ANALYSIS OF THE RELATIONSHIP BETWEEN SACRAL SKIN BLOOD FLOW AND TRANSCUTANEOUS OXYGENATION IN RESPONSE TO CAUSATIVE FACTORS OF PRESSURE ULCERS IN HEALTHY SUBJECTS

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Pressure ulcers significantly contribute to the diminished quality of life and substantial disability in people with spinal cord injury (SCI). A broad consensus among clinicians and researchers has been reached that the best approach to reducing this burden is to implement an effective preventive treatment that would greatly reduce the incidence. The preventative intervention should eliminate/diminish causative factors and pathways involved with pressure ulcer development. The objective of this thesis is to explore the relationship between sacral skin blood flow and transcutaneous oxygenation in response to causative factors of pressure ulcers (i.e. thermal stress, mechanical stress, and sympathetic modulations) in five neurologically intact subjects.

Two tests were performed to analyze the relationship between sacral skin blood flow and transcutaneous oxygenation. In test 1, skin blood flow and transcutaneous oxygenation were measured while subjects underwent orthostatic stimulation. Results from test 1 showed that both the level of heat used and the location of testing effects how skin blood flow and transcutaneous oxygenation respond to orthostatic stimulation. In test 2, skin blood flow and transcutaneous oxygenation were measured while external pressure was applied. Results from test 2 showed that a significant increase in peak skin blood flow and $TcPO_2$ ($p<0.05$) occurred on average 588 and 298 seconds, respectively, following removal of occluding pressure when the skin is heated

to 44 °C; however, at 37 °C, skin blood flow and transcutaneous oxygenation showed a significant peak increase ($p<0.05$) following removal of occluding pressure at 28 and 404 seconds, respectively.

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PREFACE

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1.0 INTRODUCTION

1.1 DISCUSSION OF PROBLEM

Insufficient blood flow and insufficient oxygen during the application of prolonged pressure to the skin can result in cell necrosis and pressure ulcerations.[1,2] Pressure ulcers significantly contribute to the diminished quality of life and substantial disability in people with spinal cord injury (SCI). The SCI population in the United States is approximately 253,000, with 11,000 new cases each year, and it is estimated that 50-85% will develop at least one pressure ulcer during their lifetimes.[3,4,5] The healthcare cost associated with the treatment of pressure ulcers in this population exceeds $1.3 billion annually, which accounts for 25% of the total cost of SCI treatment. [6] A broad consensus among clinicians and researchers has been reached that the best approach to reducing this burden is to implement an effective preventive treatment that would greatly reduce the incidence.[7] The preventative intervention should eliminate/diminish causative factors and pathways involved with pressure ulcer development.

Spinal cord injury deprives supraspinal control over the cardiovascular system which causes impaired protective vasodilatory response to stress.[8] For assessing microvascular function, laser Doppler flowmetry and transcutaneous oximetry have been used to quantify microvascular responses to causative factors related to pressure ulcers (e.g. pressure, shear, heating, and moisture).[9,1,2]

Laser Doppler flowmetry (LDF) provides noninvasive measurements of nutritional and thermoregulatory skin blood flow (SBF); and the nature of increased SBF following ischemia reflects the severity of tissue ischemia. Transcutaneous oximetry provides an estimation of arterial oxygen tension that indicates the oxygen supply to local cells. Although proven useful to assess pathological changes and effectiveness of the treatment,[10-13] laser Doppler skin perfusion and transcutaneous partial pressure of oxygen ($TcPO_2$) have been shown to be inconsistent.[14-19] Xakellis and colleagues' compared LDF skin blood flow and transcutaneous oxygenation with increasing compressive weights.[14] Their findings indicate that $TcPO_2$ values and LDF values responded differently to compressive weight.[14] $TcPO_2$ values decreased in a curvilinear pattern with increasing weight. LDF values showed a linear decline as compressive weight increased. Interestingly, their findings indicate that blood flow continued to decline beyond the point where $TcPO_2$ values reach zero; suggesting that tissue oxygenation reached minimum levels before blood flow reached biological zero.[14] These findings indicate that tissue ischemia could occur with less external pressure required to produce biological zero blood flow.

Several research studies showed that a significant decrease of transcutaneous oxygenation occurred at a higher pressure loading while significant decrease in LDF skin blood flow occurred at a lower pressure.[15-18] Transcutaneous oxygenation has been reported to reach a stable level after 20 minutes of local heating; however, after this time period skin blood flow continues to increase for up to 50 minutes of local heating.[19,20] Moreover transcutaneous oxygenation has been shown to increase after removal of occluding pressure with its peak value showing inconsistent results: similar to the baseline value in Ubbink et al.'s study,[21] and 3-5 fold of baseline value in Ewald and colleagues' study.[22] In addition the effects of sympathetic modulation on transcutaneous oxygenation are largely unknown. .

Colin and Saumet investigated the influence of external pressure on $TcPO_2$ and LDF on sacral skin blood flow.[17] Their findings indicate that external pressure as low as 40 mmHg induces a significant decrease in oxygenation and that external pressure as low as 20 mmHg induces a significant decrease in skin blood flow.[17] The external pressure needed to reach biological zero values of both LDF and $TcPO_2$ were nearly identical, 90 mmHg and 100 mmHg, respectively.[17]

Taken together, these conflicting findings hinder the clinical application of LDF and $TcPO_2$. In order to provide some insight into the relationship between transcutaneous oxygenation and LDF a series of experiments have been performed. The objective is to explore this relationship to enhance clinical applications taken from LDF and $TcPO_2$.

1.2 OBJECTIVES AND HYPOTHESES

The objective of this thesis is to explore the relationship between sacral skin blood flow and transcutaneous oxygenation in response to causative factors of pressure ulcers (i.e. thermal stress, mechanical stress, and sympathetic modulations) in neurologically intact subjects.

Two tests were performed to achieve this goal. Test 1 was a laboratory based test in which sacral skin blood flow and transcutaneous oxygenation levels were measured at 37 °C and 44 °C while subjects underwent an orthostatic stimulation. Test 1 had three objectives: (1) to test the hypothesis that decreased sacral skin blood flow during an orthostatic stimulation causes a decrease in $TcpO_2$, (2) to test the hypothesis that an orthostatic stimulation results in a larger

change of $TcpO_2$ over the glabrous skin with abundant arteriovenous shunts (i.e. right and left heels) and a smaller change of $TcpO_2$ over the non-glabrous skin with few arteriovenous shunts (i.e. right and left sacrum), and (3) to test the hypothesis that decreased sacral skin blood flow during an orthostatic stimulation is regulated by the 0.02-0.05 Hz frequency band embedded in skin blood flow oscillations.

Test 2 was a laboratory based test in which sacral skin blood flow and transcutaneous oxygenation levels were measured at 37 °C and 44 °C while externally applied pressure was supplied to the subjects right sacrum. Test 2 had two objectives: (1) to test the hypothesis that increased sacral skin blood flow during reactive hyperemia causes an increase in $TcpO_2$, and (2) to test the hypothesis that reactive hyperemia is a local response; therefore, increased $TcpO_2$ during reactive hyperemia does not change $TcpO_2$ at the skin over the left sacrum and the right and left heels.

1.3 ORGANIZATION OF THE THESIS

Five chapters follow this introduction. Chapter 2 is a review of the literature on pressure ulcer clinical research and the role that heat, orthostatic stimulation, and external pressure factor into pressure ulcer development. Chapter 3 provides the research design and methods used in the study. Chapter 4 provides the results of the experimental procedures. Chapter 5 provides the discussion of these results. Chapter 6 gives the summary, contributions, and future directions of the research.

2.0 REVIEW OF THE LITERATURE

2.1 BLOOD FLOW REGULATION

2.1.1 Healthy Subjects

Blood flow regulation can be attributed to vasomotion, which is the rhythmic constriction and dilation of blood vessels.[23-26] The dilation and constriction of blood vessels is controlled by central neurogenic, local myogenic and metabolic mechanisms.[27-32] Vasomotion can occur spontaneously[33,34] or in response to vasoactive stimuli.[35,36] Pacemakers cells may originate the constriction and relaxation of the smooth muscle cells surrounding arterioles.[37] Blood flow changes, particularly in the skin, act to meet various thermoregulatory and local nutrient needs.[23,38-40] Wavelet analysis by Brienza and colleagues identified five frequency bands embedded in the laser Doppler blood flow signal which correspond to vasomotion control patterns.[41,42]

2.1.2 Changes in Spinal Cord Injury

Following spinal cord injury many changes occur to blood flow regulation often resulting in persistent hypotension and/or episodes of uncontrolled hypertension.[43] Following spinal cord injury, the tonic activation of spinal sympathetic preganglionic neurons by descending input

from the supraspinal structures which regulate blood pressure, are disrupted and the spinal circuits become solely response for the generation of sympathetic activity.[44,45] This results in numerous cardiovascular abnormalities which, in the acute and chronic stages of SCI, are among the most common causes of death in individuals with SCI.[46-48]

In neurologically intact subjects both heart rate and blood pressure are controlled by inputs from two components of the autonomic nervous system, the sympathetic and parasympathetic nervous systems. These two components generally have opposing roles, depending on the needs and stresses of the individual. The parasympathetic is predominate during rest and acts to decrease heart rate. As opposed to the sympathetic nervous system which is largely excitatory and prepares the body for an emergency (fight or flight reaction), typically counteracting the parasympathetic nervous system. Activation of the sympathetic nervous system results in increased heart rate, force of myocardial contractions, and peripheral vascular resistance, resulting in increased blood pressure. In neurologically intact subjects the insula and hypothalamus contribute to the autonomic regulation of cardiovascular control.[49] Spinal cord injury leads to disruption of the descending spinal cardiovascular pathways which leads to hypoactivity of the sympathetic nervous system and unopposed prevalence of the intact parasympathetic control.[50] Thus individuals with SCI display low resting blood pressure, loss of regular adaptability of blood pressure, and disturbed reflex control.[8]

Immediately following spinal cord injury (hours to days) there is a transient state of hypoexcitability of the isolated spinal cord, a condition known as spinal shock. Spinal shock is associated with flaccid paralysis of the muscles, absent tendon reflexes, impairment of spinal autonomic function, dilation of blood vessels particularly in the skin, and profound hypotension.[51-53] Over time the signs and symptoms of spinal shock resolve, but SCI patients are

often plagued with sudden falls in blood pressure during postural change or following prolonged periods of sitting.[53,54] SCI patients can also present with sudden attacks of hypertension triggered by afferent stimuli below the level of the spinal cord lesion (autonomic dysreflexia), which causes severe headaches and upper body flushing.[8]

2.2 PRESSURE ULCERS IN SCI PATIENTS

2.2.1 Definition

The National Pressure Ulcer Advisory Panel in 2007 revised their definition of pressure ulcerations as, "A pressure ulcer is a localized injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure, or pressure in combination with shear and/or friction. A number of contributing or confounding factors are associated with pressure ulcers; the significance of these factors is yet to be elucidated."[55] Pressure ulcers are commonly referred to as bed sores, decubitus ulcers, or pressure sores. The skin overlying the sacrum and heel is most often affected, but pressure ulcers may also be observed over the occiput, elbows, greater trochanter, and lower extremities.[56] Pressure ulcers can develop on any part of the body where sustained pressure and compressive forces are maintained for a sufficient period of time.[56]

2.2.2 Clinical Diagnosis and Staging

Pressure ulcers are clinically diagnosed by visual inspection and palpation. In the United States, the National Pressure Ulcer Advisory Panel (NPUAP) staging system is the most

commonly used set of guidelines used in diagnosis.[57] The NPUAP's staging system has been revised throughout the years, the most recent revision (2007) accounts for deep tissue injuries. The European Pressure Ulcer Advisory Panel (EPUAP) has published its own staging system for pressure ulcers. In a study to assess the reliability of the proper staging of pressure ulcers using the EPUAP definitions, there was significant confusion amongst the clinicians.[58] No study to date has assessed the current reliability of staging pressure ulcers using the new NPUAP staging definitions amongst clinicians. The NPUAP classification for stage 1 pressure ulcer has changed considerably throughout the years and differs remarkably from the original 1989 NPUAP Consensus Conference definition of, "Non-blanchable erythema of intact skin; the heralding lesion of skin ulceration."[59] The stage 2 definition has also changed remarkably since the 1989 NPUAP Consensus Conference which defined a stage 2 pressure ulcer as, "Partial-thickness skin loss involving epidermis and/or dermis. The ulcer is superficial and presents clinically as an abrasion, blister, or shallow crater."[59] Stage 3 and 4 pressure ulcers have undergone little to no change since their original NPUAP 1989 definitions. Deep tissue injury was not recognized in the NPUAP staging system until 2007.[55] The NPUAP staging system also allows for the classification of unstageable pressure ulcers, which present with an ulceration base covered by slough and/or eschar in the wound bed.[55] The current NPUAP staging definitions are depicted in Table 1.

Table 1. 2007 NPUAP staging system

Stage	2007 NPUAP Definitions
1	Intact skin with non-blanchable redness of a localized area usually over a bony prominence. Darkly pigmented skin may not have visible blanching; its color may differ from the surrounding area.
2	Partial thickness loss of dermis presenting as a shallow open ulcer with a red pink wound bed, without slough. May also present as an intact or open/ruptured serum-filled blister.
3	Full thickness tissue loss. Subcutaneous fat may be visible but bone, tendon or muscle are not exposed. Slough may be present but does not obscure the depth of tissue loss. May include undermining and tunneling.
4	Full thickness tissue loss with exposed bone, tendon or muscle. Slough or eschar may be present on some parts of the wound bed. Often include undermining and tunneling.
Suspected Deep Tissue Injury	Purple or maroon localized area of discolored intact skin or blood-filled blister due to damage of underlying soft tissue from pressure and/or shear. The area may be preceded by tissue that is painful, firm, mushy, boggy, warmer or cooler as compared to adjacent tissue.
Unstageable	Full thickness tissue loss in which the base of the ulcer is covered by slough (yellow, tan, gray, green or brown) and/or eschar (tan, brown or black) in the wound bed.

2.2.3 Etiology

The exact etiology of pressure ulcers is not fully understood. However, a broad consensus of researchers agree that prolonged exposure to high-pressure gradients cause tissue necrosis via occlusion of capillary blood flow.[60] Pressure is defined as a perpendicular force that compresses tissues, typically between a bony prominence and an external surface.[61] Prolonged pressure can thus lead to ischemia by decreased tissue profusion which denies oxygen and other nutrients to the cells and allows a toxic build up of cell metabolites. An inverse relationship between the intensity of the external pressure and time required for ulcer formation has been demonstrated.[62] Thus high external pressure may cause ulceration in a shorter amount of time, and lower pressures require a longer time to cause ulceration. Shear forces may also play a role

in pressure ulcer development. A shear force is a force parallel to the skin. A shear force can compromise blood supply resulting in ischemia, cellular death, and necrosis.[61] Shear forces have been shown to amplify the effects of pressure.[63] An example of a shear force is when the head of the bed is titled upwards causing the weight of the upper body to produce shear forces towards the foot of the bed. Shear stress is thought to impair blood flow to deeper tissues.[64] Frictional forces are yet another force which may lead to pressure ulcers. Friction, which resists shearing forces, may lead to shedding of the epidermis (top to bottom model of pressure ulcer development, discussed below).[64]

There are primarily two theories detailing the mechanisms and progression and formation of pressure ulcers. One theory states that pressure ulcers form deep in the bone and move outward to the skin (deep tissue injury theory). It has been suggested that deep tissue injury occurs first near the bone. If unrelieved, ischemic injury and tissue necrosis can continue in an outward fashion, until reaching the outer layer of the skin.[61] Recent ultrasonic findings in deep tissue injury support this theory.[61] The second theory, top to bottom model, describes pressure ulcer formation resulting from skin destruction that occurs at the epidermis and proceeds inward to deeper tissue.[61] This is the less favored model based on current research[61]

2.2.4 Predisposing Factors

Due to loss of sensation, impairment of mobility, reduction of soft tissue thickness, decreased vasomotor tone, and incontinence people, with SCI are a high risk group for pressure ulcer development.[65] Risk factors in the SCI population for pressure ulceration include variables such as injury completeness, presence of additional medical conditions, prior history of ulcers, advanced age, lack of high school education, unemployment, smoking, unhealthy dietary habits,

lack of fitness, and difficulty performing skin care procedures.[66] SCI deprives supraspinal control over the cardiovascular and microvascular systems.[65] The degree of autonomic nervous system impairment is dependent upon the level of injury. SCI at T6 or above may result in low blood pressure, decreased heart rate, orthostatic hypotension, autonomic dysreflexia, and low blood flow that may predispose a person to an abnormal response to loading.[65] A spinal cord injury below T6 allows adequate sympathetic innervation for the cardiovascular system.[65]

2.2.5 Incidence and Prevalence

The incidence and prevalence of pressure ulcers varies per setting and patient population. Reports estimate the prevalence of pressure ulcers in inpatient hospital patients to surpass 15% with over 60,000 deaths each year associated with complications from pressure ulcers.[67] Approximately 57% to 60% of pressure ulcers occur in the hospital setting within the first 2 weeks of admission.[68-70] Approximately 70% of pressure ulcers occur in individuals over the age of 70.[71] Incidence rates in the long term care setting have been reported to be from 2.2% to 23.9%.[57] The SCI population in the United States is approximately 250,000. It is estimated that 50%-85% of the SCI population will develop at least one pressure ulcer during their lifetimes.[57,5]

2.2.6 Financial Impact

Although studies vary on the cost to treat pressure ulcers they are nonetheless very substantial. Healthcare costs associated with the treatment of pressure ulcers in just the spinal cord injury population, exceeds $1.3 billion annually.[6] This cost is estimated to be 25% of the total cost of SCI treatment.[6] In 1999 Beckrich and colleagues' reported there were 1.6 million

pressure ulcers developed in hospitals annually in the United States, with treatment costs estimated to be $2.2 to $3.6 billion annually.[6] Costs of treatment for pressure ulcers is associated with the severity of the wound. It is estimated that each stage III or stage IV pressure ulcer can add $14,000 to $23,000 to the cost of patients' care.[72] Zhan and Miller report that development of a pressure ulcer adds $10,845 to the cost of care, prolongs hospital stays by approximately 4 days, and increases mortality by 7.23%.[73] The total cost of wound care is estimated at $125-$451 for Stage I or II ulcers and $14,000-$23,000 for Stage III or IV ulcers.[74,72]

2.3 TECHNOLOGY TO ASSESS PRESSURE ULCER RISK

2.3.1 Interface Pressure

Non-invasive measurements of the skin microcirculation have been used extensively in the field of tissue viability. For many years, interface pressure has been used as an indicator of tissue loading tolerance.[42] While interface pressure can identify areas of high pressure and evaluate the pressure distribution achieved for a particular individual using a support surface, physiological responses cannot be detected.[42] Thirty two mmHg is traditionally used as a value which causes capillary closure;[75] however, the range of interface pressures capable of occluding capillary blood flow varies widely.[76] Because it is the microcirculation which actually supplies the skin with blood and nutrients (oxygen) it is important to have non-invasive measurements of the microcirculation. Laser Doppler flowmetry and transcutaneous oxygenation provide non-invasive means of monitoring cutaneous microcirculation.

2.3.2 Laser Doppler Flowmetry

Laser Doppler flowmetry (LDF) is a noninvasive measure of microcirculatory blood flow.[77] Monochromatic laser light is transmitted to the skin by a probe. Although 93-97% is absorbed by various structures, the remaining 3-7% is reflected. Another optical fiber collects the backscattered light from the tissue and returns it to the monitor, resulting in an output signal that is proportional to the microvascular perfusion.[77] LDF measures skin blood flow at approximately 1 mm depth of the skin. The area of skin sampled by LDF is small, and is estimated to be about 1 mm^2.[78] LDF requires no heating, but can be used along with heating, to acquire accurate measurements. The principle method of LDF is to measure the Doppler shift by moving objects, such as red blood cells.[77] LDF has been used in both healthy subjects and those thought to be at risk for pressure ulcer development during pressure, heating, postural changes, and the effects of various support surfaces.[79,80]

2.3.3 Wavelet Analysis

Physiological rhythms associated with blood flow control mechanisms are embedded within the laser Doppler blood flow signal.[42] Researchers have attempted to decompose the laser Doppler blood flow signal using various spectral analysis techniques. Traditionally Fourier transforms have been used to study the frequency components of skin blood flow.[42] However, Fourier transforms do not provide sufficient time resolution for nonstationary signals (such as blood flow).[81,82] A windowed Fourier transform permits time frequency analysis, but does not allow adequate time and frequency resolution.[83] Wavelet analysis has been used to overcome these shortcomings.[41,42] Using wavelet analysis Stefanovska and Bracic identified five frequency

bands in the blood flow signal, corresponding to heart rate, respiratory activity, vascular myogenic response, neurogenic response, and metabolic responses.[84] This technique has subsequently been used by Geyer and colleagues to investigate the effects of heat on skin blood,[42] by Brienza and colleagues to compare skin blood flow's response to heating and indentation,[41] by Jan and colleagues to overcome temporal variability in skin blood flow measurements[85], and by Li and colleagues to compare skin blood flow oscillations in individuals with and without spinal cord injury.[86]

2.3.4 Transcutaneous Oxygenation

Measuring blood oxygen levels transcutaneously is possible because oxygen, as well as carbon dioxide, can diffuse across the skin.[77] However, the skin is not very permeable to gases under unheated conditions. At higher temperatures the ability of the skin to transport gases is greatly improved. Transcutanous oxygenation minimally requires the skin to be heated to 37 ˚C. The $TcPO_2$ monitor has a Clark polarographic electrode that has been modified to include a heating element. The electrode tip is covered with a thin membrane which oxygen diffuses through to the cathode where a reduction of oxygen occurs as a result: $O_2 + 2H_2O + 4e^- \rightarrow 4\ OH$. At the anode the following reaction takes place: $4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$. The reduction of oxygen at the electrode's cathode generates a current that is converted into a voltage and digitized. This digitized signal is then passed to the microcomputer where it is reconverted to display pO_2 in mmHg.

