressure profiles of constant loading and alternating pressure.

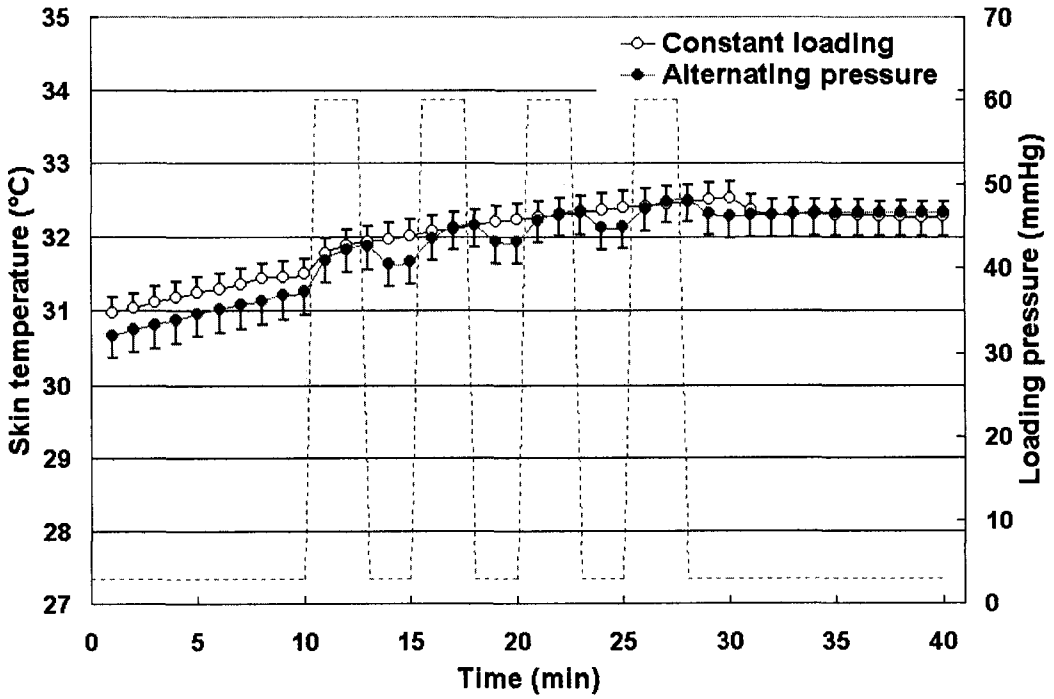

Figure 6-3. A comparison of mean skin temperature during alternating pressure and constant loading (values are mean ± S.E.).

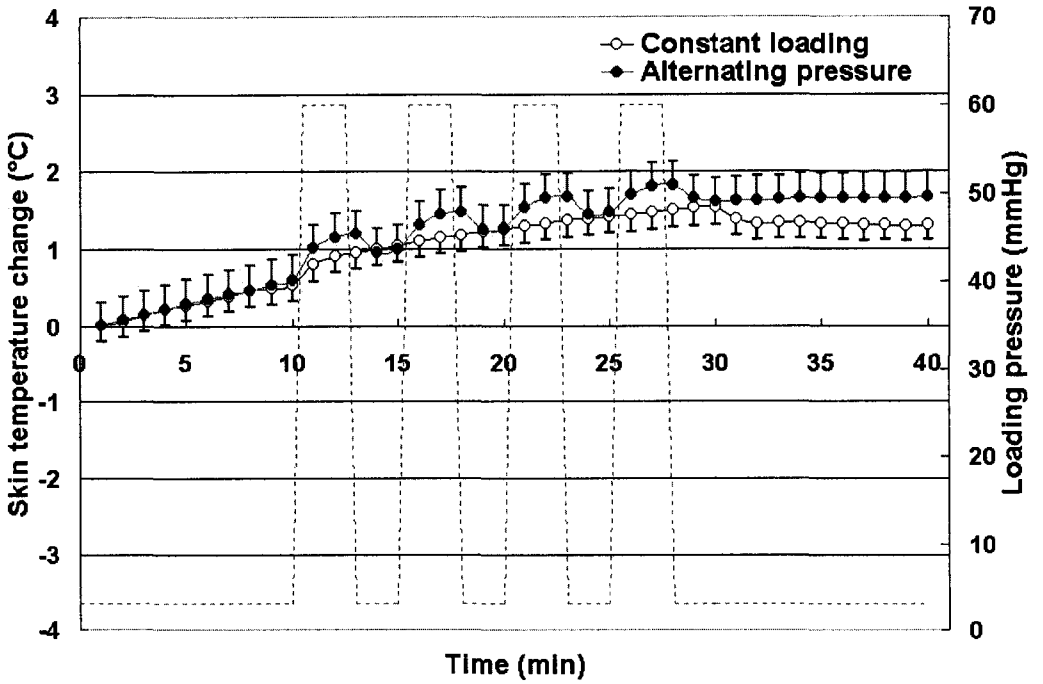

Figure 6-4. A comparison of relative changes of mean skin temperature during alternating pressure and constant loading (values are mean ± S.E.).

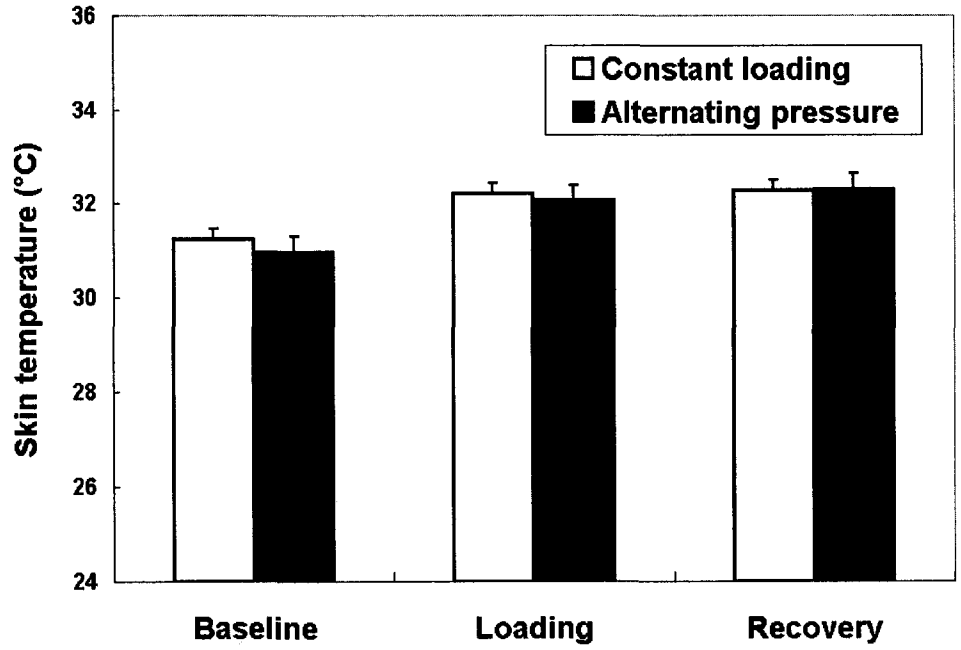

Figure 6-5. A comparison of mean sacral skin temperature during pre-loading (baseline), constant loading, alternating pressure and post-loading (recovery) periods (values are mean ± S.E.).