Heat causes three effects on the skin's surface oxygen tension, thus making measurement of $TcPO_2$ possible. First, heating the stratum corneum beyond 40 ˚C changes its structure, which allows oxygen to diffuse faster. Secondly, heat causes the oxygenation dissociation curve to

shift to the right, thus more oxygen is released from hemoglobin. Lastly, dermal capillary hyperemia is induced by the heat.[77]

Clinically $TcPO_2$ has been used extensively in neo-natal care due to the thin epidermis of the infant, allowing easier diffusion of gases through the skin.[87] Arterial blood oxygen values are well correlated to $TcPO_2$ values in infants, making $TcPO_2$ a valuable, non-invasive tool for monitoring respiratory gas status.[88] It has also been used extensively to estimate surgical amputation levels.[89] Upon review of the literature Wutschert concludes that 20 mmHg should be the cutoff value in determining amputation level.[89] $TcPO_2$ has been used to determine patients that would benefit from hyperbaric oxygen therapy,[90]and is used extensively in the diagnosis and prognosis of peripheral vascular disease.[21,91-96]

$TcPO_2$ has been used to predict wound healing following surgical wounds.[97] $TcPO_2$ has also been used as a measurement technique in pressure ulcer research. Several researchers have investigated $TcPO_2$ levels on externally applied pressure,[98] the effects of various support surfaces,[80,76] and in the differences between healthy and pressure damaged skin .[19] Sacral $TcPO_2$ values have been shown to be lower in SCI patients than in neurologically intact patients.[11]

2.4 TEMPERATURE IN PRESSURE ULCER DEVELOPMENT

Local heating produces vasodilation which is regulated by both neurogenic and locally released metabolic mechanisms.[96] The complex interaction of these mechanisms is very poorly understood and research has shown inconsistent findings because of the different temperatures used, length of heat applied, and rate of heat application.[20] Minson described the response of skin blood flow to heat as a biphasic response. Local heating initially produces a rapid increase

in skin blood flow, followed by a brief nadir, and then a slowly increasing secondary dilation until it plateaus.[20] Minson and colleagues concluded that the initial vasodilation is predominantly mediated by an axon reflex mechanism and the secondary vasodilation until a plateau is reached is attributed to the release of nitric oxide.[20] The metabolic response to heat has been further studied by Brienza and colleagues which determined that increased skin blood flow was in the metabolic frequency band of .008 to .02 Hz when using wavelet analysis to analyze the skin blood flow signal.[41]

Shear stress is the primary stimulus which regulates the release of nitric oxide.[100] However, by blocking the axon reflexes during heating Minson and colleagues found that the secondary rise to plateau phase of skin blood flow was not altered.[20] Geyer and colleagues did not observe a typical biphasic response to heat, but rather only the secondary peak.[42] They attributed this to the slower rate of heating used to achieve maximum vasodilation.[42] This peak extended into the post-heating recovery period. Geyer and colleagues' heating protocol used a very different rate of heating than Minson. In Geyer's study, blood flow was monitored for 10 minutes without heat, followed by 3 minutes at 35 ˚C followed by an increase of 1˚C per minute for 9 minutes, and a final period of 3 minutes at 45 ˚C with a 10 minute post-heating period. Minson and colleagues used a much faster rate of heating. Skin blood flow was monitored for 30 minutes unheated followed by a rise of 0.5 ˚C every 5 seconds to a temperature of 42 ˚C and held constant for 50 to 80 minutes.

The measurements of skin blood flow and transcutaneous oxygenation in response to heating have shown mixed results. Shubert found a biphasic response of skin blood flow during heating while the $TcPO_2$ showed a gradual increase throughout the heating protocol.[19] However, Ewald *et al* found that $TcPO_2$ values mirrored trends in skin blood flow, however the $TcPO_2$

values were delayed.[22] Very little literature exists examining the relationship between skin blood flow and transcutaneous oxygenation during a heating protocol.

2.5 ORTHOSTATIC MODULATION ON SKIN BLOOD FLOW

In 1953 Gaskell, based on Girling's work on rats, suggested that postural vasoconstriction in human limbs was due to a local "veno-vasomotor" reflex.[101] Hassan used laser Doppler flowmetry and local anesthesia to conclude that posture induced vasoconstriction was mediated mainly by sympathetic efferent nerves.[102] Mayrovitz studied if the effects of posture induced vasoconstriction were transient or maintained, and concluded that posture induced vasoconstriction is maintained throughout the entire time of gravity dependent posture, and that no vasodilator escape was observed.[103] Mayrovitz also concluded that the magnitude of the response is somewhat dependent on anatomical site, suggesting that sites with more arteriovenous shunts respond more to postural changes.[103]

Postural changes stimulate a change in intravascular blood volume and pressure which produces vascular responses.[104] Postural changes force the distal vasculature to compensate and respond to the subsequent increase in volume and pressure.[103] Normally, in unheated skin, individuals free of distal vasculature problems, show a decrease in blood perfusion when in a dependent position.[101] The physiological mechanisms associated with causing a decrease in skin blood flow in the dependent position have been described as local neurogenic reflexes with smaller contributions of local myogenic and central effects,[102] although controversy exists on the exact mechanism. Abnormalities in skin blood flow's response to dependency have been reported in diabetics[105] and in peripheral vascular disease.[106,16]

Reduction in the postural vasoconstriction response at elevated temperatures in healthy subjects has been reported by Rendell and colleagues.[104] Complete elimination of the postural vasoconstriction response is a clinical evaluation tool for those with peripheral vascular disease. However, this response has been reported in healthy subjects who are subjected to orthostatic stimulation with skin heating.[107]

The effects of orthostatic stimulation on transcutaneous oxygen have not been studied extensively. Caspary took separate measurements of LDF and $TcPO_2$ in patients with peripheral arterial occlusive disease during an orthostatic stimulation. He concluded that during an orthostatic stimulation, patients with peripheral arterial occlusive disease will notice a reverse effect of increasing blood flow and increasing $TcPO_2$ when changed to a gravity dependent position.[16] However, he observed that some patients will only increase $TcPO_2$ and not LDF, and some will increase LDF and not $TcPO_2$. He did see a significant difference in patients showing a rise in $TcPO_2$ but not in LDF.[16] The sitting/supine ratio values of LDF and $TcPO_2$ were significantly larger for $TcPO_2$.[16] Ubbink reported $TcPO_2$ to be a valid measure of peripheral vascular disease at 37°C but not at 44°C.[107]

Although posture induced vasoconstriction is often used clinically, the exact physiological mechanism is not fully understood. Vasoconstriction appears to be induced mainly by local neurogenic reflexes[101] with smaller contributions of local myogenic and central effects.[102] It has been proposed by investigators that both cardiopulmonary and arterial baroreceptors play an important role in cutaneous adjustments to upright posture. [108] However, Vissing *et al.* conclude that baroreceptors do not initiate cutaneous vasoconstriction, but rather activation of a local neurogenic, presumably veno-arteriorlar, reflex is responsible mechanism triggering cutaneous vasoconstriction in dependent limbs.[109] The exact mechanism of the local

veno-arteriorlar reflex is not known, Vissing *et al.* hypothesized that a local neural impulse travels in the axonal sympathetic fibers in response to increase vascular transmural pressure.[109] The myogenic response may also contribute to the changed in systemic vascular resistance during orthostasis[110-113] and to vasoconstriction during limb dependency.[114-116]

As previously noted, there is a strong connection between SCI and orthostatic hypotension. As many as 74% of SCI patients were clinically diagnosed with orthostatic hypotension while performing orthostatic maneuvers during physical therapy.[117] The precise mechanisms responsible for orthostatic hypotension in SCI patients are still uncertain.[43] Lesions above T6 disrupt supraspinal control of the splanchnic bed, predisposing them to orthostatic instability. In addition, the disruption of spinal sympathetic pathways would likely affect the vascular resistance responses to orthostasis.[43] In addition to a dysfunctional sympathetic nervous system, SCI patients also have altered baroreflex function, lack of skeletal muscle pumping activity, cardiovascular deconditioning, and altered salt and water balance which result in orthostatic instability.[43]

2.6 EXTERNAL PRESSURE IN PRESSURE ULCER DEVELOPMENT

Many factors contribute to the production of pressure ulceration; however, most researchers agree that prolonged, unrelieved pressure can reduce blood flow, causing ischemia and subsequent pressure ulceration. Landis, in 1930, found the average pressure in the fingernail capillary bed was 32 mmHg.[75] Kosiak, in 1961, demonstrated that roughly 80 percent of externally applied pressure is transmitted to the skin microvasculature.[118] Therefore, manufactures of support surfaces have focused on keeping interface pressure values below 40

mmHg. However, when using physiological measures, such as skin blood flow and transcutaneous oxygenation, the amount of pressure to occlude blood flow varies.[76] Moreover, in support of physiological measures over interface pressure Frantz and Xakellis conclude that, "by using perfusion as the assessment parameter, variation in individual physiological response to compressive surface pressure could be identified more accurately and high risk conditions averted."[119]

Pressure induced reduction of skin blood flow affects the skin breakdown process in numerous ways, dependent upon the tissue's tolerance for pressure.[120] An inverse relationship between the intensity of the external pressure and the time required for ulcer formation has been demonstrated by Dinsdale[62] and supported by Kemp who reported patients with longer surgical procedures were more likely to develop pressure ulcers.[120] Both normal forces as well as shear forces can contribute to pressure ulceration.[121] Dinsdale found a lower pressure was sufficient to cause ulceration in animals when pressure was combined with shear than compared to pressure alone.[122] Laser Doppler Flowmetry has been used extensively to study the effects of compressive loading on skin blood flow.[119,79,17,123]

Clinically, a common intervention to prevent pressure ulcers in bedridden patients is to turn the patients every 2 hours. In addition provocation of the blanching response is clinically used in the diagnosis of pressure ulcers. Subsequently, many research studies have examined what effect this pressure relieving maneuver has on skin blood flow. A rapid reduction or elimination of ischemia inducing external pressure results in a transient increase in skin blood flow, an event termed reactive hyperemia.[41] The assessment of reactive hyperemia can provide insight into changes in microcirculation affecting either reduced vasodilator bioavailability or

enhanced vasoconstriction in response to hypoxia.[124] In addition endothelial damage may be indicative of a reduced hyperemic response.[125]

Reactive hyperemia is mediated mainly by local control mechanisms with evidences occurring in denervated tissue.[126] Brienza and colleagues concluded that the myogenic response causes an increase in skin blood flow both during loading (pressure induced vasodilation) and after loading (reactive hyperemia).[41] Bayliss, in 1902, characterized the myogenic response as a decrease in vessel diameter after an increase of transmural pressure, and by an increase in vessel diameter after a decrease of transmural pressure.[127] Both the magnitude and duration of the reactive hyperemia has shown to be related to the magnitude and duration of the external pressure.[128] The transient increases in skin blood flow seen during reactive hyperemia is considered to be a protective response by numerous researchers.[129-131]

Several animal studies report on the effect of occlusion time on the hyperemic response.[132,133] Using occlusion times of 1, 2, or 3 minutes, Tee *et al.* had similar results of occlusion time affecting the hyperemic response. Their results support that the magnitude of reactive hyperemia is dependent on the occlusion duration.[124]

As previously noted, there is evidence of reactive hyperemia in denervated tissue.[126] Hagisawa *et al.* found no significant difference in peak blood flow between able bodied and SCI subjects during reactive hyperemia.[134] Other research studies have supported these results;[135-136] however, Shubert and Fagrell found a dimished reactive hyperemia in SCI subjects.[137]

Age also has an effect on the hyperemic response.[138] The aging process causes changes in the quality of collagen and a decrease of elastin and ground substance in the skin.[139] Pressure damage is thus more likely because these changes allow more direct transfer of mechanical load to the underlying tissue.[138] In addition there is also a modest reduction of subcutaneous tissue in

the ages skin, resulting in less resistance to mechanical load.[139] A decrease in maximum flow during reactive hyperemia in older subjects has been reported by investigators.[138]

The myogenic response has been found in a variety of vessels, including vein, arteries, and arterioles.[140-143] Most studies have focused on small arteries and arterioles because of their importance in blood flow distribution. Vessels of very large and very small diameters have the weakest myogenic response, while intermediate size vessels have the largest myogenic response.[144] Upon a review of the literature Shubert concludes that the existence of the myogenic response is well established in a variety of vessels from different vascular beds, but the strength of the myogenic response varies.[31]

Numerous theories exist on the mechanism of the myogenic response. There is some evidence that the pressure induced alteration of vessel wall tension, and not the pressure induced cell length or the pressure itself is the stimulus for the myogenic response.[145] This evidence has support based on intracellular calcium concentration and the level of myosin light chain phosphorylation are significantly correlated with vessel wall tension and not with vessel diameter.[146]

Many researchers believe that the myogenic response has sensor elements which detects the stimulus, and initiates the myogenic response.[31] This theory is tested frequently with stretch-activated cation channels, but direct experimental evidence for this theory is lacking.

The vessel wall has three components which are exposed to differences in transmural pressure; endothelial cells, smooth muscles cells, and nerve endings in the adventia.[31] Based on studies in which the nerve endings are blocked with numerous drugs, Shubert concluded that the myogenic response is not mediated by these nerve endings.[31]

Some controversy exists on what the role of the endothelium has on the myogenic response. Some studies have shown altered myogenic response with the removal of the endothelium, and others have shown that the endothelium has no influence on the myogenic response.[147] However, this may have been due to the methods in which endothelial regulation has been removed.[147] Engelke *et al.* demonstrated that blocking endothelial NO release does not eliminate the maximal vasodilation during reactive hyperemia.[148] Brienza and colleagues found that metabolic control (release of nitric oxide by endothelial cells) has a minor role in skin blood flow's response to mechanical stress, thus supporting Engelke's work.[41]

Numerous animal studies have shown that an increase in transmural pressure produces a membrane depolarization of the smooth muscle cells.[149-155] The exact mechanism for this membrane depolarization in response to an increase in transmural pressure is not known; however, several theories exist involving stretch activated channels,[156,157] calcium activated potassium channels,[155,158,159] chloride channels,[160] calcium channels,[161] intracellular calcium concentration,[162] and intracellular secondary messengers.[163]

The myogenic response is demonstrated in both reactive hyperemia and pressure induced vasodilation (non-occluding pressure).[39,41,164] Abraham *et al.* demonstrated that non-occluding local pressure leads to a slow developing and long lasting pressure induced vasodilation in the hand.[164]

Studies on transcutaneous oxygenation following occluding pressures have had mixed results. In general transcutaneous oxygenation has been shown to increase following removal of pressure, with peak values mixed. Ubbink *et al.* showed that transcutaneous oxygenation post occlusion values were similar to pre-occlusion values.[21] However, in Ewald and colleagues study post occlusion transcutaneous oxygenation values were three to five times the value of pre-

occlusion transcutaneous oxygenation values, and delayed by 10 to 20 seconds compared to the laser Doppler blood flow values.[22] In another study by Ewald transcutaneous oxygenation showed a transient increase following removing of occluding pressure while performed at temperatures between 35-37 °C; however, at higher temperatures, >39 °C, Ewald reported transcutaneous oxygenation levels to return to baseline levels only .[165]

The use of cyclic loading patterns has also been studied. Bader studied cyclic loading in SCI and healthy subjects and concluded that cyclic loading enhances sacral oxygenation levels in healthy subjects, but not in SCI subjects.[76] Using laser Doppler flowmetry, Mayrovitz et al. demonstrated enhanced heel skin blood flow under cyclic loading in elderly women but not in healthy subjects.[166] Jan demonstrated that LDF skin blood flow was enhanced under cyclic loading as compared to constant loading.[167]

3.0 RESEARCH DESIGN AND METHODS

3.1 SUBJECTS

Five neurologically intact subjects (4 males, 1 female) were recruited into this study. The demographic data were as follows (values are mean $\pm$ SD): age 22.4 $\pm$ 1.5 years, height 177.4 $\pm$ 14.2 cm, weight 75.16 $\pm$ 16.1 kg, BMI 23.8 $\pm$ 3.9 kg/m^2. The following conditions constituted exclusion criteria: diabetes, vascular disease, hypertension, the presence of pressure ulcers on the sacrum or heels, use of vasoactive medication, BMI >30, or BMI <20.

3.2 RESEARCH DESIGN

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 Tissue Integrity Management Laboratory at the University of Pittsburgh. Subjects were acclimated to room temperature for at least 20 minutes prior to testing to achieve a steady baseline blood flow.

The test subjects lay prone on a standard treatment table. Blood pressure and heart rate were recorded prior to the start of testing and at the end of testing. With subjects lying prone, a laser Doppler flowmetry (LDF) probe was adhered to the right sacrum with a double sided adhesive ring. $TcPO_2$ probes were calibrated before use, according to manufacturer's guidelines.

Four transcutaneous oxygenation probes were then connected to the subject. One $TcPO_2$ probe was adhered to the right sacrum, another to the left sacrum, and one each to the left and right heels (see Figure 1). Often calibration failed on the $TcPO_2$ unit. All recommendations by the manufacturer were followed, such as replacing the membrane on the probe. However, if re-membraning the electrode failed twice to produce a positive calibration, then that probe was dropped from the test, resulting in some subjects having fewer than 4 probes placed on them. $TcPO_2$ probes were held in place using adhesive fixation rings supplied by the manufacturer. Contact liquid supplied by the manufacturer was placed between the skin/probe interface, according to manufacturer's guidelines. Temperature on the LDF and $TcPO_2$ probes was set at 37 °C. Skin blood flow and $TcPO_2$ levels were recorded for 10 minutes. At the end of the ten minute period subjects would switch from a prone position to a seated position on the edge of the treatment table (see Figure 2). The maneuver took approximately 30 to 60 seconds to complete for each subject. Skin blood flow and $TcPO_2$ were then recorded for an additional 10 minutes in the seated position.

After a 20 minute washout period, this test was repeated at 44 °C. The same testing procedures were followed: subjects lay prone for 10 minutes while LDF skin blood flow was recorded on the right sacrum. $TcPO_2$ was measured on the left and right sacrum and the left and right heel. After 10 minutes the subjects changed posture to a seated position in which skin blood flow and $TcPO_2$ were recorded for an additional 10 minutes.

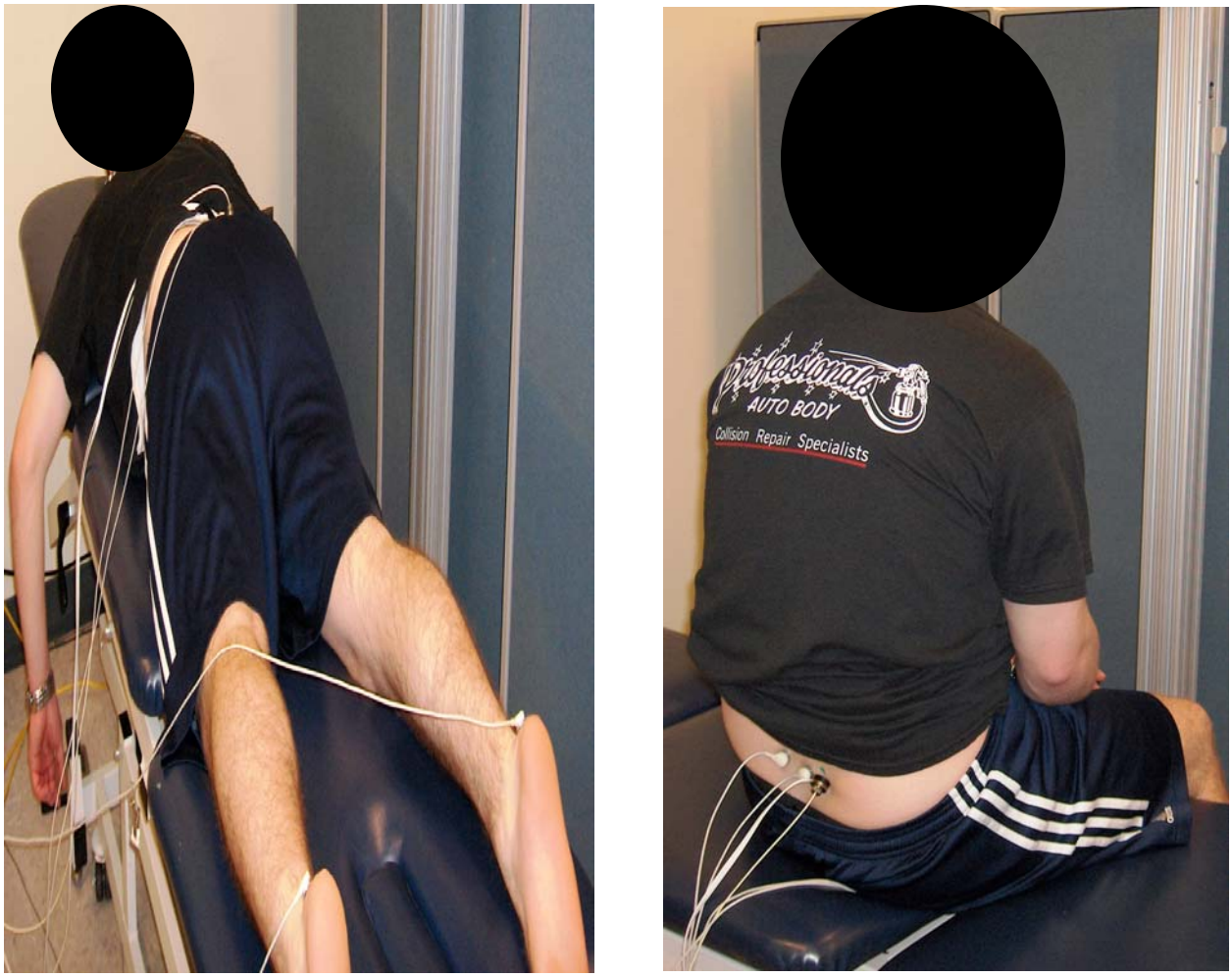

Figure 1. Subject before orthostatic stimulation (left) and after orthostatic stimulation (right)

Upon a second day of testing, subjects underwent an occluding pressure protocol to test the hyperemic response. After being acclimated to room temperature for at least 20 minutes to achieve a steady baseline blood flow, subjects lay prone on a standard treatment table. A combined laser Doppler flowmetry and a transcutaneous oxygenation probe was designed for the study (see Figure 3). A custom-designed, computer-controlled indenter was used to apply loading pressure on the skin over the sacrum (see Figure 4). The laser Doppler flowmetry probe and the transcutaneous oximetry probe were incorporated into a rigid indenter with features

similar to those described by Bader,[76] Schubert and Fagrell,[137] and Herrman et al.[126] including a force transducer to control loading pressure with simultaneous measurements of skin blood flow and oxygenation. The LDF and $TcPO_2$ probes had a diameter of 19 mm and 22 mm, respectively. The distance between the measure sites of the two probes was separated by 17 mm.

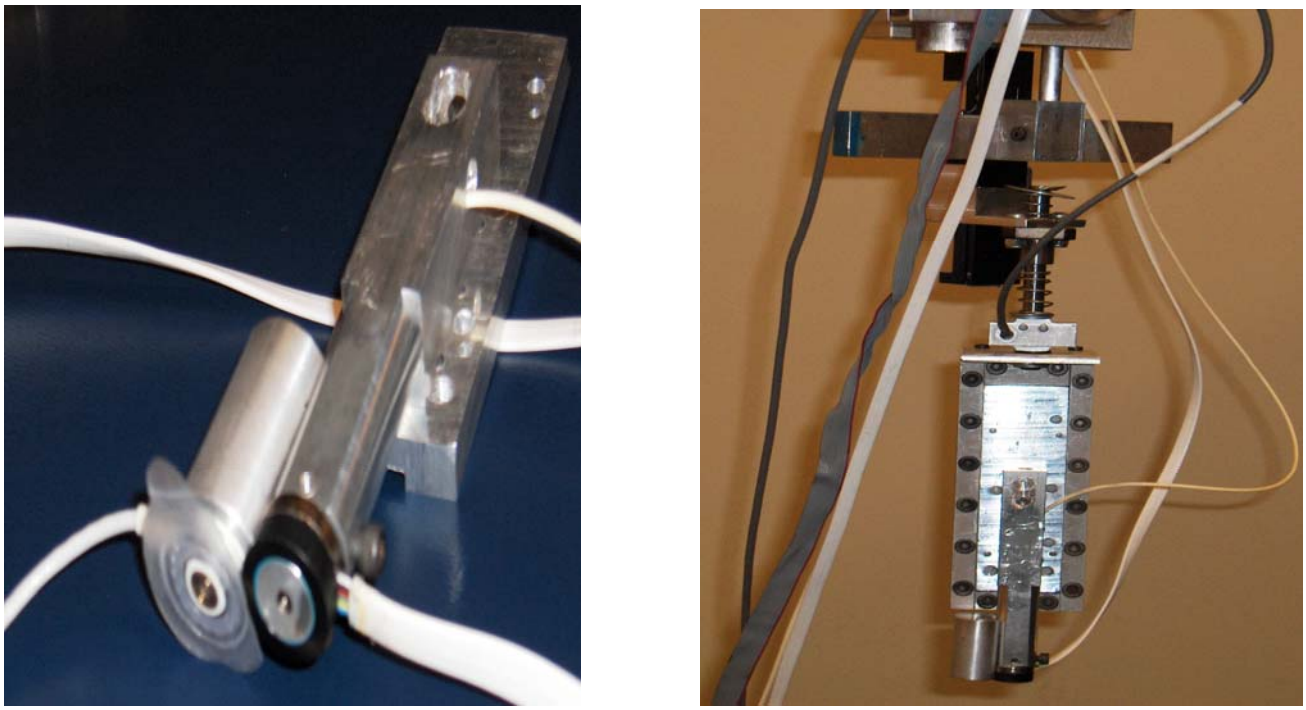

Figure 2. Combined indenter head (left) on computer controlled indenter (right)

Additional transcutaneous oxygenation probes were attached to the left sacrum, and the left and right heels. All transcutaneous oxygenation probes were calibrated according to manufacturer's guidelines. Transcutaneous oxygenation probes were held in place using adhesive rings supplied by the manufacturer, and contact liquid was placed between the skin/probe interface, according to manufacturer's guidelines. Temperature on all four transcutaneous oxygenation probes and the LDF was held constant at 37 °C. The combined

indenter was held onto the right sacrum with slight pressure (3 mmHg). Ten minutes of baseline data was recorded at this pressure. After 10 minutes, the computer controlled indenter increased the pressure to 150 mmHg. This pressure was held for 5 minutes. Skin blood flow and transcutaneous oxygenation were then recorded for an additional 15 minutes following the high pressure phase, in which pressure was held at 3 mmHg.

After a 30 minute washout period, this testing protocol was repeated; however, the heat for the LDF and the transcutaneous oxygenation was set at 44 °C. All other aspects of the test were identical to the previous test.

Local pressure and local heating were utilized in this study. Pressure ulcers are considered a local phenomena, in which local effects lead to the formation of pressure ulcers. Other investigator have used indirect heating and indirect pressure while studying skin blood flow and transcutaneous oxygenation. Because pressure ulcers are considered to be caused by local stimulus, local heating and local pressure were utilized in this thesis.

3.3 INSTRUMENTATION

The Laserflow (Blood Perfusion Monitor 2; Vasamedics, Eden Prairie, MN, USA) and Softip pencil probe (P-435; Vasamedics) were used to measure capillary blood perfusion (ml/min per 100 g tissue). The light source of the Laserflow is the Helium-Neon laser with wavelength of 760-800 nm. This wavelength is minimally absorbed by melanin, and can be used to measure blood flow in individuals with a variety of skin colors. A temperature control module (TCO; Vasamedics) with heater probe (P-422; Vasamedics) was used to heat the skin to 37 and 44 °C. Laser Doppler skin blood flow signal was sampled at 20 Hz throughout testing. Real time

recording of the data was achieved using the LabVIEW Software 7.1 (National Instruments, Austen, TX, USA)

The TCM 400 Transcutaneous pO_2 Monitoring System (Radiometer Medical, Denmark) was used to measure transcutaneous oxygenation. The TCM 400 Transcutaneous pO_2 Monitoring System allows 1 to 6 probes to be used to measure $TcPO_2$. All probes were calibrated according to manufacturer's recommendation. The TCM 400 Transcutaneous pO2 Monitoring System contains a built in barometer which affords the user to calibrate the probes to room air. Each probe contains a heating element which allows for temperatures to be set between 37 °C to 45 °C in increments of 0.5 °C, with accuracy better than $\pm$ 0.1 °C. The skin is not very permeable to gases under unheated conditions. At higher temperatures the ability of the skin to transport gases is greatly improved. Heat causes three effects on the skin's surface oxygen tension, thus making measurement of $TcPO_2$ possible. First, heating the stratum corneum beyond 40 ˚C changes its structure, which allows oxygen to diffuse faster. Secondly, heat causes the oxygenation dissociation curve to shift to the right, thus more oxygenation is released from hemoglobin. Lastly, dermal capillary hyperemia is induced by the heat.[76]

The TCM 400 monitor has a Clark polarographic electrode. The electrode tip is covered with a thin membrane which oxygen diffuses through to the cathode where a reduction of oxygen occurs as a result: $O_2 + 2H_2O + 4e^- \rightarrow 4\ OH^-$. At the anode the following reaction takes place: $4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$. The reduction of oxygen at the electrode's cathode generates a current that is converted into a voltage and digitized. This digitized signal is then passed to the microcomputer where it is reconverted to display pO_2 in mmHg. The TCM 400 takes a recording every 10 seconds, and stores this information on the machine, which can then be exported to an external PC.

The left and right sacrum and the left and right heel were chosen as testing sites to monitor systemic changes in $TcPO_2$. These two sites, sacrum and heel, have high incidence of pressure ulcerations and different microvasculature. Glabrous skin, such as the heel, contains many arteriovenous shunts, whose main role is that of thermoregulation.[104] Non-glabrous skin, such as the sacrum, contains fewer arteriovenous shunts and its blood supply is mainly nutritive in nature.

3.4 WAVELET ANALYSIS

Wavelet analysis was performed on the skin blood flow data measured at 20 Hz. Wavelet analysis provides a multi-resolution, time-frequency analysis of sacral skin blood flow. Wavelet transform decomposes a signal (i.e. sacral skin blood flow) over dilated and translated wavelets.[168] Continuous wavelet transform of a signal *f(u)* was defined as:[169]

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

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

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

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 transformed data are easier to interpret and/or are amenable to pattern recognition because their complete scales tend to reinforce the traits and make all information more visible than data from discrete wavelet transform.[170]

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

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

where ω_o will be designated as 2π.[85] The relative contribution of each frequency band will be used to determine the dominant control mechanism.[42] Matlab 6.0 and Wavelet Toolbox (The MathWorks Inc., Natick, MA) was used to perform wavelet transforms and normalization.

The characteristic frequency bands associated with the individual control mechanisms are as follow: endothelial nitric oxide (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 rationale for designation of a frequency range for each characteristic frequency band's control mechanism is described in previous work.[42]

The power of each of the five frequency bands was averaged from the 2 minute to the 10 minute period. The first two minutes were eliminated to diminish any edge effects. The change in posture took anywhere from 30 to 60 seconds for each subject. Wavelet analysis was then performed on the 8 minutes following complete change in posture, with the last two minutes not used to eliminate any edge effects.

3.5 STATISTICAL ANALYSIS

Skin blood flow data was re-sampled from 20 Hz to 0.1 Hz by averaging the data over this time period to match the sampling of the $TcPO_2$. A Shapiro-Wilk test was performed to check the normality of the data. A Spearman's rho test of correlation was subsequently

performed to get correlation coefficients between right sacral skin blood flow and right sacral transcutaneous oxygenation. A two-tailed t-test was performed to see if the correlation was significant. A Wilcoxon Signed Ranks Test was performed to see if skin blood flow and transcutaneous oxygenation values before and after orthostatic stimulation and occlusion were significantly different.

4.0 RESULTS

4.1 ORTHOSTATIC STIMULATION AT 44 °C

Skin blood flow data over the right sacrum and transcutaneous oxygenation data over the right sacrum, both at 44 °C, was plotted against time for each subject (Figures 3-7). Skin blood flow data was reduced from 20 Hz to 1 data point for every 10 seconds by averaging the blood flow data over this time period.

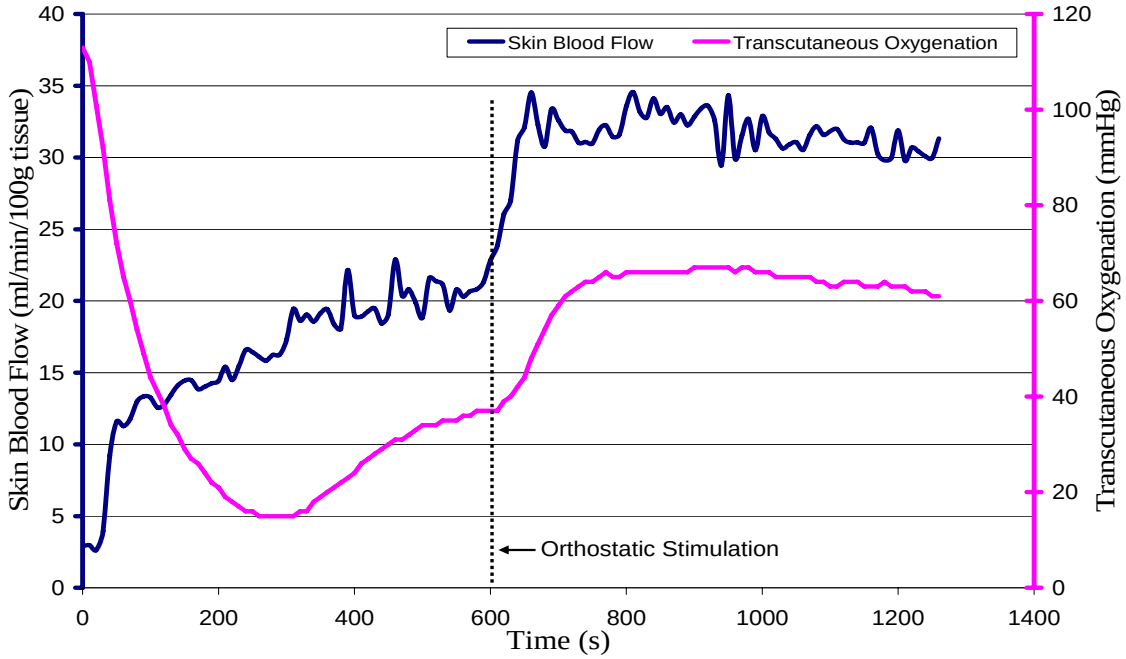

Figure 3. Subject 1, skin blood flow and $TcPO_2$ during orthostatic stimulation at 44 °C

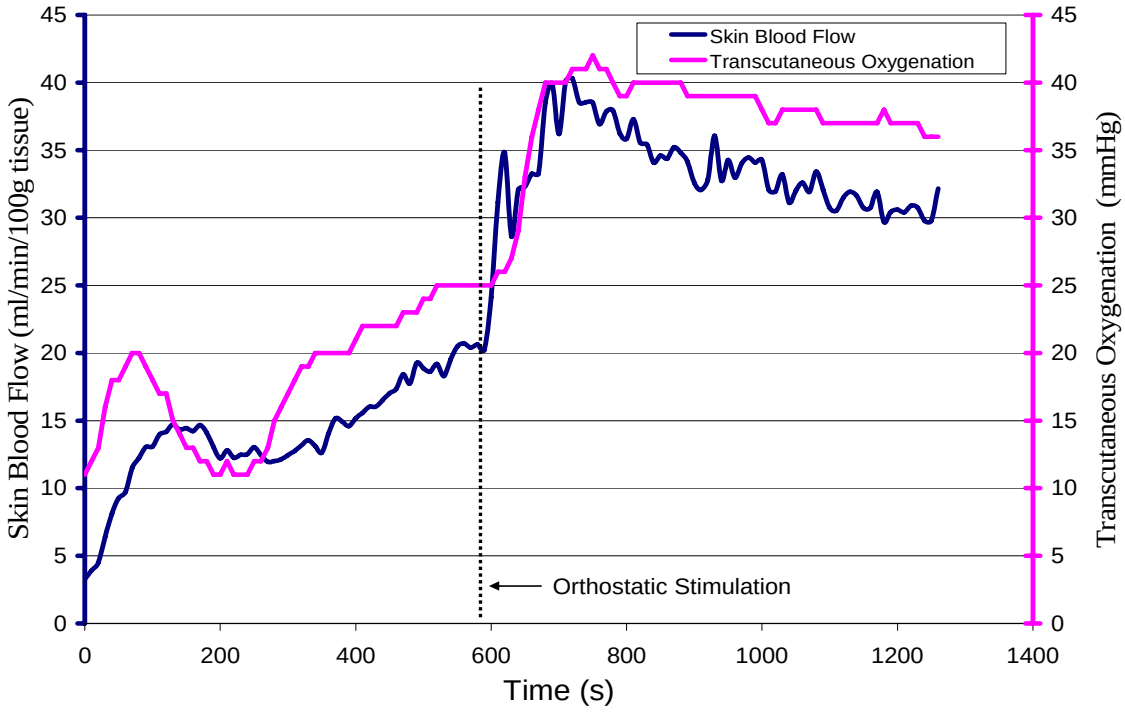

Figure 4. Subject 2, skin blood flow and $TcPO_2$ during orthostatic stimulation at 44 °C

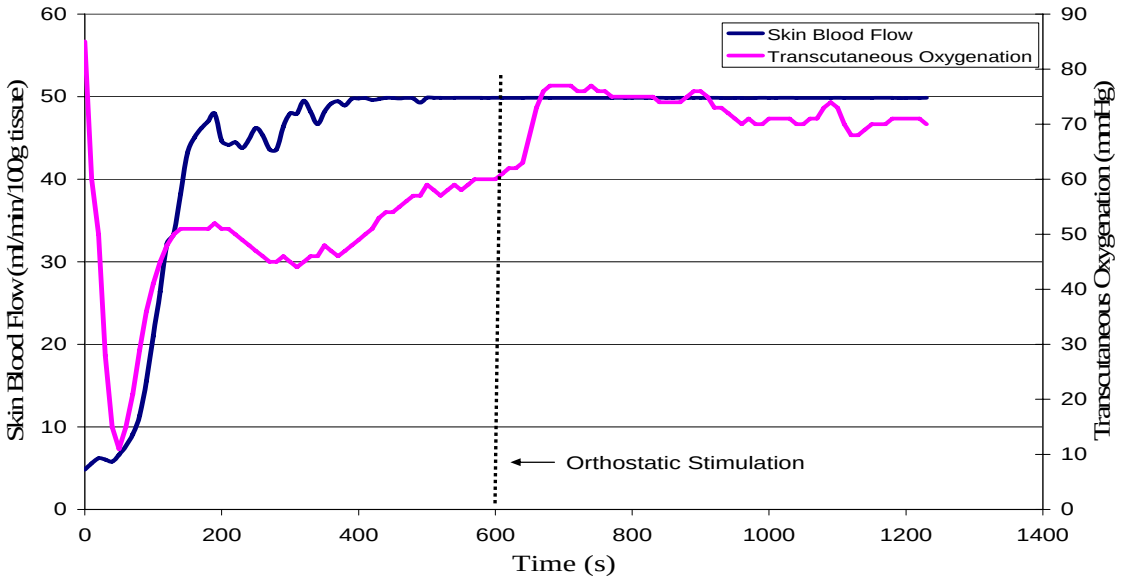

Figure 5. Subject 3, skin blood flow and $TcPO_2$ during orthostatic stimulation at 44 °C

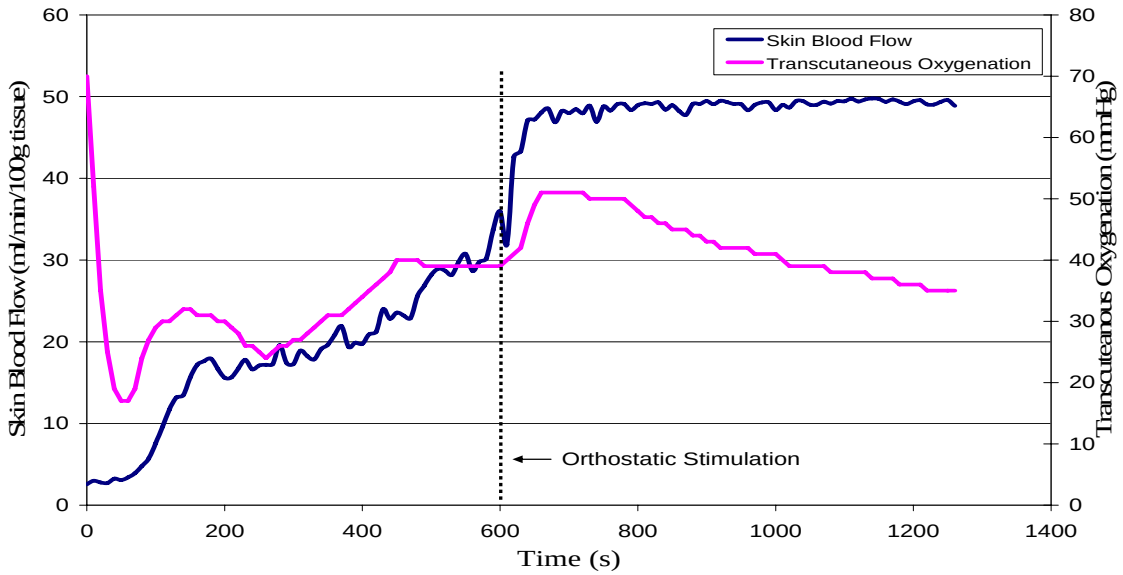

Figure 6. Subject 4, skin blood flow and $TcPO_2$ during orthostatic stimulation at 44 °C

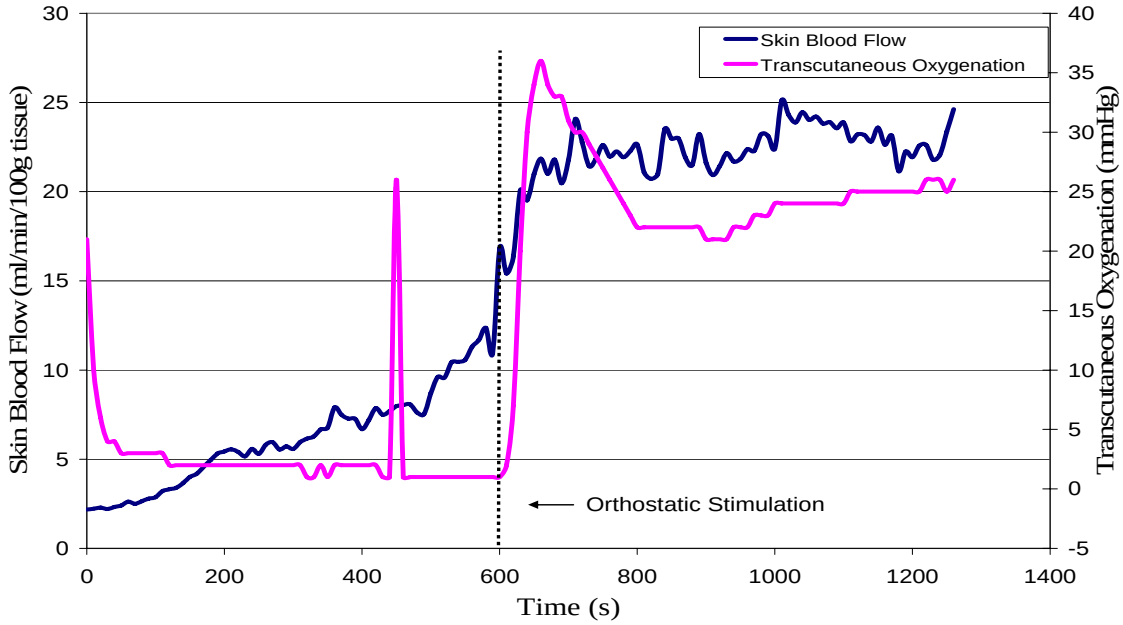

Figure 7. Subject 5, skin blood flow and $TcPO_2$ during orthostatic stimulation at 44 °C

A nonparametric test of correlation, Spearman's rho, was performed on each subject's right sacrum skin blood flow and transcutaneous oxygenation data. Correlation coefficients were obtained and a two tailed t-test was performed for significance; results are depicted in Table 2. The average correlation coefficient between the five subjects was 0.661.