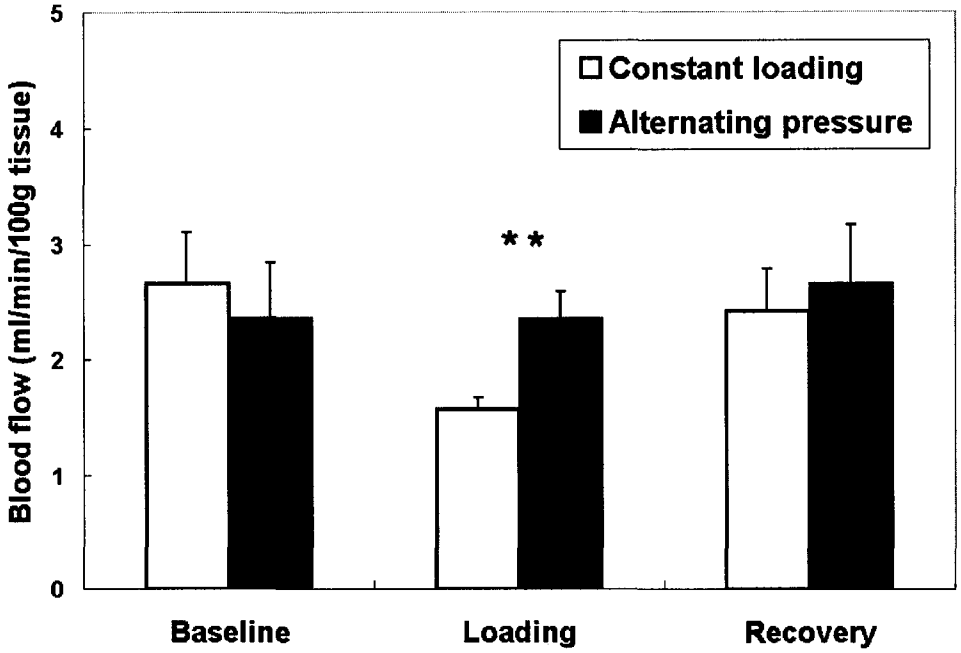

Figure 6-6. A comparison of mean skin blood flow during pre-loading (baseline), constant loading and alternating pressure and post-loading (recovery) periods (values are mean ± S.E.) (** indicates $p<0.01$).

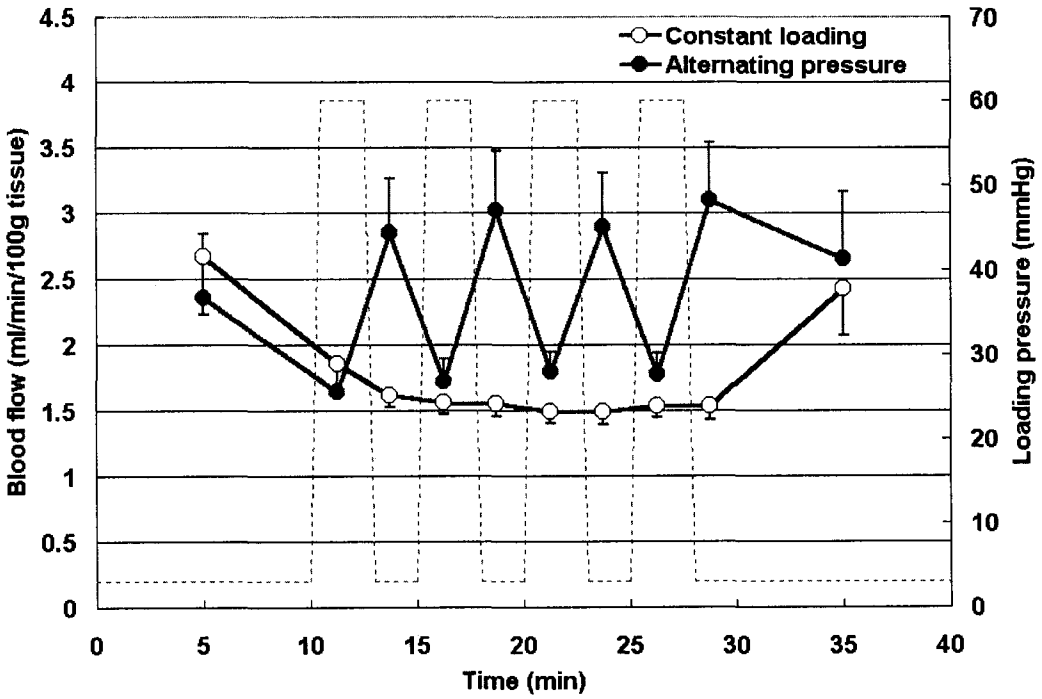

Figure 6-7. A comparison of mean skin blood flow during 4 cycles of alternating pressure and constant loading (values are mean ± S.E.).