Table 2 Orthostatic stimulation correlation coefficients

Subject	Correlation Coefficient	Significance level
1	0.539	.01
2	0.949	.01
3	0.635	.01
4	0.525	.01
5	0.657	.01
Average	0.661	---

Skin blood flow data at 44 °C for each subject was then averaged before orthostatic stimulation and after orthostatic stimulation. The period of time in which subjects changed posture was eliminated from analysis because of the artifact that was created in the laser Doppler skin blood flow signal. The percent differences between the two were then calculated. Results are depicted in Table 3.

Table 3 44°C orthostatic stimulation skin blood flow

Subject	Skin Blood Flow Pre-Orthostatic Stimulation	Skin Blood Flow Post Orthostatic Stimulation	% Difference
1	16.29	31.79	95.09
2	14.31	33.80	136.17
3	39.96	49.83	24.72
4	18.08	48.95	170.62
5	6.31	22.58	257.65
Average	18.99	37.39	96.86

Skin blood flow at 44 °C following orthostatic stimulation shows a remarkable increase, as compared to pre-orthostatic stimulation skin blood flow values. On average, skin blood flow increased by 96.86% following orthostatic stimulation. A nonparametric test of significance, Wilcoxon Signed Ranked Test was performed and this difference is statistically significant

($p<0.05$). Following orthostasis skin blood flow values either remained elevated or slightly decreased throughout the seating period; however, skin blood flow values remained significantly higher than pre-orthostatic values.

Transcutaneous oxygenation at 44 °C over the right sacrum was averaged for each subject both before and after orthostatic stimulation. The transitional time period from prone to seated position was eliminated from the data analysis. The results are depicted in Table 4.

Table 4. 44°C orthostatic stimulation $TcPO_2$

Subject	$TcPO_2$ Pre-Orthostatic Stimulation	$TcPO_2$ Post Orthostatic Stimulation	% Difference
1	35.19	63.77	81.18
2	18.36	38.55	110
3	48.60	72.45	49.07
4	32.52	42.49	30.64
5	2.7	24.85	802.38
Average	27.48	48.42	76.16

In general, $TcPO_2$ values over the right sacrum showed a significant increase after change in posture. On average, $TcPO_2$ values increased 76.16 % after orthostasis. A nonparametric test of significance, Wilcoxon Signed Ranks Test, was performed and this difference is statistically significant ($p<0.05$). $TcPO_2$ values appear to be delayed by 20 seconds in response to orthostatic stimulation.

Transcutaneous oxygenation values over all 4 sites (left/right sacrum and left/right heel) were plotted against time for each subject (Figures 8-12). In certain instances all 4 probes could not be calibrated. In this case the left heel was not used as a test site. If two probes could not be calibrated both the left heel and the left sacrum were not tested. This allowed for comparison of glabrous (heel) versus non-glabrous (sacrum) skin.

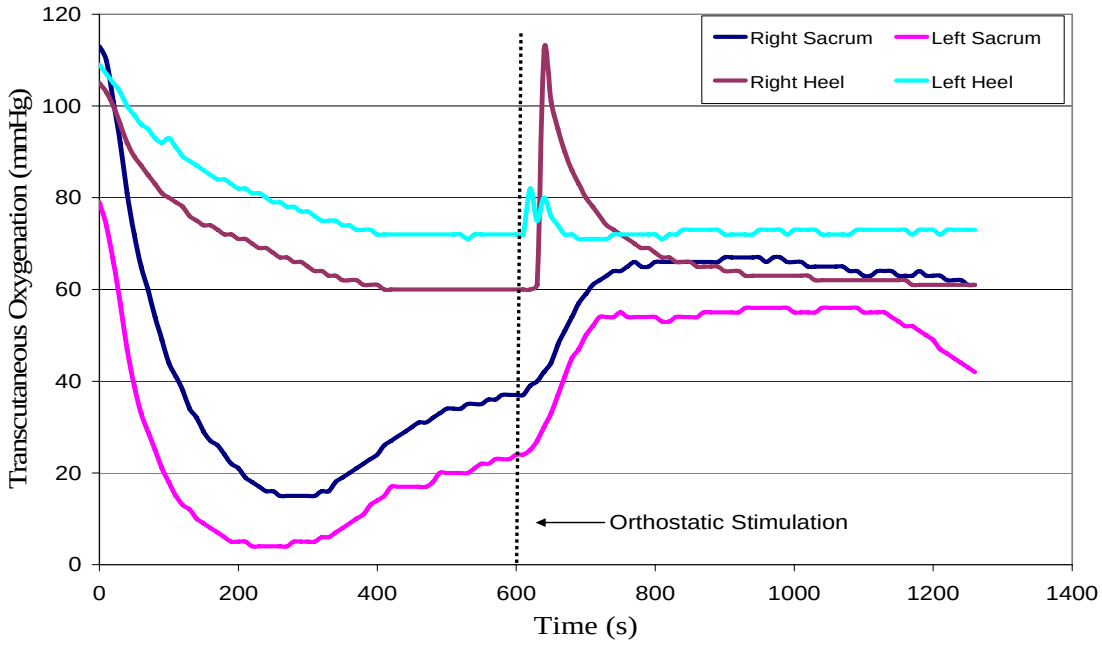

Figure 8. Subject 1, $TcPO_2$ during orthostatic stimulation at 44 °C

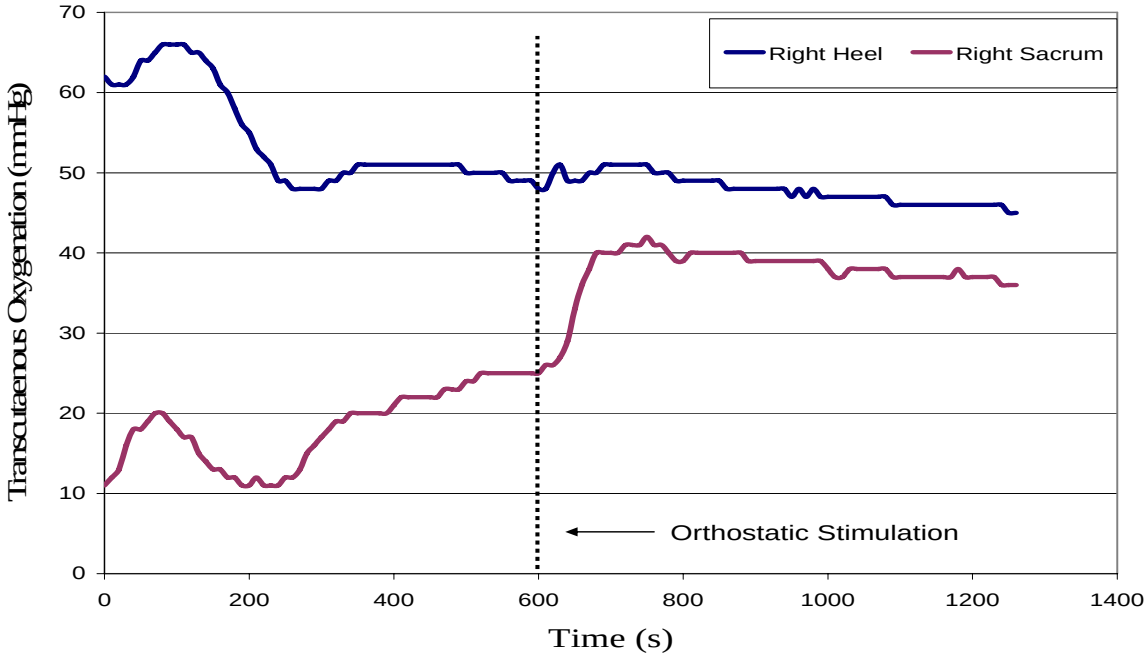

Figure 9. Subject 2, $TcPO_2$ during orthostatic stimulation at 44 °C

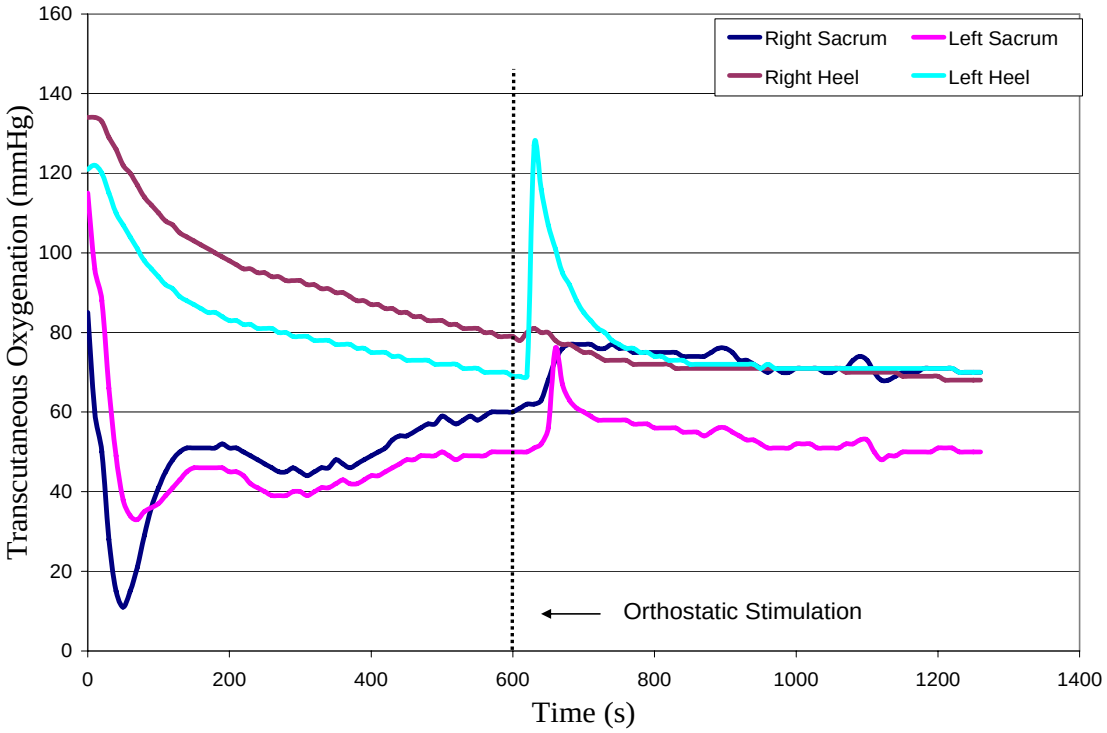

Figure 10. Subject 3, $TcPO_2$ during orthostatic stimulation at 44 °C

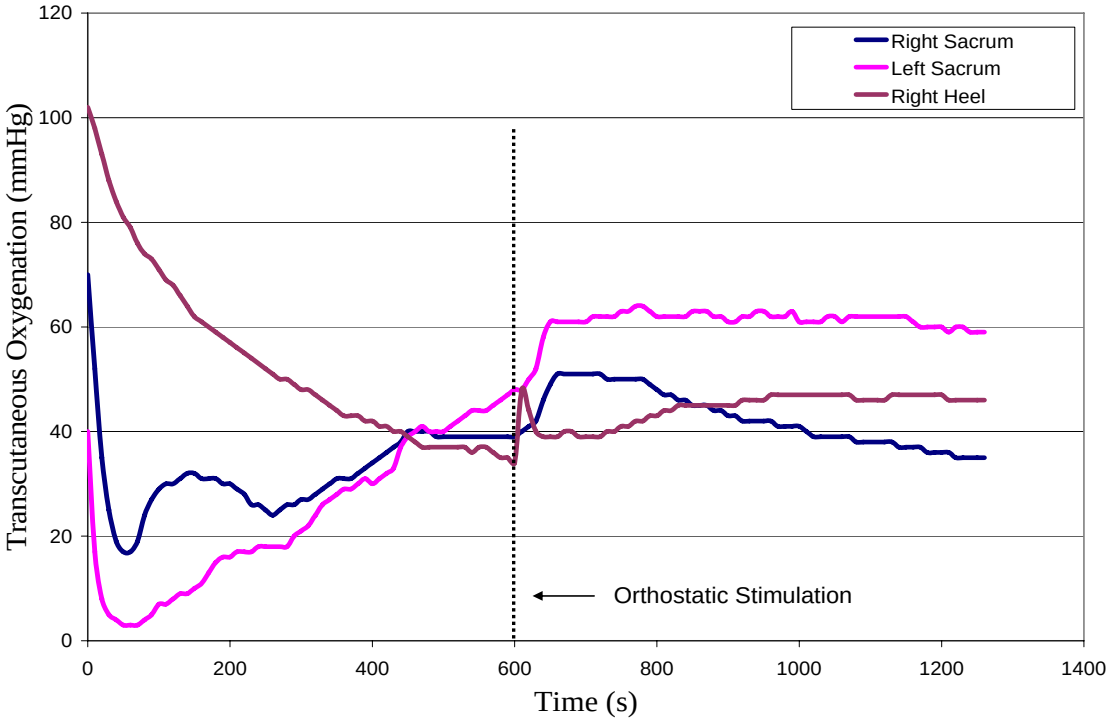

Figure 11. Subject 4, $TcPO_2$ during orthostatic stimulation at 44 °C

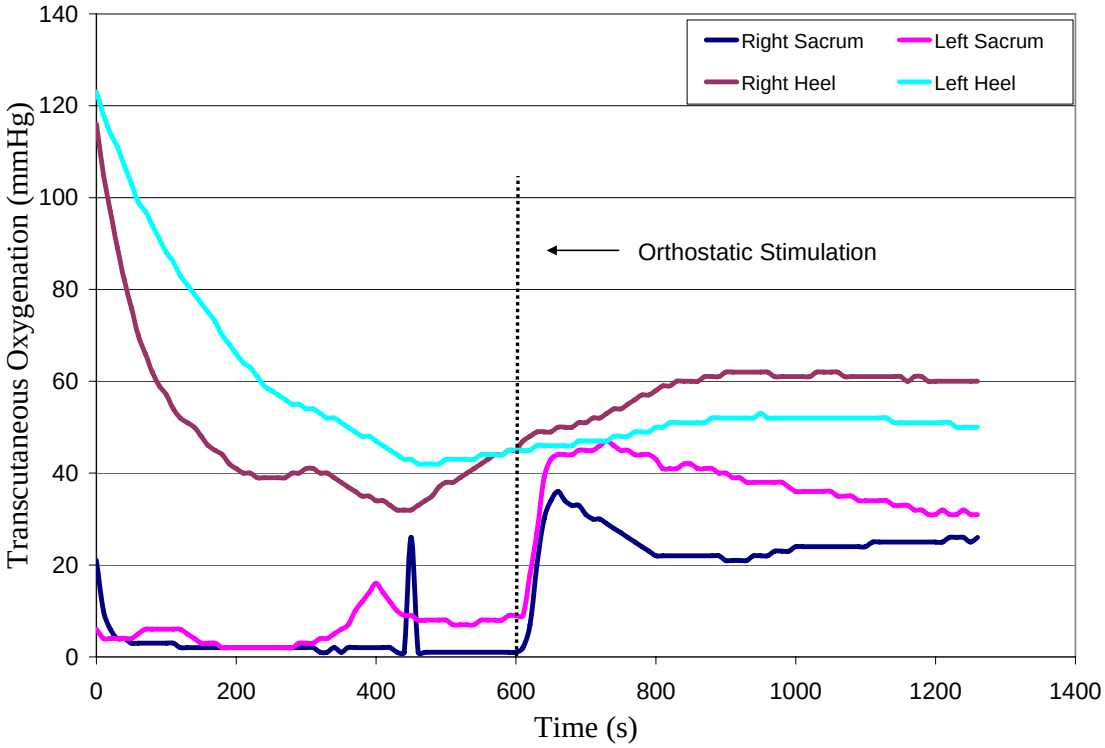

Figure 12. Subject 5, $TcPO_2$ during orthostatic stimulation at 44 °C

Visual inspection of the data shows that sacral $TcPO_2$ values tended to mirror each other throughout testing. Heel $TcPO_2$ values also shared similar characteristics throughout the testing protocol for all subjects. Sacral $TcPO_2$ values were on average 20 seconds delayed following orthostasis. Heel $TcPO_2$ values were delayed on average 10 seconds following orthostasis. However, sacral $TcPO_2$ values responded in greater magnitude to the orthostatic stimulation. Each subjects' average $TcPO_2$ values at each test site was plotted both before and after orthostatic stimulation (Figures 13-16).