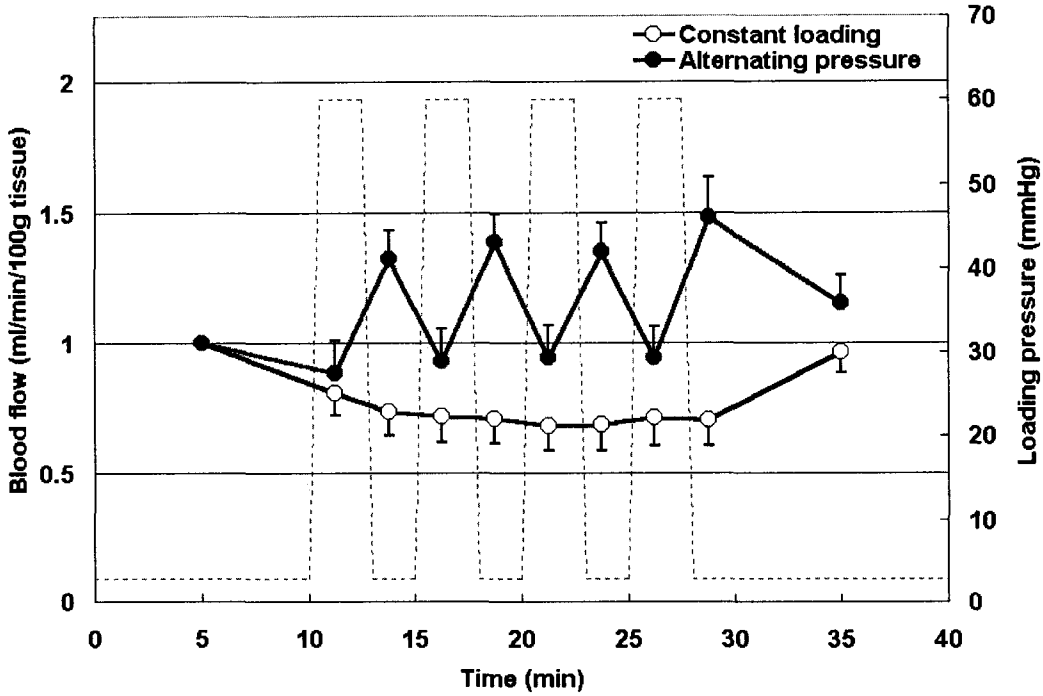

Figure 6-8. A comparison of ratio of skin blood flow during 4 cycles of alternating pressure and constant loading (values are mean ± S.E.) (* indicates $p<0.05$; ** indicates $p<0.01$).

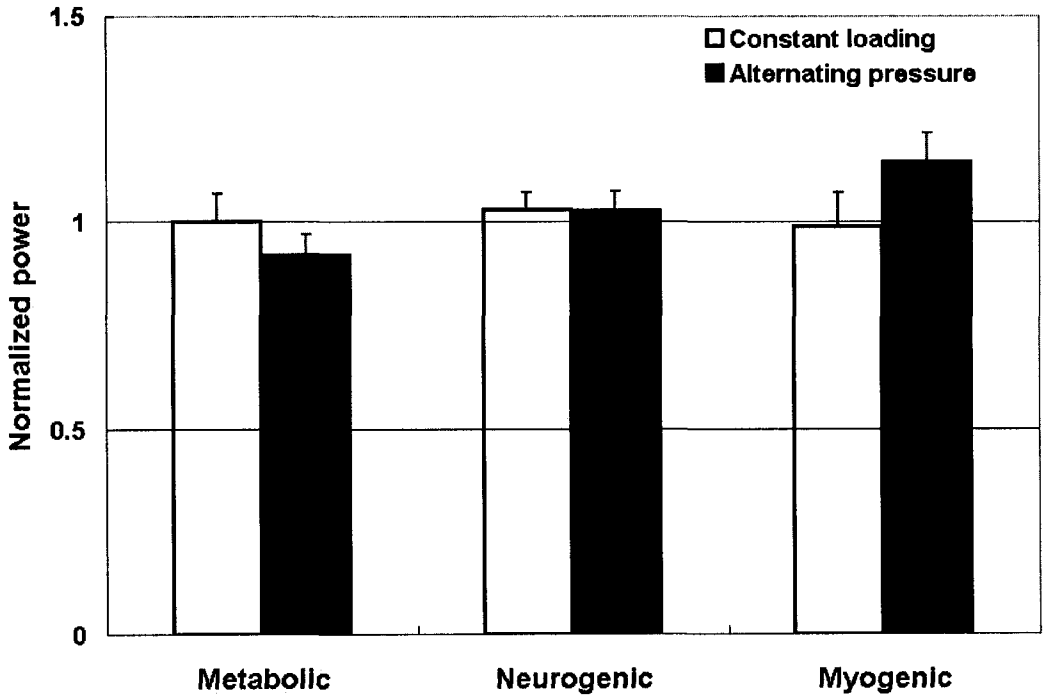

Figure 6-9. A comparison of power of 3 characteristic frequency bands during recovery period after applications of alternating pressure and constant loading.

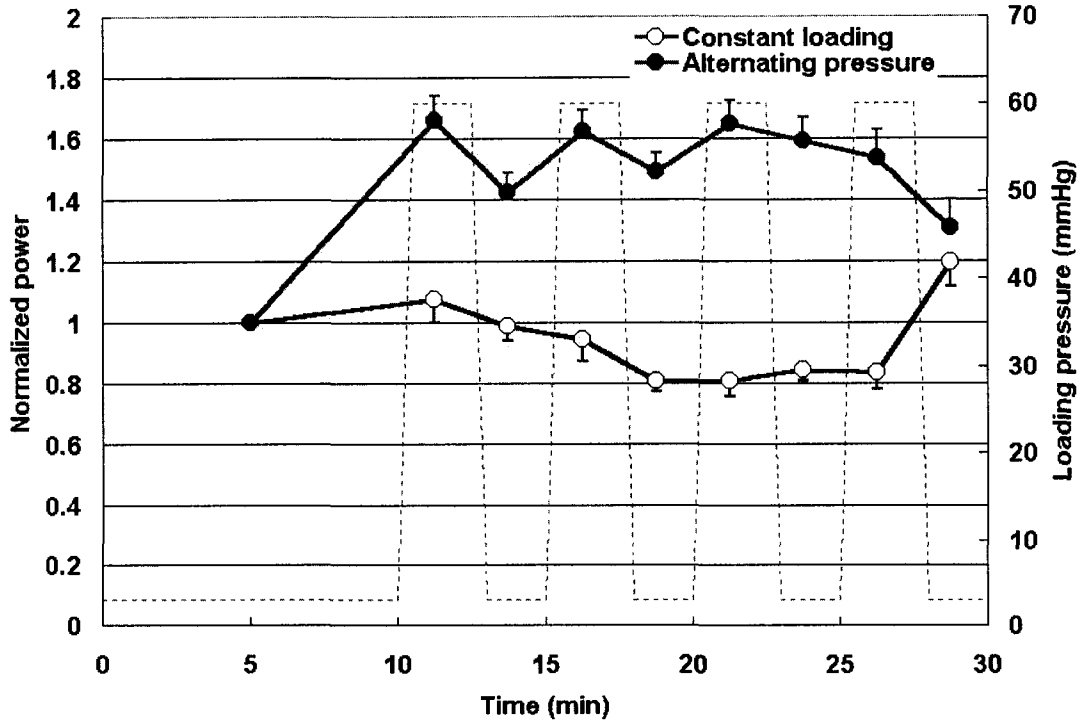

Figure 6-10. A comparison of normalized power of metabolic frequency during alternating pressure and constant loading.