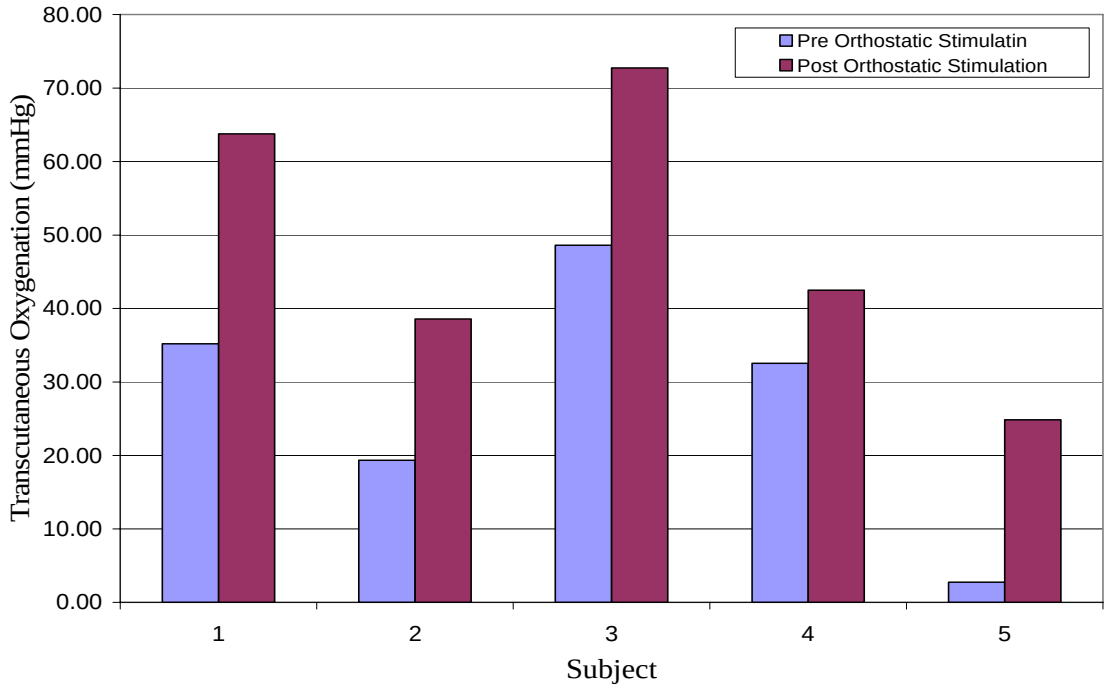

Figure 13. Right sacrum orthostatic stimulation at 44 °C

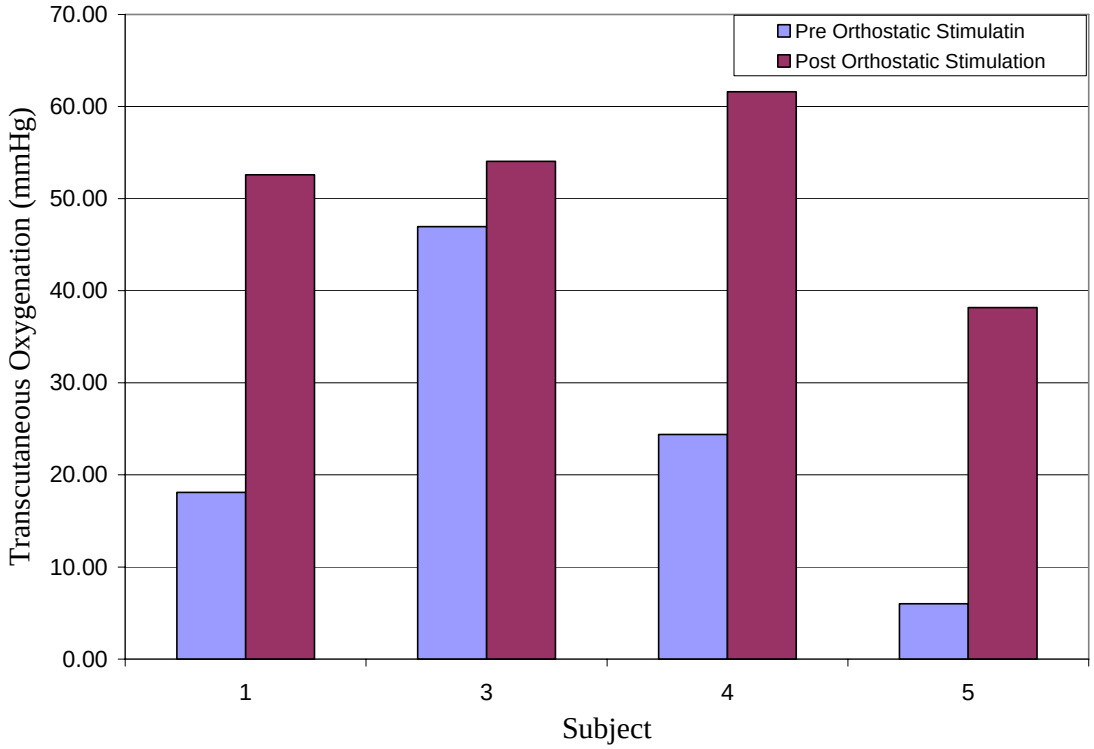

Figure 14. Left sacrum orthostatic stimulation at 44 °C

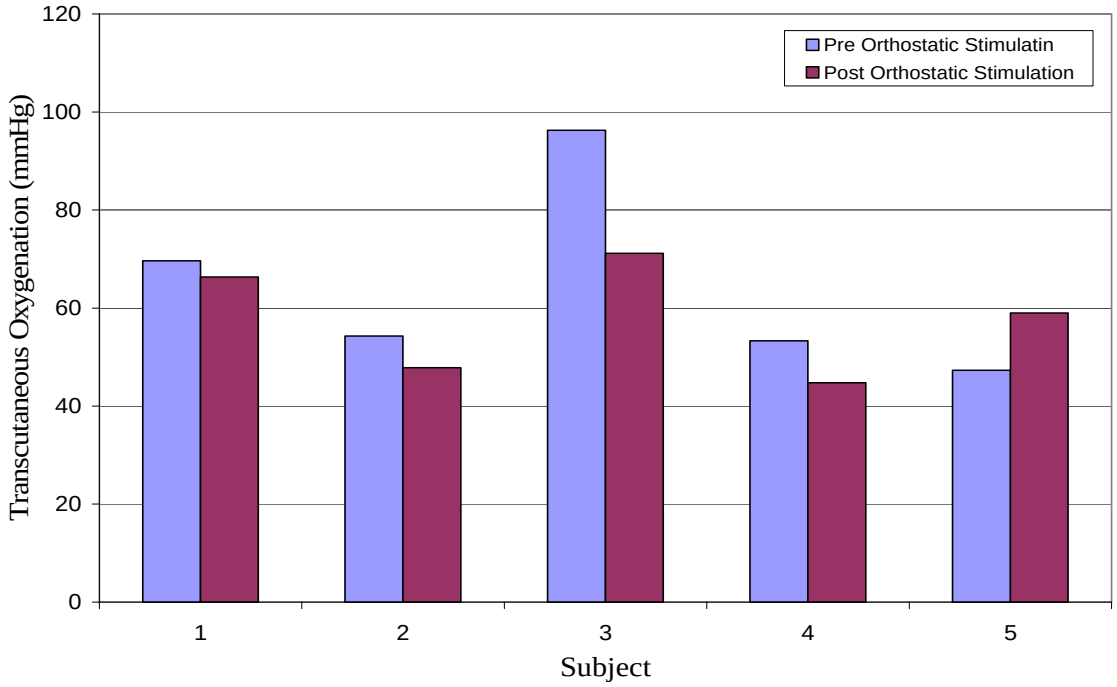

Figure 15. Right heel orthostatic stimulation at 44 °C

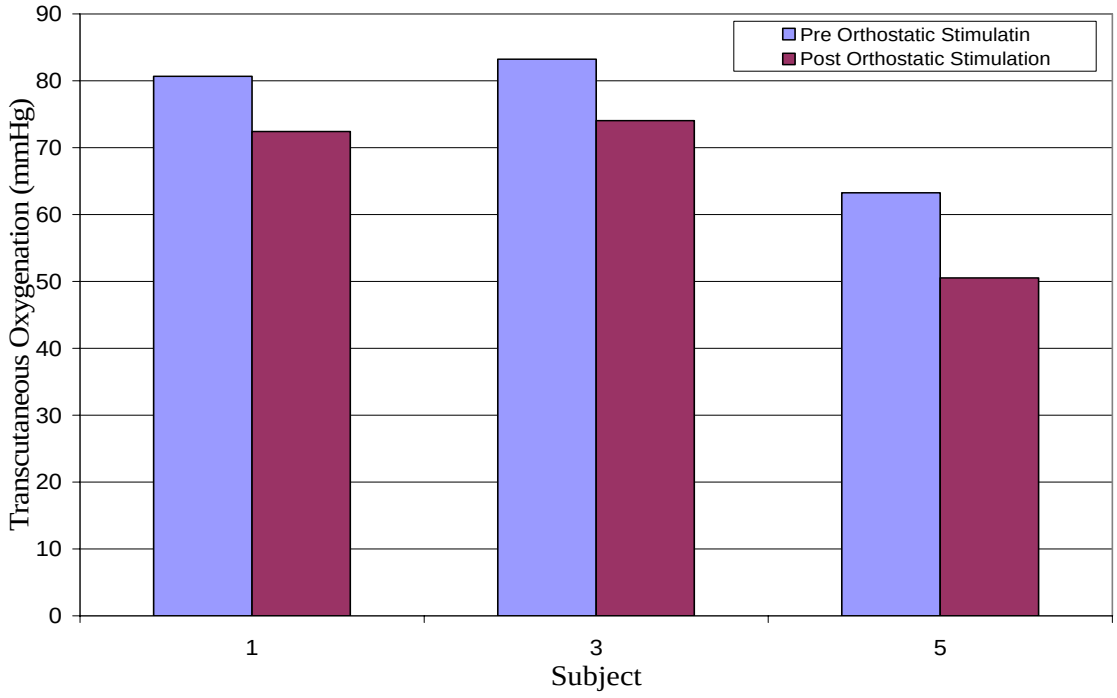

Figure 16. Left heel orthostatic stimulation at 44 °C

All of the subjects showed an increased in average transcutaneous oxygenation over their left and right sacrum following orthostatic stimulation. However, over the left and right heels, the opposite is true. All data for the left and right heels shows a decrease in average transcutaneous oxygenation following orthostatic stimulation, except for subject 5's right heel, which shows an increase. Only the right sacrum data shows a significant difference between pre and post $TcPO_2$ levels ($p < 0.05$) using a Wilcoxon Signed Ranks Test. This is summarized in Table 5.

Table 5. 44°C orthostatic stimulation $TcPO_2$ at each anatomical site

	Pre RS	**Post RS**	**% Diff**	**Pre LS**	**Post LS**	**% Diff**	**Pre RH**	**Post RH**	**% Diff**	**Pre LH**	**Post LH**	**% Diff**
1	35.19	63.77	81.18	18.10	52.57	190.48	69.61	66.33	-4.74	80.67	72.43	-10.22
2	18.36	38.55	110	------	------	--------	54.30	47.82	-11.93	------	------	------
3	48.60	72.45	49.07	46.95	54.05	15.11	96.26	71.18	-26.06	83.23	74.07	-11.01
4	32.52	42.49	30.64	24.39	61.61	152.55	53.31	44.75	-16.05	------	------	------
5	2.7	24.85	802.38	6.00	38.16	536.06	47.28	59.02	24.83	63.26	50.52	-20.13
Avg	27.48	48.42	76.16	23.86	51.60	116.24	64.15	57.82	-9.87	75.72	65.67	-13.27

Abbreviations: Pre = TcPO2 before orthostatic stimulation; Post = $TcPO_2$ after orthostatic simulation. RS = Right Sacrum; LS = Left Sacrum; RH = Right Heel; LH = Left Heel

Wavelet analysis was performed on the skin blood flow data for each subject. The power was normalized and averaged for all five subjects, the results of which are depicted in Figure 15. No significant increase or decrease in power was detected ($p < 0.05$).

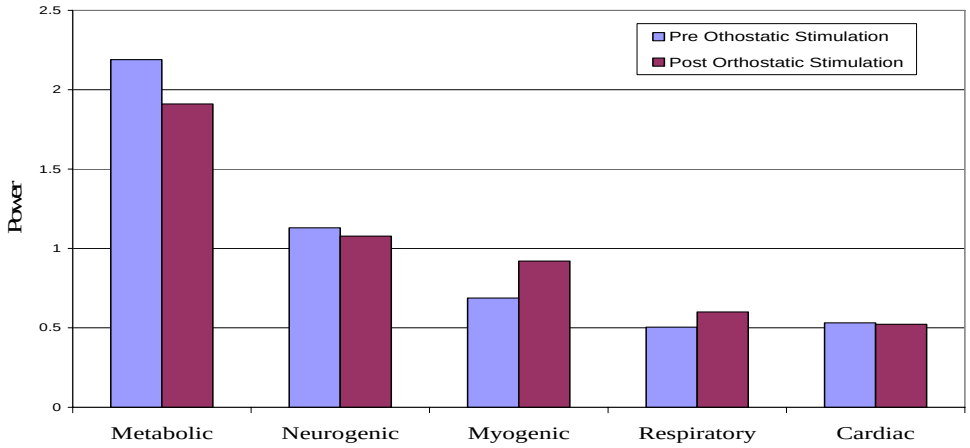

Figure 17. Normalized power comparison of the five characteristic frequency bands

4.2 ORTHOSTATIC STIMULATI ON AT 37 °C

Skin blood flow data over the right sacrum and transcutaneous oxygenation data over the right sacrum, both at 37 °C, was plotted against time for each subject (Figures 18-22). Skin blood flow data was reduced from 20 Hz to 1 data point for every 10 seconds by averaging the blood flow data over this time period.

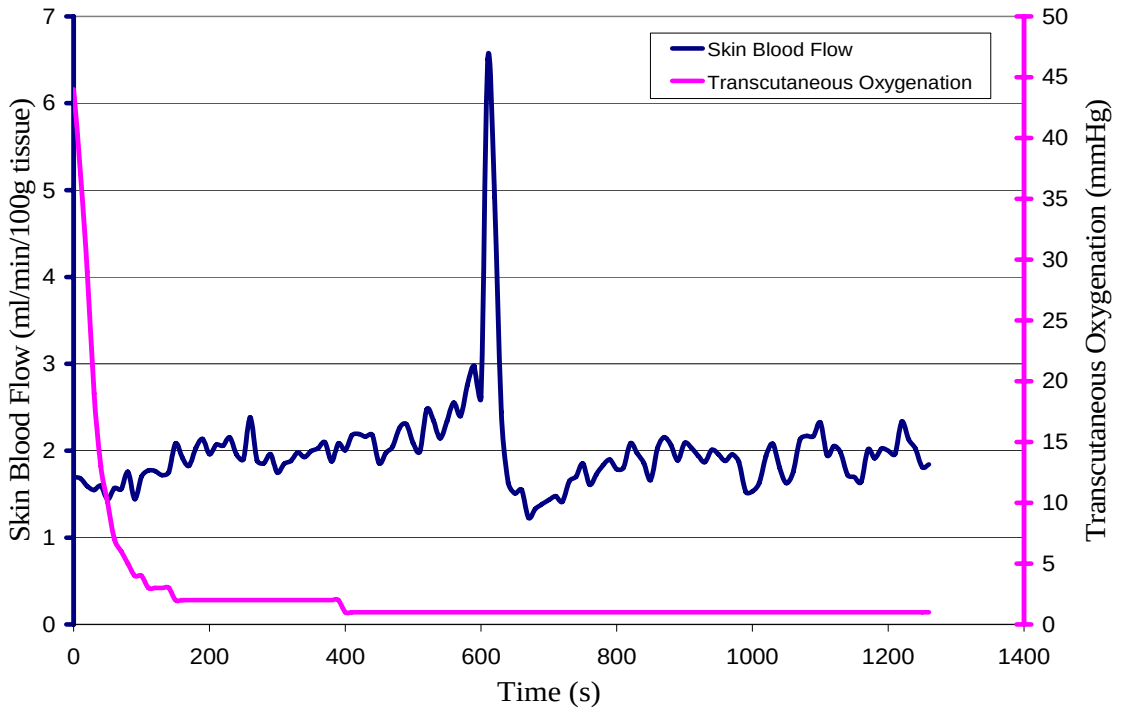

Figure 18. Subject 1, skin blood flow and $TcPO_2$ orthostatic stimulation at 37 °C

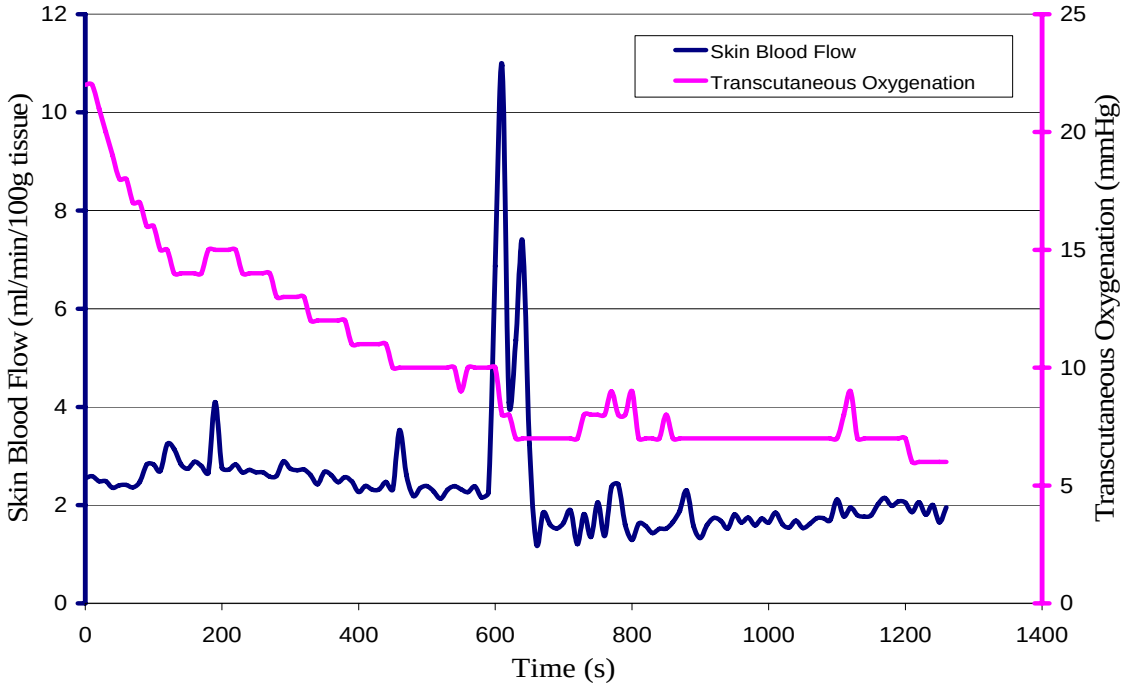

Figure 19. Subject 2, skin blood flow and $TcPO_2$ orthostatic stimulation at 37 °C

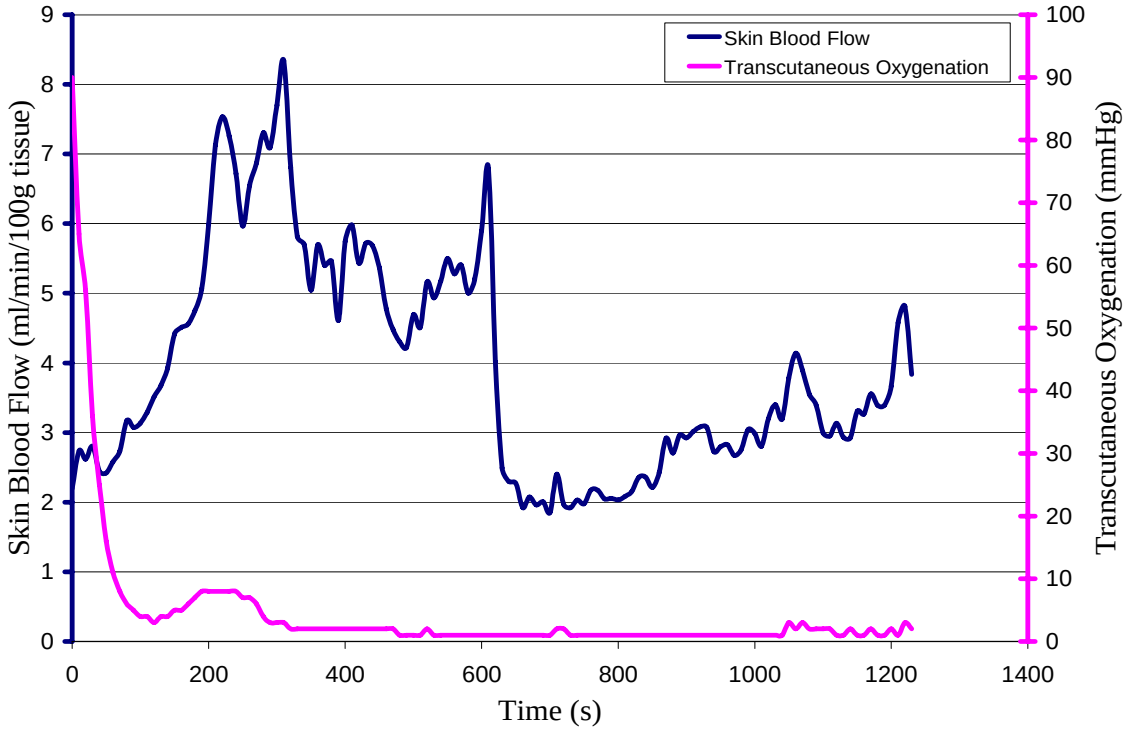

Figure 20. Subject 3, skin blood flow and $TcPO_2$ orthostatic stimulation at 37 °C

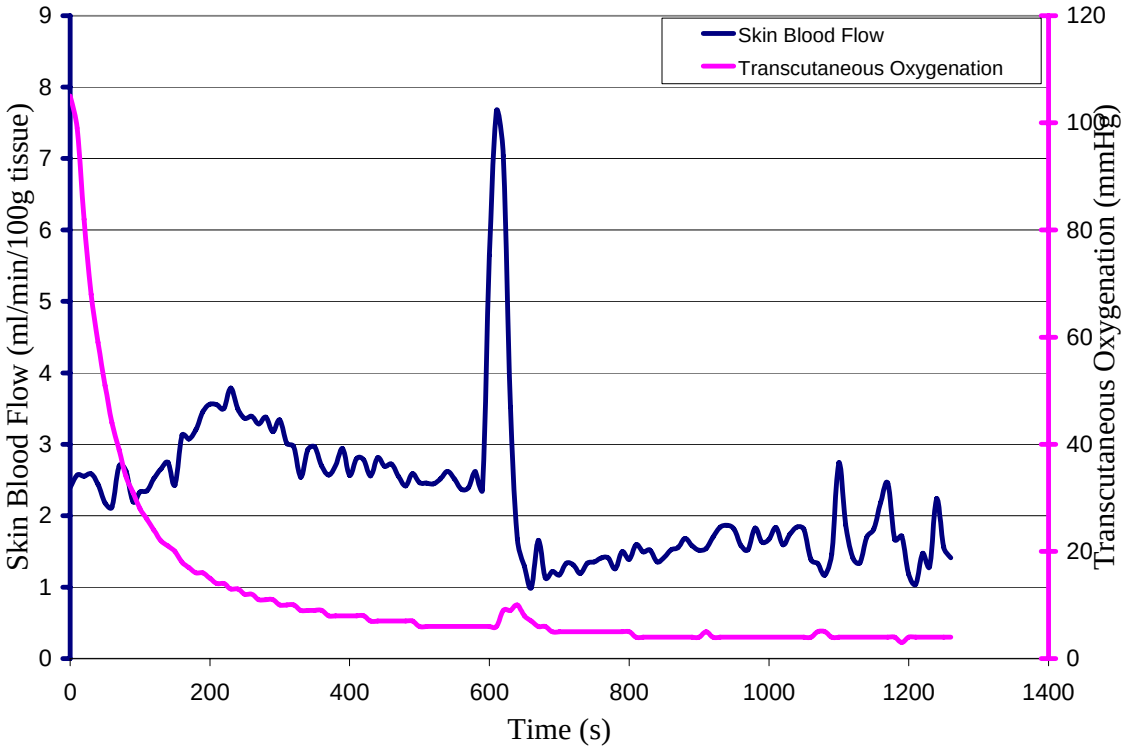

Figure 21. Subject 4, skin blood flow and $TcPO_2$ orthostatic stimulation at 37 °C

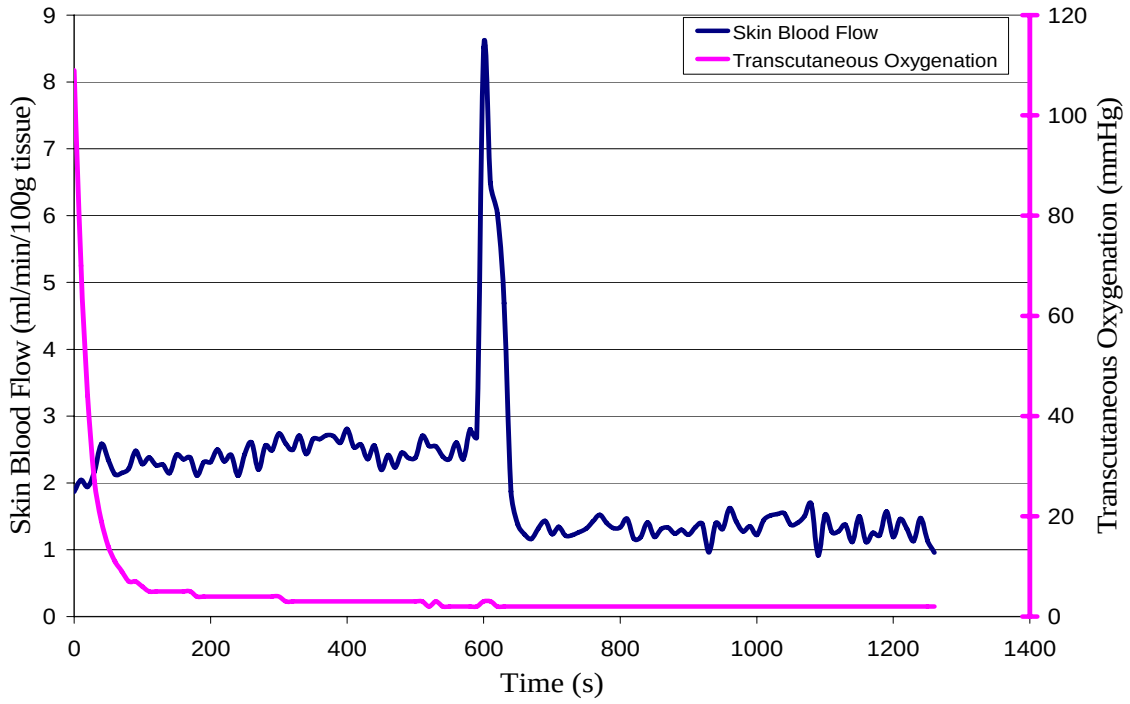

Figure 22. Subject 5, skin blood flow and $TcPO_2$ orthostatic stimulation at 37 °C

A nonparametric test of correlation, Spearman's rho, was performed on each subject's right sacrum skin blood flow and transcutaneous oxygenation. Correlation coefficients were obtained and a two tailed t-test was performed for significance; results are depicted in Table 6. The average correlation coefficient between the five subjects was 0.423.

Table 6. 37°C orthostatic stimulation correlation coefficients

Subject	Correlation Coefficient	Significance level
1	-.244	.01
2	.710	.01
3	.390	.01
4	.650	.01
5	.610	.01
Average	.423	---

Skin blood flow data at 37 °C for each subject was then averaged before orthostatic stimulation and after orthostatic stimulation. The period of time in which subjects changed posture was eliminated from analysis because of the artifact that was created in the laser Doppler skin blood flow signal. The percent differences between the two were then calculated. Results are depicted in Table 7.

Table 7. 37°C orthostatic stimulation skin blood flow

Subject	Skin Blood Flow Pre Orthostatic Stimulation	Skin Blood Flow Post Orthostatic Stimulation	% Difference
1	2.00	1.84	-7.63
2	2.66	1.73	-34.70
3	5.00	2.81	-43.67
4	2.82	1.55	-44.77
5	2.51	1.32	-47.47
Average	2.99	1.85	-38.12

Skin blood flow at 37 °C following orthostatic stimulation shows a remarkable decrease, as compared to pre-orthostatic stimulation skin blood flow values. On average, skin blood flow decreased by -38.12% following orthostatic stimulation. A nonparametric test of significance, Wilcoxon Signed Ranks Test, was performed and this difference is statistically significant to ($p<0.05$). Following orthostasis skin blood flow values remained decreased throughout the seating period.

Transcutaneous oxygenation at 37 °C over the right sacrum showed a general decline throughout the testing period, with orthostasis causing no significant effect on $TcPO_2$ values. Transcutaneous oxygenation values over all 4 sites (left/right sacrum and left/right heel) were plotted against time for each subject (Figures 23-27). In certain instances all 4 probes could not be calibrated. In this case the left heel was not used as a test site. If two probes could not be

calibrated both the left heel and the left sacrum were not tested. This allowed for comparison of glabrous (heel) versus non-glabrous (sacrum) skin.

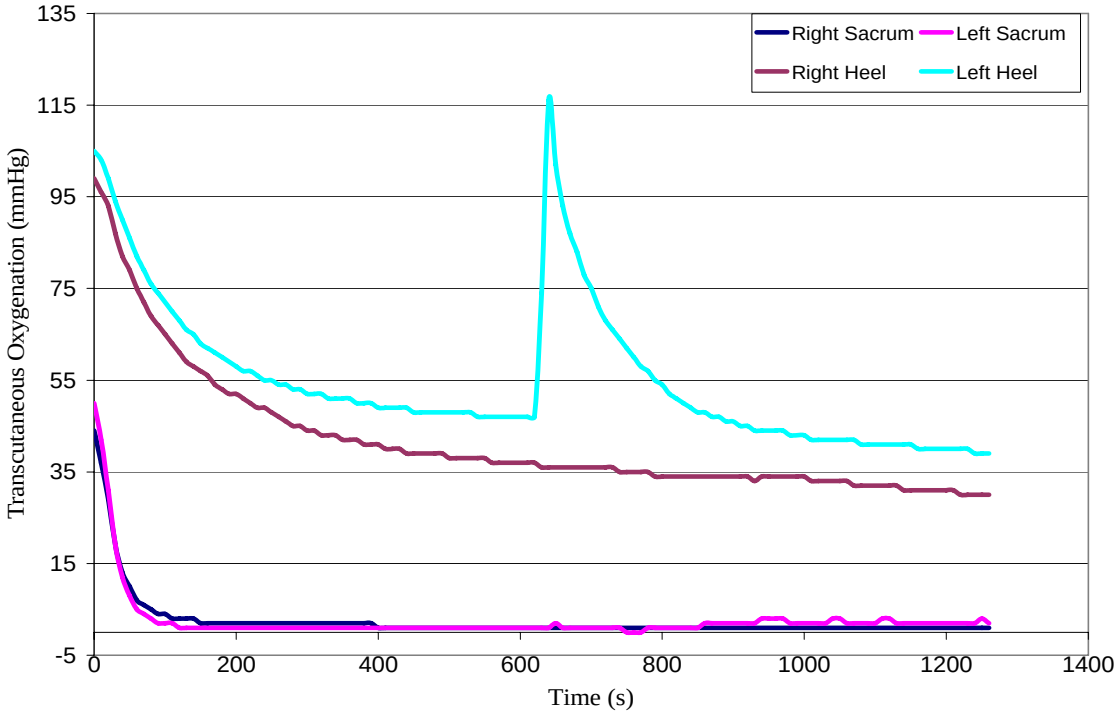

Figure 23. Subject 1, $TcPO_2$ during orthostatic stimulation at 37 °C

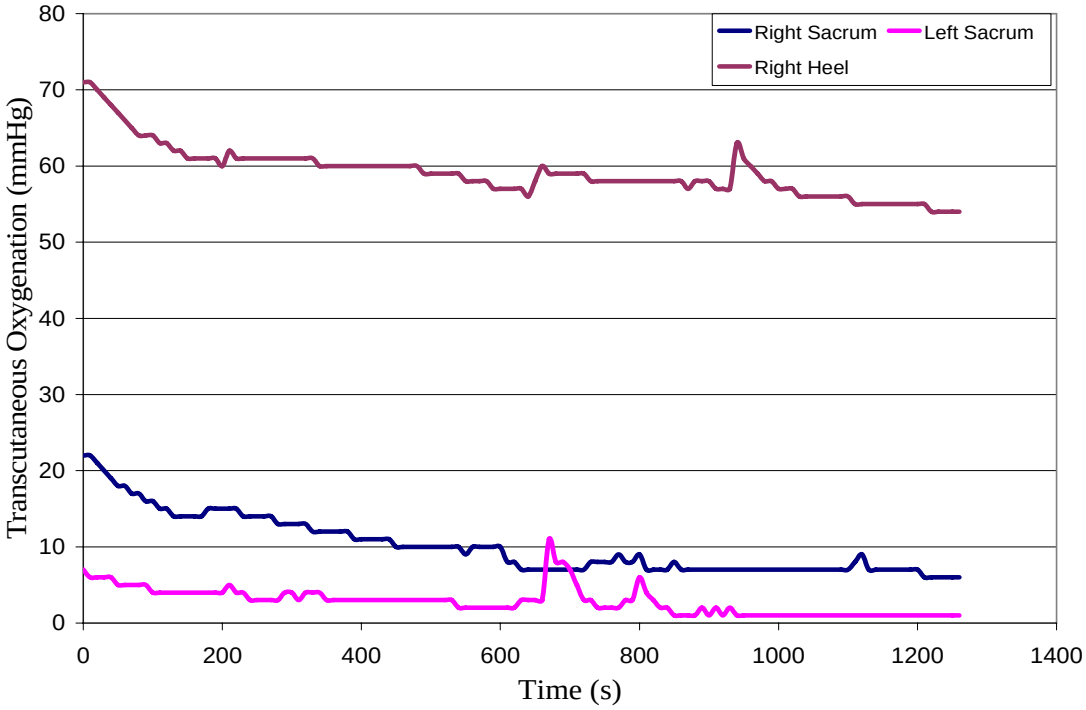

Figure 24. Subject 2, $TcPO_2$ during orthostatic stimulation at 37 °C

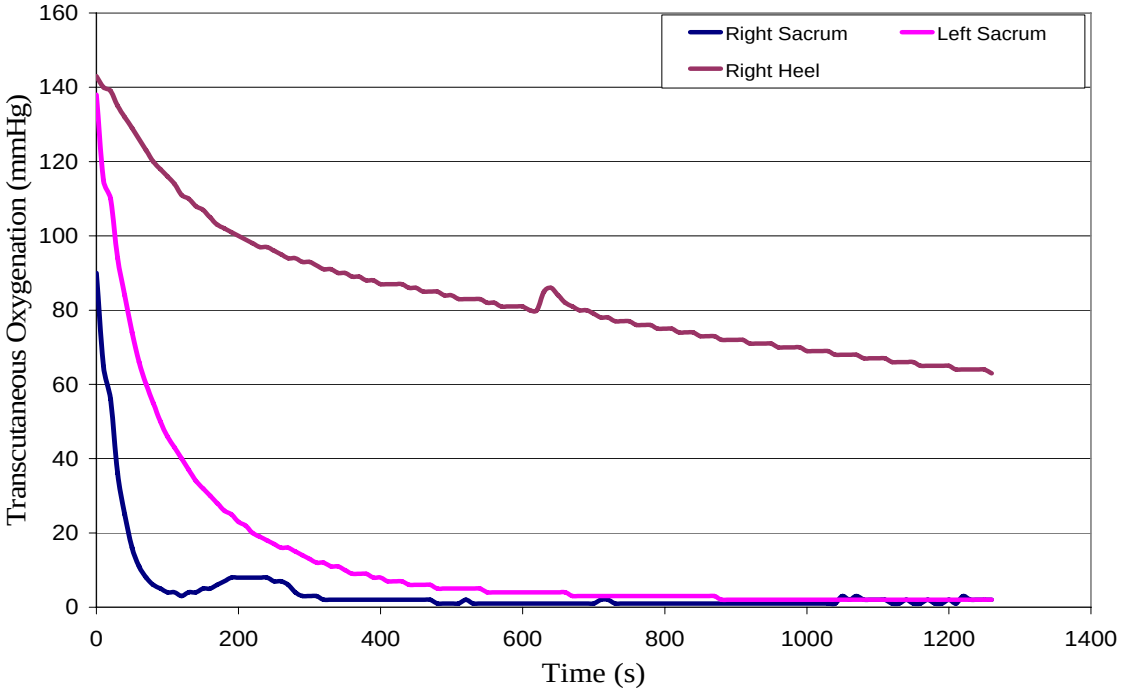

Figure 25. Subject 4, $TcPO_2$ during orthostatic stimulation at 37 °C

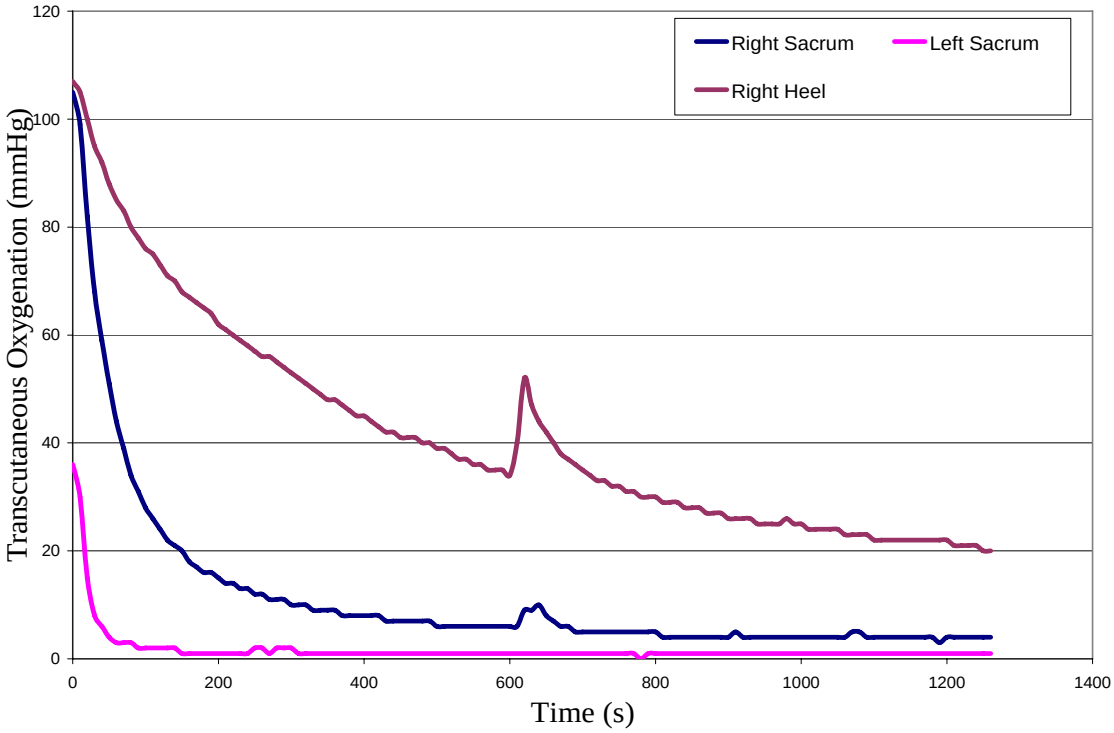

Figure 26. Subject 4, $TcPO_2$ during orthostatic stimulation at 37 °C

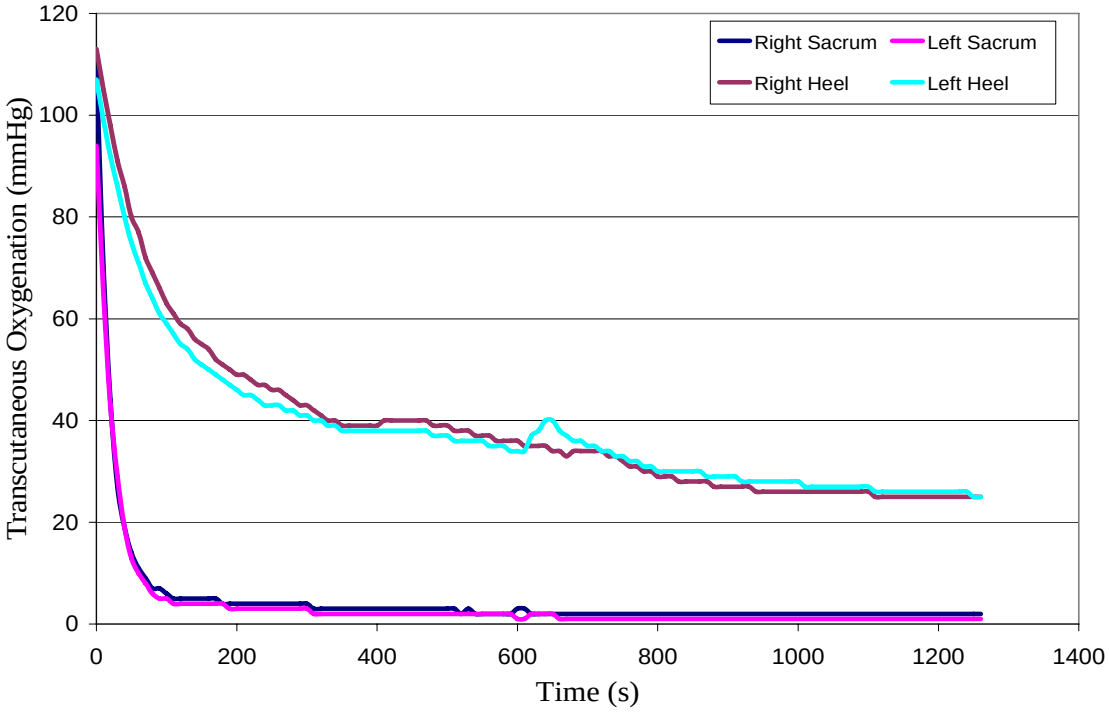

Figure 27. Subject 5, $TcPO_2$ during orthostatic stimulation at 37 °C

$TcPO_2$ data at 37 °C shows a general negative trend, with orthostasis causing no effect on values (aside from artifact during movement). Due to the general downward trend seen in the data, with orthostasis providing no effect, pre and post orthostatic stimulation values of $TcPO_2$ were not compared.

Wavelet analysis was performed on the skin blood flow data for each subject. The power was normalized and averaged for all five subjects, the results of which are depicted in Figure 25. No significant increase or decrease in power was detected (p=.05).

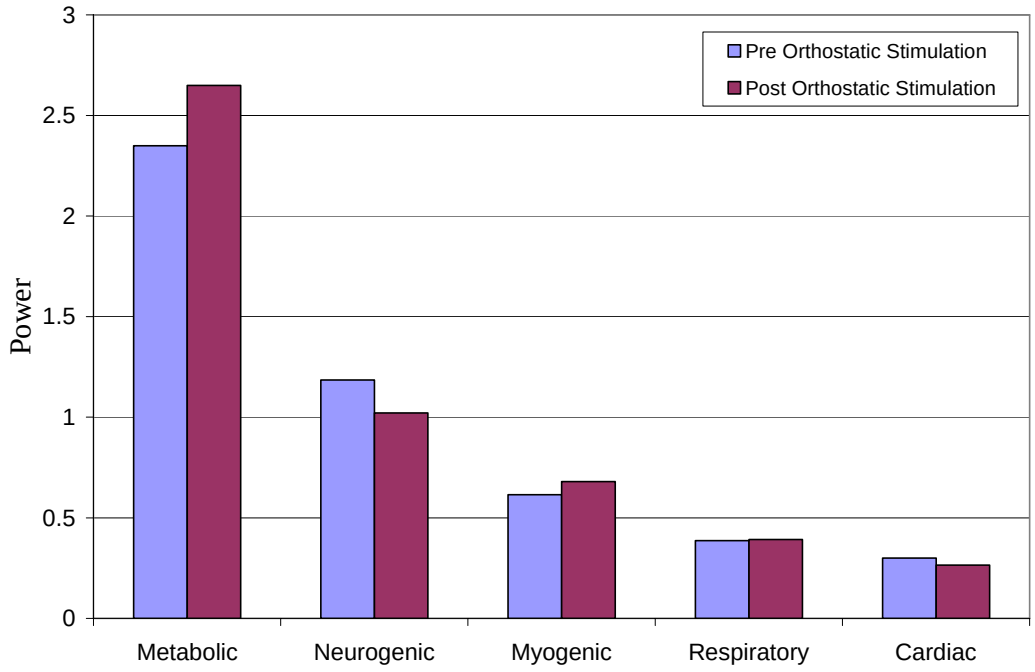

Figure 28. Normalized power comparison of the five characteristic frequency bands

4.3 HYPEREMIC RESPONSE AT 44 °C

Skin blood flow data over the right sacrum and transcutaneous oxygenation data over the right sacrum, both at 44 °C, was plotted against time for each subject (Figures 29-33). Skin blood flow data was reduced from 20 Hz to 1 data point for every 10 seconds by averaging the blood flow data over this time period.