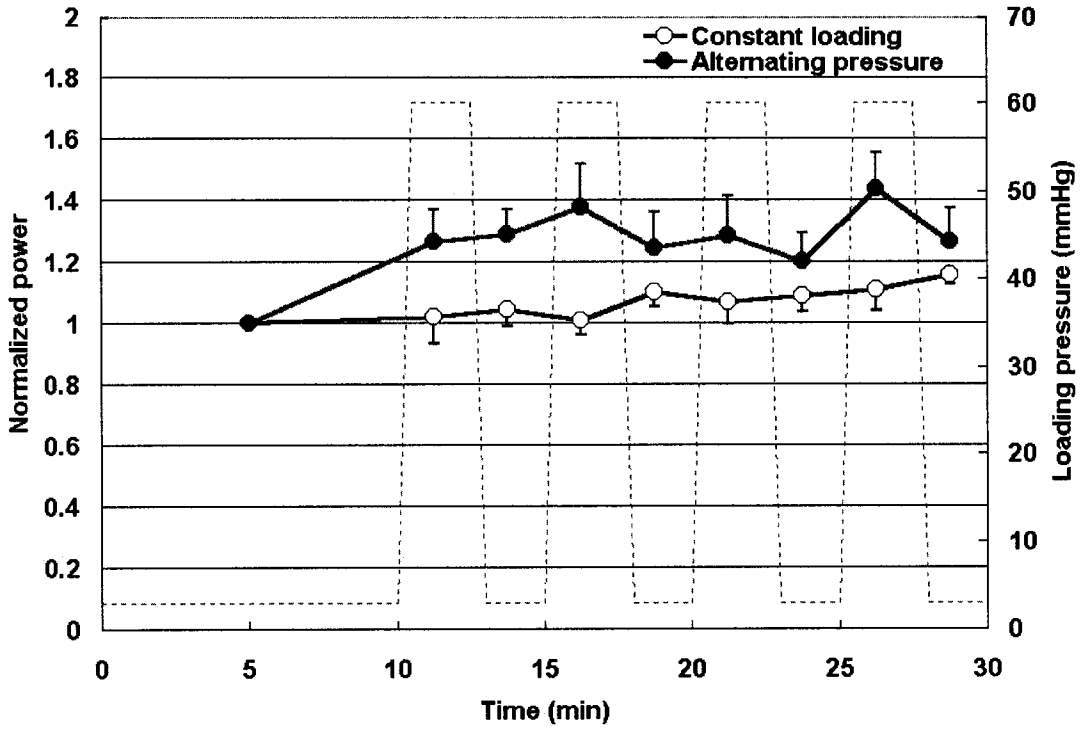

Figure 6-11. A comparison of normalized power of neurogenic frequency during alternating pressure and constant loading.