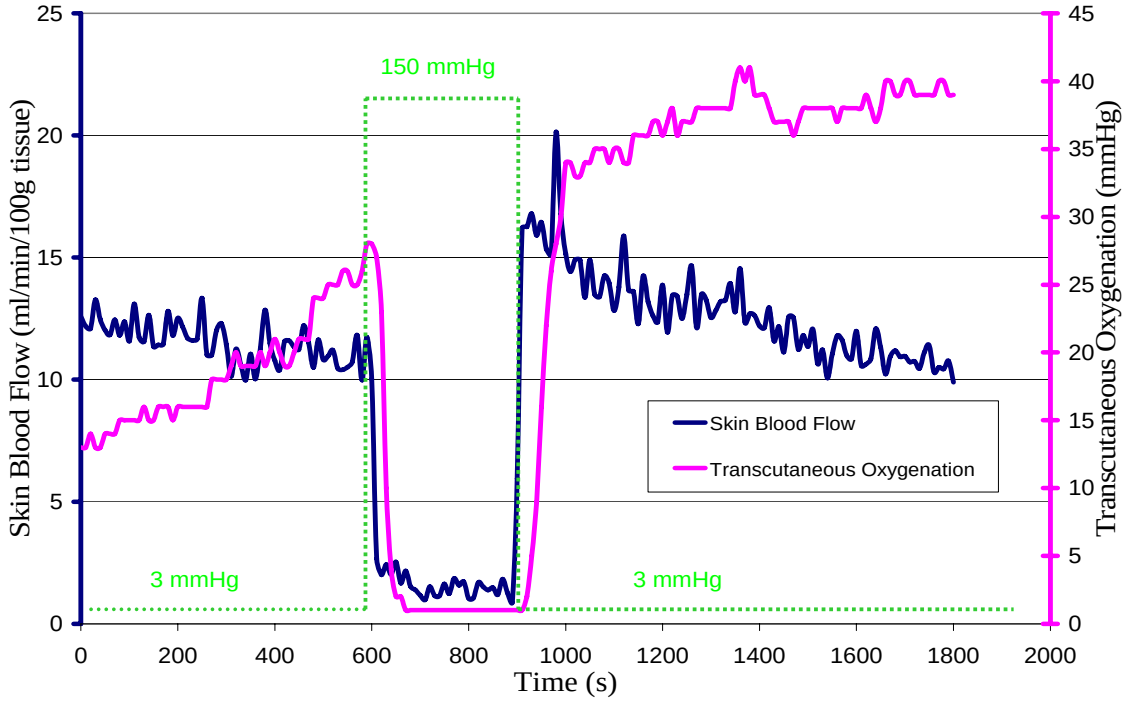

Figure 29. Subject 1, skin blood flow and $TcPO_2$ during hyperemic response at 44 °C

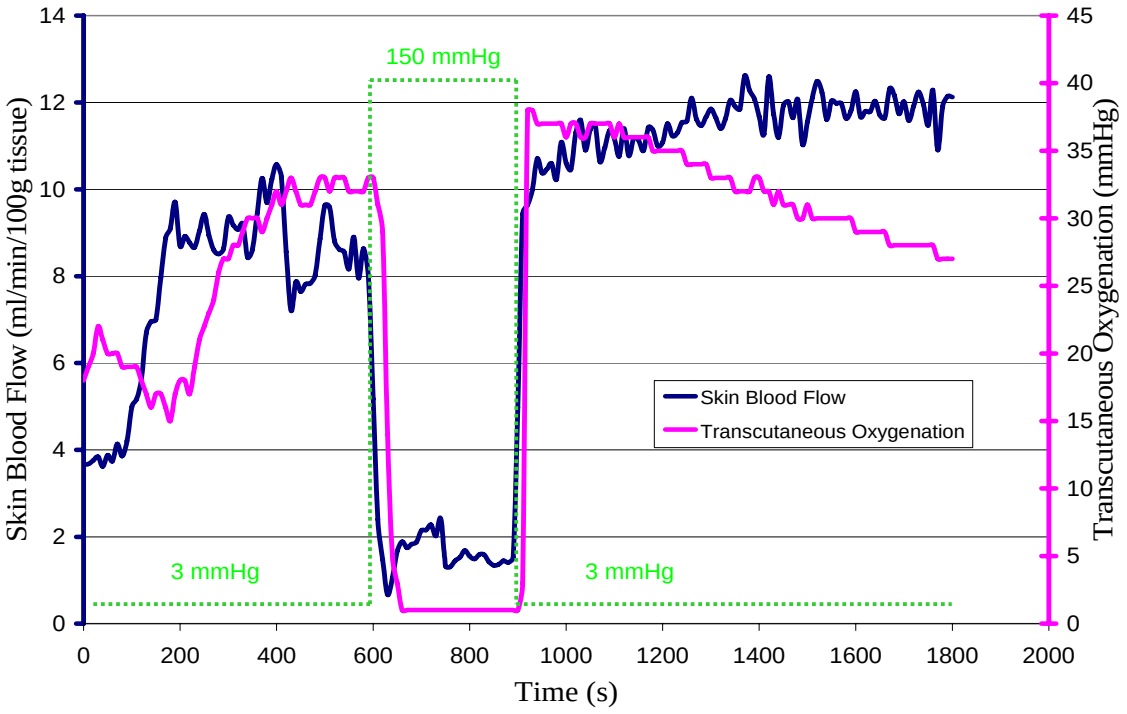

Figure 30. Subject 2, skin blood flow and $TcPO_2$ during hyperemic response at 44 °C

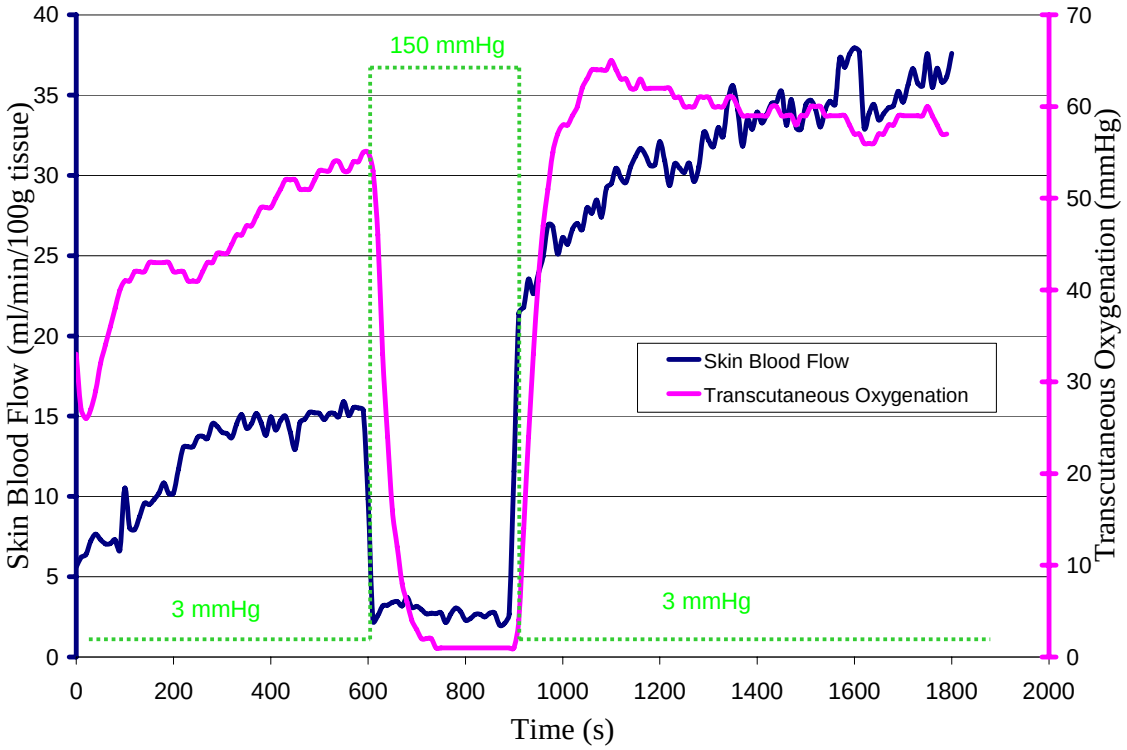

Figure 31. Subject 3, skin blood flow and $TcPO_2$ during hyperemic response at 44 °C

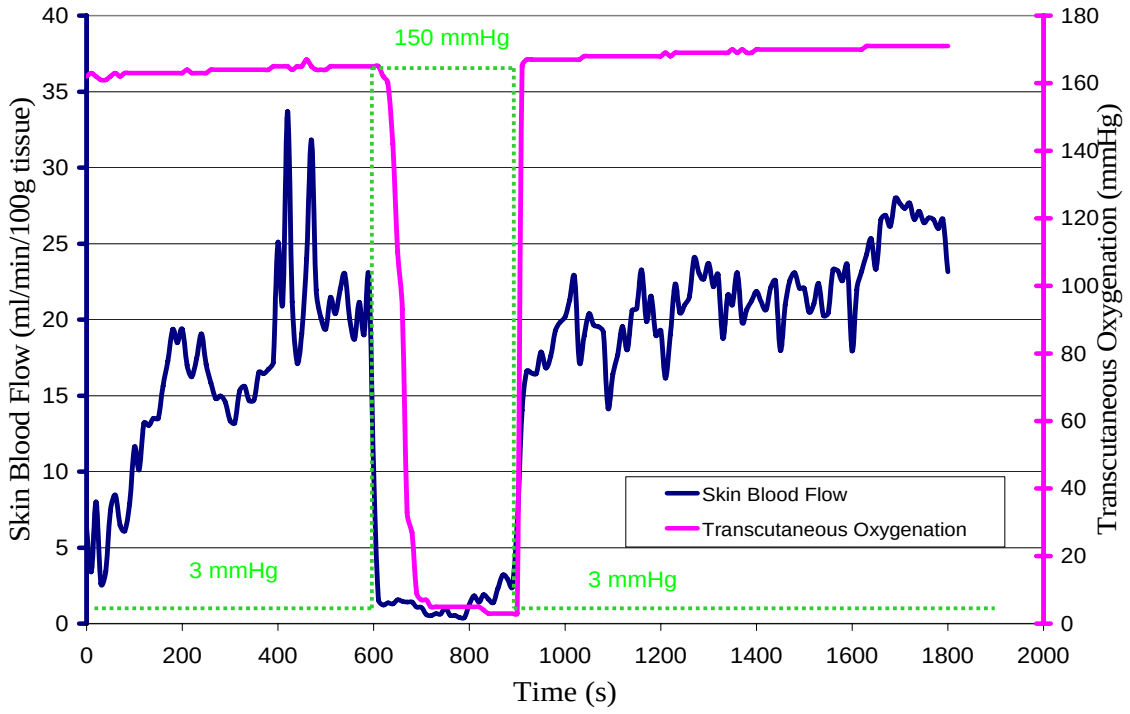

Figure 32. Subject 4, skin blood flow and $TcPO_2$ during hyperemic response at 44 °C

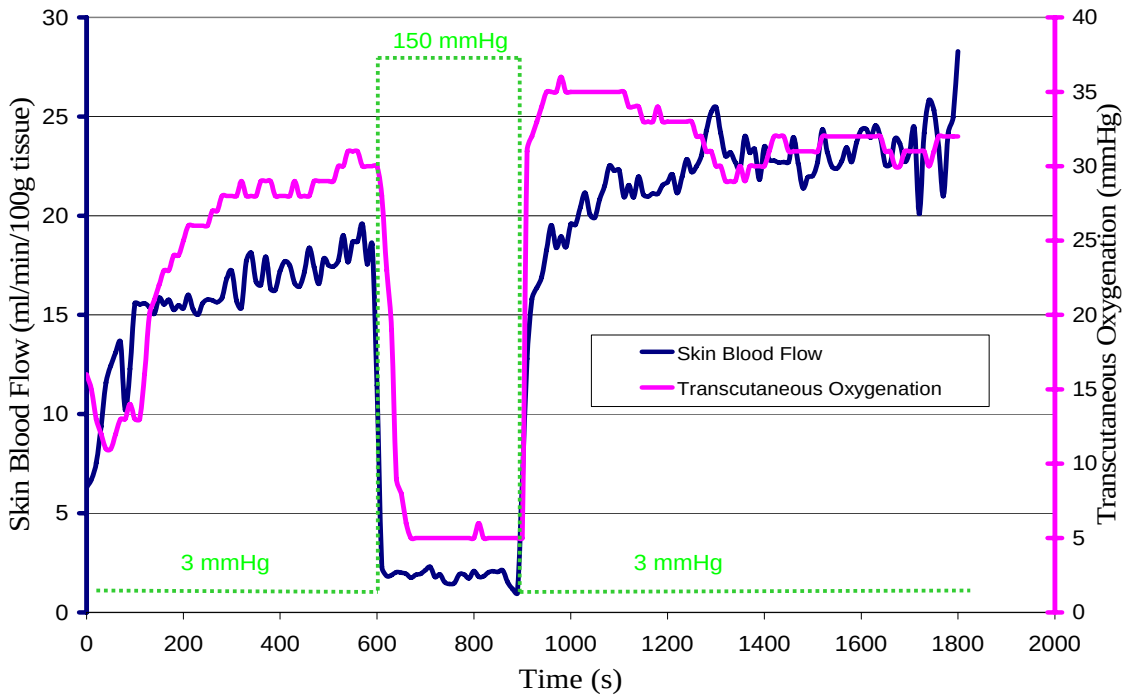

Figure 33. Subject 5, skin blood flow and $TcPO_2$ during hyperemic response at 44 °C

A nonparametric test of correlation, Spearman's rho, was performed on each subject's right sacrum skin blood flow and transcutaneous oxygenation. Correlation coefficients were obtained and a two tailed t-test was performed for significance; results are depicted in Table 8. The average correlation coefficient between the five subjects was 0.6526.

Table 8. 44°C hyperemic response correlation coefficients

Subject	Correlation Coefficient	Significance Level
1	.302	.01
2	.544	.01
3	.818	.01
4	.853	.01
5	.746	.01
Average	.6526	--

To characterize the skin blood flow's response to occluding pressure the following variables have been calculated. They are:

Resting Skin Blood Flow- The average skin blood flow value (ml/min/100g tissue) before indentation calculated by averaging the 200 seconds before indentation.

Peak Skin Blood Flow- The average value of skin blood flow (ml/min/100g tissue) following occlusion calculated by averaging the 200 seconds following occlusion.

Percent Difference- Percent difference between peak skin blood flow and resting skin blood flow.

Time to Peak- The amount of time (s) following occlusion needed to reach the maximum skin blood flow value.

These values are depicted in Table 9.

Table 9. 44°C hyperemic response skin blood flow

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Average
Resting Skin Blood Flow	11.02	8.41	14.66	21.58	16.12	14.36
Peak Skin Blood Flow	15.16	10.72	26.18	18.21	19.35	17.93
Percent Difference	37.55	27.39	78.46	-15.63	10.85	22.52
Time to Peak	80	470	700	790	900	588

Four of the five subjects displayed a peak blood flow response following release of occluding pressure. This peak is a significant increase using the Wilcoxon Signed Ranks Test ($p<0.05$). Subject 4 displayed a non significant decrease following release of occluding pressure; however, this subject did show an increasing trend in skin blood flow in the post occlusion time period. Blood flow tended to increase throughout the post occlusion time period, showing an upward trend of blood flow from release of occluding pressure. This however was not the case for one subject, subject 1, who displayed a return to baseline levels following transient increases in skin blood flow.

Similar variables were analyzed to determined transcutaneous oxygenation's response during the occluding pressure protocol. They are:

Resting $TcPO_2$- The average transcutaneous oxygenation value (mmHg) before indentation calculated by averaging the 200 seconds before indentation.

Peak $TcPO_2$- The average value of $TcPO_2$ (mmHg) following occlusion calculated by averaging the 200 seconds following occlusion.

Percent Difference- Percent difference between peak transcutaneous oxygenation and resting transcutaneous oxygenation.

Time to Peak- The amount of time (s) following occlusion needed to reach the maximum $TcPO_2$ value.

The values of these variables are depicted in Table 10.

Table 10. 44°C hyperemic response transcutaneous oxygenation

	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Average**
Resting $TcPO_2$	23.47	32.19	52.47	164.90	29.28	60.47
Peak $TcPO_2$	26.19	35.29	51	167.29	34.57	62.87
Percent Difference	11.56	9.62	-2.81	1.44	18.05	3.97
Time to Peak	460	20	200	730	80	298

Subjects' transcutaneous oxygenation values displayed unique characteristics following release of occluding pressure. Three subjects (2,3,5) have transcutaneous oxygenation values which peak following a removable of occluding pressure and trend to baseline or near baseline levels. Two subjects (1,4) had increasing trends of transcutaneous oxygenation following removal of occluding pressure. In all transcutaneous oxygenation values were increased by 3.97% following removal of occluding pressure. This is a significant increase, measured using the Wilcoxon Signed Ranks Test, ($p<0.05$). In order to get to this peak level, it took on average 298 seconds, with a range from 20 seconds to 730 seconds.

Each subjects' transcutaneous oxygenation values over each anatomical site was plotted against time (Figures 34-38).