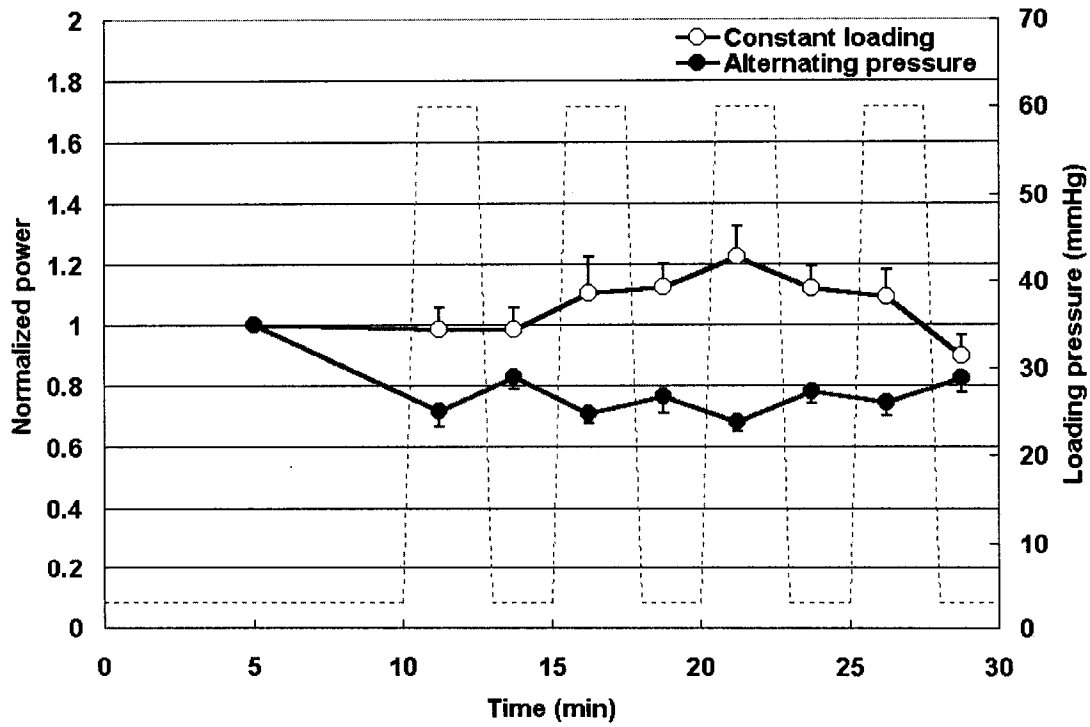

Figure 6-12. A comparison of normalized power of myogenic frequency during alternating pressure and constant loading.

7.0 SUMMARY AND RECOMMENDATIONS

7.1 Summary of Work

To best of our knowledge this is the first study using wavelet analysis to characterize blood flow control mechanisms' response to thermal stress or various loading patterns. We successfully identified that the metabolic control mechanism responds to thermal stress while myogenic response is sensitive to mechanical stress. A novel method to assess beneficial responses associated with alternating pressure has been established. We demonstrated that alternating pressure stimulates an increase in skin blood flow. Our study suggests that optimization of alternating pressure amplitude, frequency and offset parameters to compensate for impaired control mechanisms in pathological populations may be possible using wavelet analysis of blood flow oscillations. The specific accomplishments of this work are described as follows:

1. A computer-controlled system to apply heat and various loading conditions on the sacrum with simultaneous measurement of physiological responses (i.e. skin blood flow and temperature) was developed.
    a. A program for force feedback control of the indenter using LabVIEW 5.0 was developed.
    b. A non-linear contact finite element model to determine an optimal edge radius for the indenter head in order to minimize edge effects was developed and used for the indenter design.

c. Matlab routines for implementing wavelet analysis on the blood flow oscillation data sets were developed.

2. Methods to study the relative contributions of the various blood flow control mechanisms' response to various stimuli were developed. Rationale for the designation of specific frequency bands associated with specific blood flow control mechanisms was given (i.e. metabolic (0.008-0.02 Hz), neurogenic (0.02-0.05 Hz), myogenic (0.05-0.15 Hz), respiratory (0.15-0.4 Hz) and cardiac (0.4- 2.0 Hz)).
3. Metabolic control mechanisms were found to be stimulated more by incremental heating of the skin than by incremental pressure applied to the skin ($p<0.01$).
4. Myogenic control mechanisms were found to be stimulated more by incremental pressure applied to the skin than by incremental heating of the skin ($p<0.01$).
5. Both reactive hyperemia and pressure-induced vasodilation were shown to be associated with the myogenic control mechanism ($p<0.05$).
6. The variability in the blood flow signal power for individual frequency bands generated using wavelet analysis was shown to be smaller than the variability of either the combined time-domain blood flow signal or the maximal blood flow normalized signal ($p<0.05$).
7. Our results indicated alternating pressure stimulates an increase in skin blood flow compared to constant loading ($p<0.01$).

8. Our study using wavelet analysis of blood flow oscillations suggests that optimization of alternating pressure amplitude, frequency and offset parameters to compensate for impaired control mechanisms in pathological populations may be possible. If a relationship between loading patterns and blood flow regulation could be identified, alternating pressure technology may greatly reduce the risk of a pressure ulcer by enhancing tissue viability.

7.2 Limitations of This Study

Analysis of blood flow oscillations has been used to study microvascular control mechanisms for the past twenty years. Findings from these studies suggest that blood flow oscillations may be associated with heart rate, respiration, myogenic activity of vascular smooth muscle cells, neurogenic control, and metabolic related controls (Salerud et al., 1983; Kastrup et al., 1989; Hoffmann et al., 1990; Intaglietta, 1990; Muck-Weymann et al., 1996; Kvernmo et al., 1999; Stefanovska and Bracic, 1999; Stefanovska et al., 1999; Kvandal et al., 2003; Soderstrom et al., 2003). However, the relationships between these oscillations, the underlying physiological mechanisms and the frequency content of the skin blood flow signal have not been definitively investigated, especially in lower frequency ranges. We demonstrated that frequency content changes in specific ranges are related to heating and various loading conditions. Our interpretations of these results are heavily dependent on the assumption that there is a correspondence between the identified frequency bands and specific control mechanisms. Although we are encouraged by the consistency between our results and the results of Stefanovska and colleagues, with regard to the clustering of data within the five ranges, there is

considerable variability in our data (Figure 3-3 and 5-3). More work should be performed to confirm these associations and validate the results. The measurement and analysis technique developed in this work appears to offer considerable advantages over traditional spectral analysis, especially in the low frequency ranges where we believe the metabolic, neurogenic and myogenic control mechanisms mediate blood flow oscillations. Broad use of the technique by others performing research in this area could produce more definitive results. Such results could help answer some of the important research questions posed here with regard to the relationship between mechanical stresses and metabolic, neurogenic and myogenic control mechanisms.

The second limitation of our methodology is that blood flow responses are assessed at the skin level instead of the skeletal muscle level. Although pressure ulcers may develop first in muscle tissue (Daniel et al., 1981; Alterescu and Alterescu, 1988), our method for assessing skin blood flow responses to mechanical stress is still highly relevant in pressure ulcers prevention. Our general hypothesis is that AP elicits protective microvascular responses that modulate vasomotion and enhance blood flow. The hypothesis is based on the responses of arteriolar vasomotion of the microvascular system both in the skin and the skeletal muscle. Laser Doppler flowmetry cannot detect blood flow below the skin level. Alternative techniques would be necessary for non-invasive monitoring of microcirculation within skeletal muscles.

The third limitation of our research concerns the use of a single rigid indenter to assess blood flow response to alternating pressure. Alternating pressure support surface contains air-filled chambers to change the location of the contact pressure of the weight-bearing tissues (Brienza and Geyer, 2000). Our results suggest that increased metabolic

control is responsible for an increase in skin blood flow during alternating pressure. These metabolic-related vasodilators can be transported up or down-stream into adjacent microvascular networks (Rivers, 1995; Beach et al., 1998). Thus blood perfusion responses are not only determined by loading patterns imposed on it, but also are affected by adjacent microvasculature. Because we used a single indenter for our research, we were unable to simulate a multi-chamber alternating pressure device and thus could not assess the potential interaction of neighboring microcirculatory networks under such conditions.

7.3 Recommendations

Individuals with SCI are at high risk for developing pressure ulcers (Defloor, 1999; Garber et al., 2000; Krause et al., 2001). As the group, they may stand to be in the best position to benefit from our findings. Impaired neurogenic control over cardiovascular system has been considered to be the main cause of increased risk of developing pressure ulcers in people with SCI (Byrne and Salzberg, 1996; Teasell et al., 2000). In this study we demonstrated that locally applied pressure induces myogenic mediated vasodilation as measured from wavelet analysis of blood flow oscillation (see Chapter 4). We also demonstrated that alternating pressure stimulates an increase in skin blood flow due to enhanced metabolic responses as measured from wavelet analysis of blood flow oscillation (see Chapter 6). The metabolic and myogenic control mechanisms are local control mechanisms of the cutaneous microcirculation and are spared in individuals with SCI. Thus, our findings have high potential to be reproduced in SCI

population. A logical next step in this line of research is to repeat these experiments using subjects with spinal cord injuries.

A novel method to assess beneficial mechanisms associated with alternating pressure has been established. Using wavelet analysis with quantification methods we demonstrated the possibility of using wavelet analysis to optimize parameters of alternating pressure for enhancing tissue viability. Future research should vary parameters of alternating pressure (i.e. air cell configurations and pressure parameters) and compare their effects on the skin blood flow and control mechanisms.

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