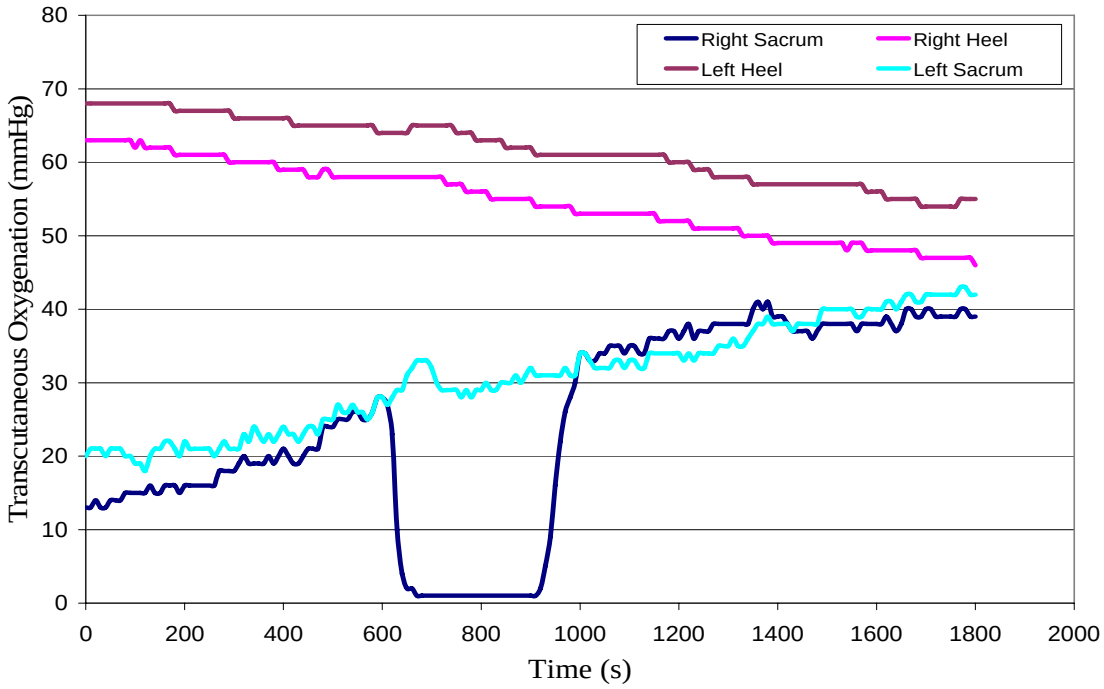

Figure 34. Subject 1, $TcPO_2$ during hyperemic response at 44 °C

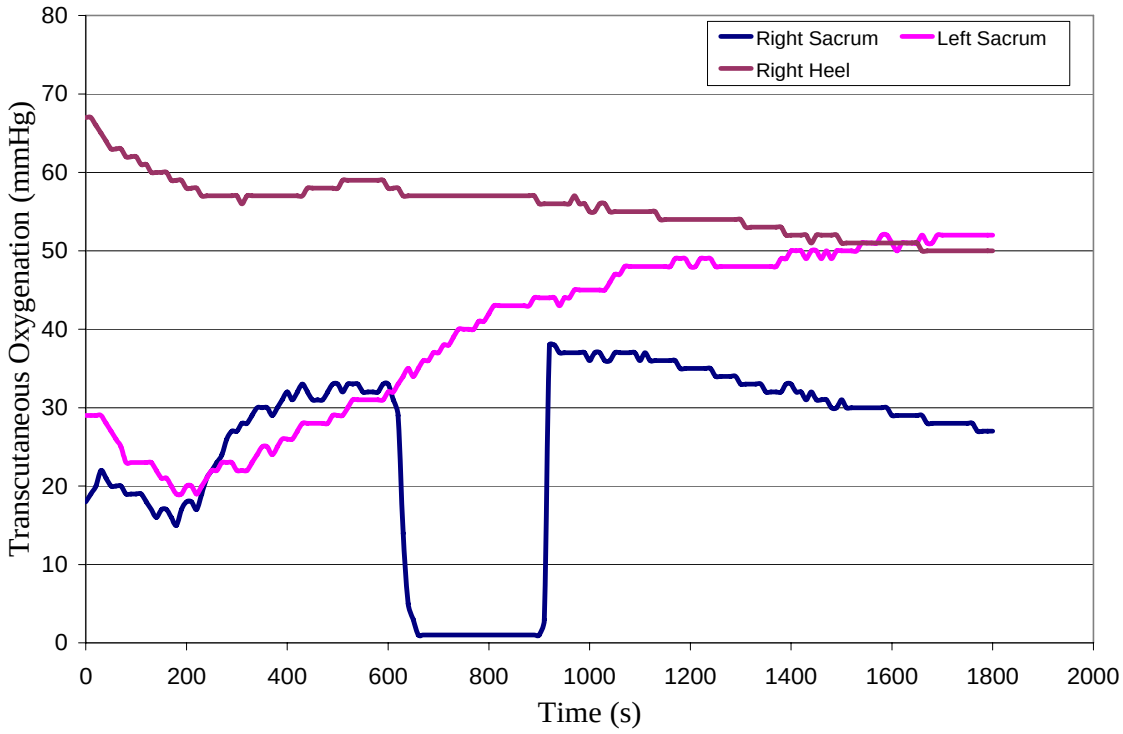

Figure 35. Subject 2, $TcPO_2$ during hyperemic response at 44 °C

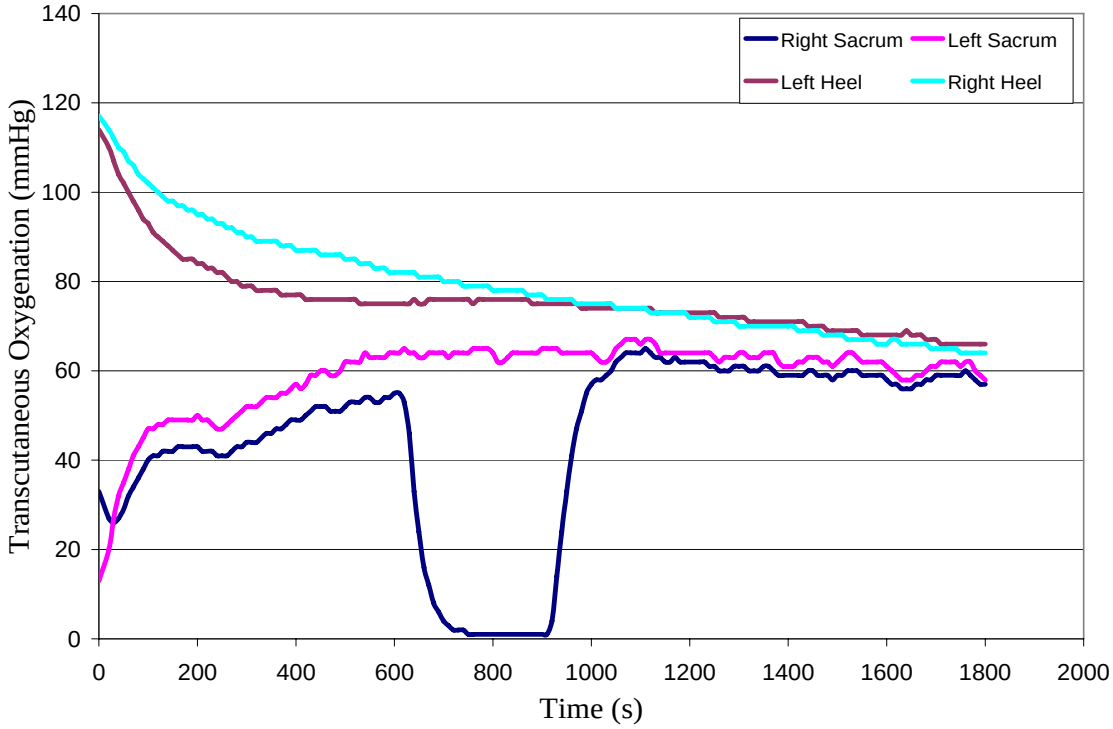

Figure 36. Subject 3, $TcPO_2$ during hyperemic response at 44 °C

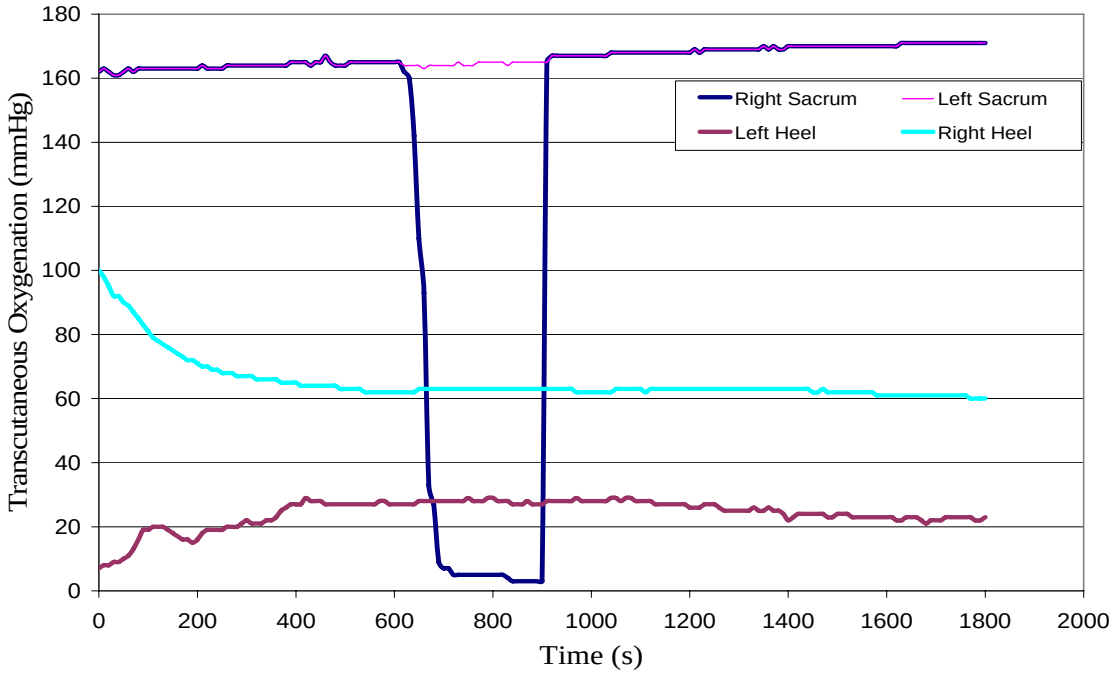

Figure 37. Subject 4, $TcPO_2$ during hyperemic response at 44 °C

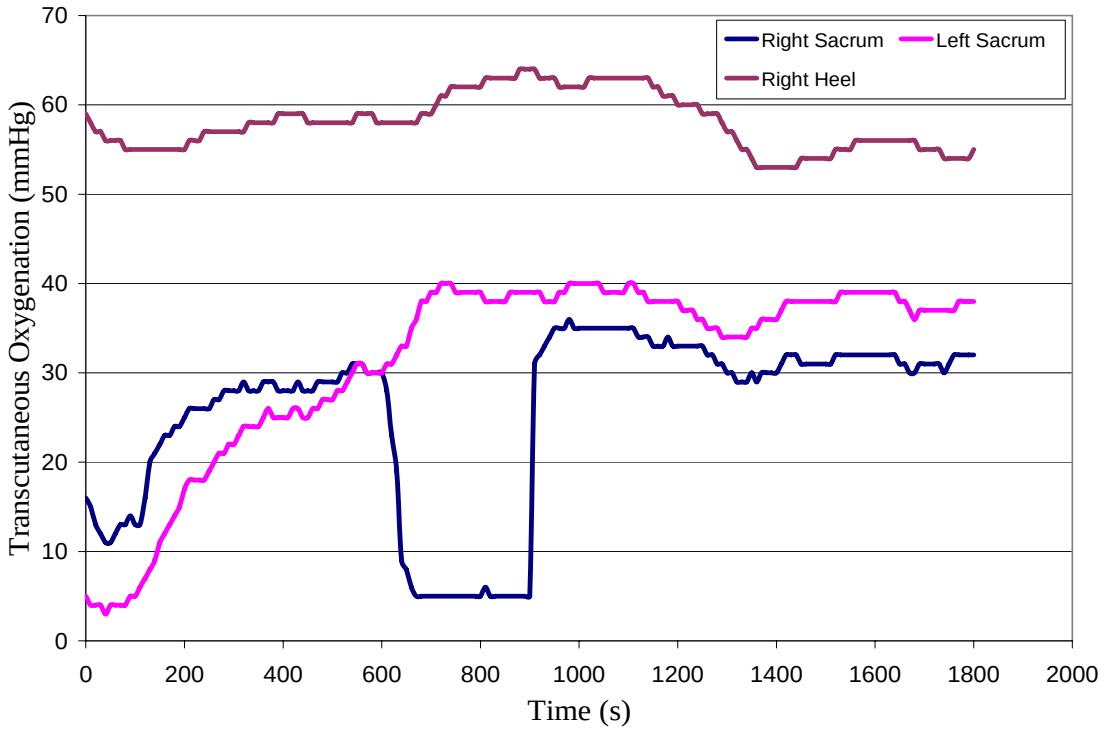

Figure 38. Subject 5, $TcPO_2$ during hyperemic response at 44 °C

Within subjects there appears to be a strong relationship between each sacrum and each heel's levels of transcutaneous oxygenation. This holds true for each subject, except for Subject 2's sacral data.

Wavelet analysis was performed on the skin blood flow data for each subject. The data was broken up into two segments, pre occlusion skin blood flow and post occlusion skin blood flow. The first two minutes of pre occlusion skin blood was eliminated from analysis to eliminate any edge effects. The last two minutes of post occlusion skin blood flow was also eliminated to eliminate any edge effects. The power was normalized and averaged for all five subjects, the results of which are depicted in Figure 37. A two-tailed test of significance was performed on post and pre occlusion values. No significant increase or decrease in power was detected (p=.05).

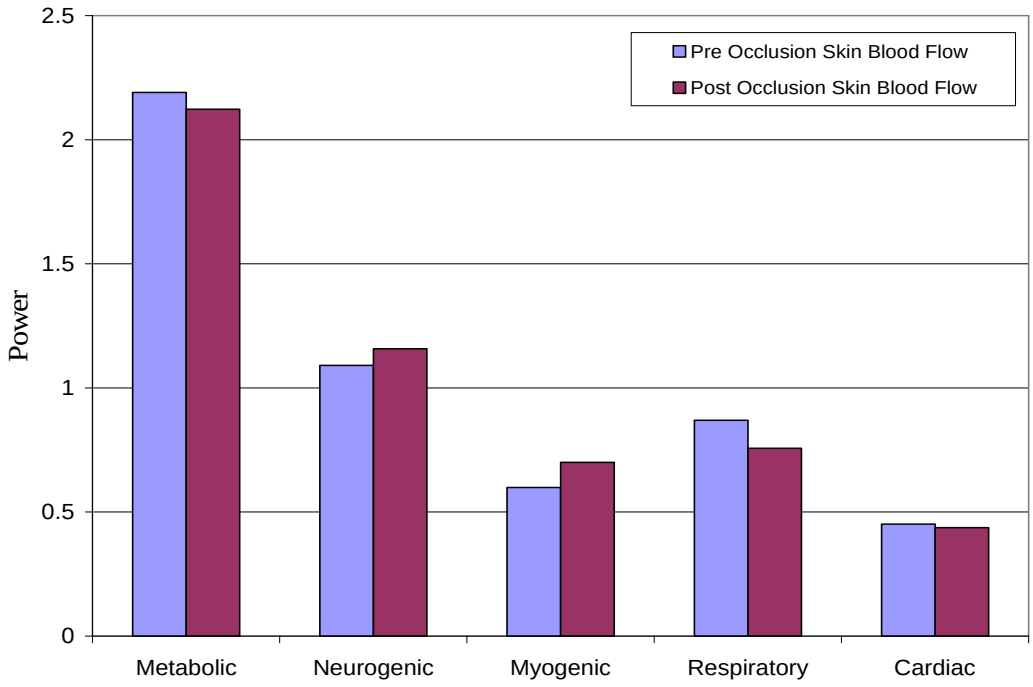

Figure 39. Normalized power comparison of the five characteristic frequency bands

4.4 HYPEREMIC RESPONSE AT 37 °C

Skin blood flow data over the right sacrum and transcutaneous oxygenation data over the right sacrum, both at 37 °C, was plotted against time for each subject (Figures 40-44). Skin blood flow data was reduced from 20 Hz to 1 data point for every 10 seconds by averaging the blood flow data over this time period.

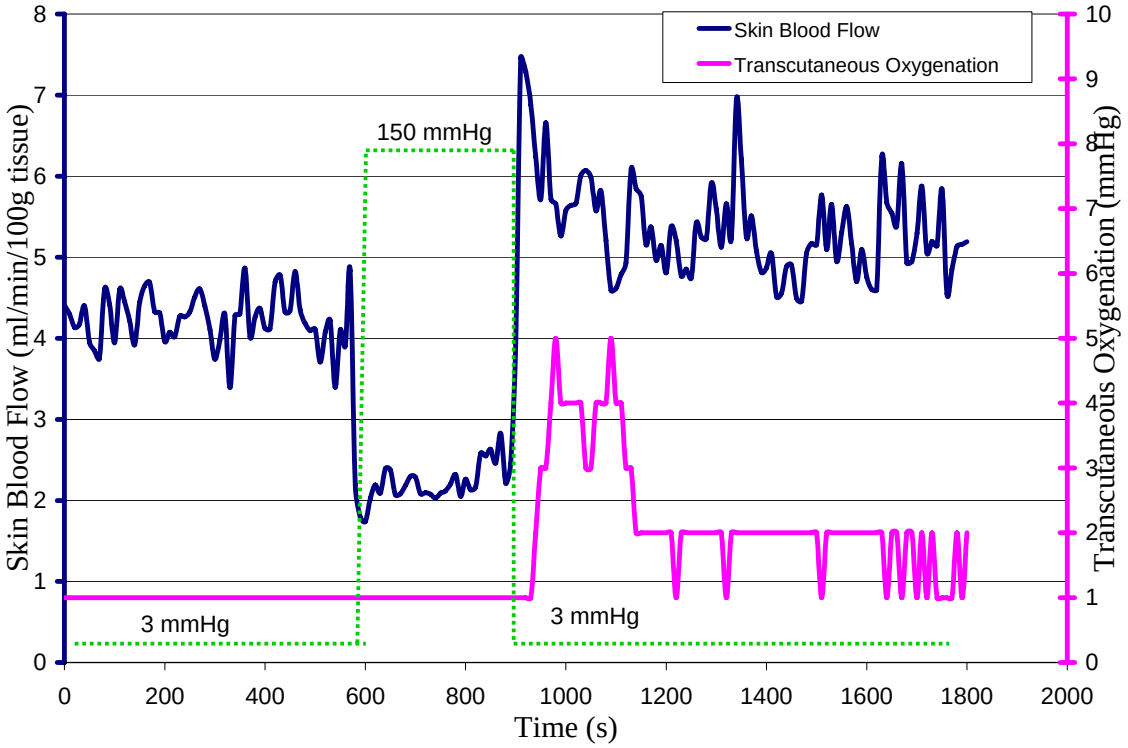

Figure 40. Subject 1, skin blood flow and $TcPO_2$ during hyperemic response at 37 °C

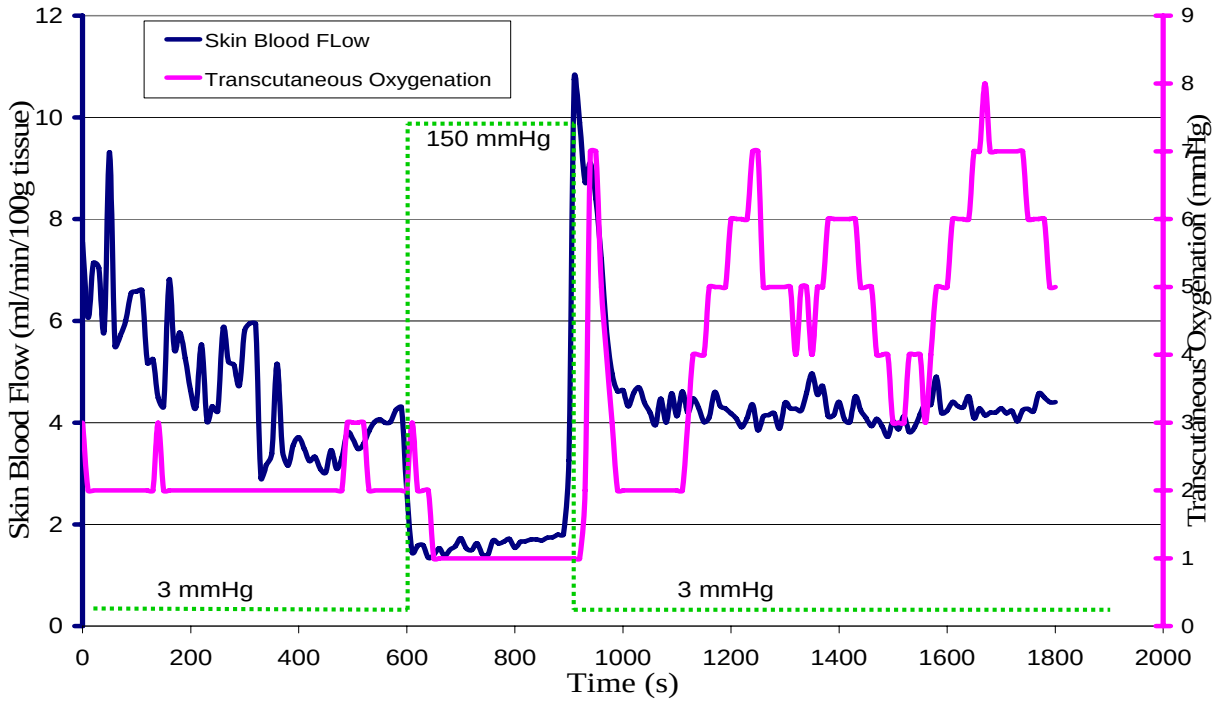

Figure 41. Subject 2, skin blood flow and $TcPO_2$ during hyperemic response at 37 °C

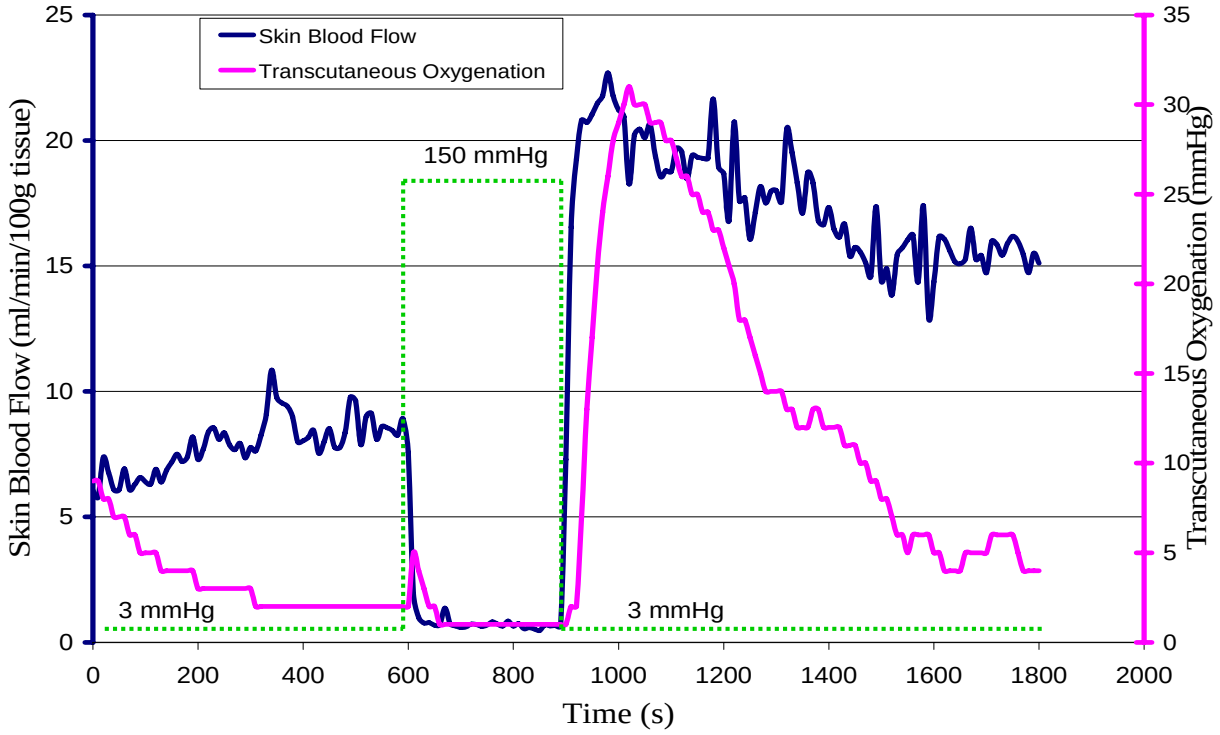

Figure 42. Subject 3, skin blood flow and $TcPO_2$ during hyperemic response at 37 °C

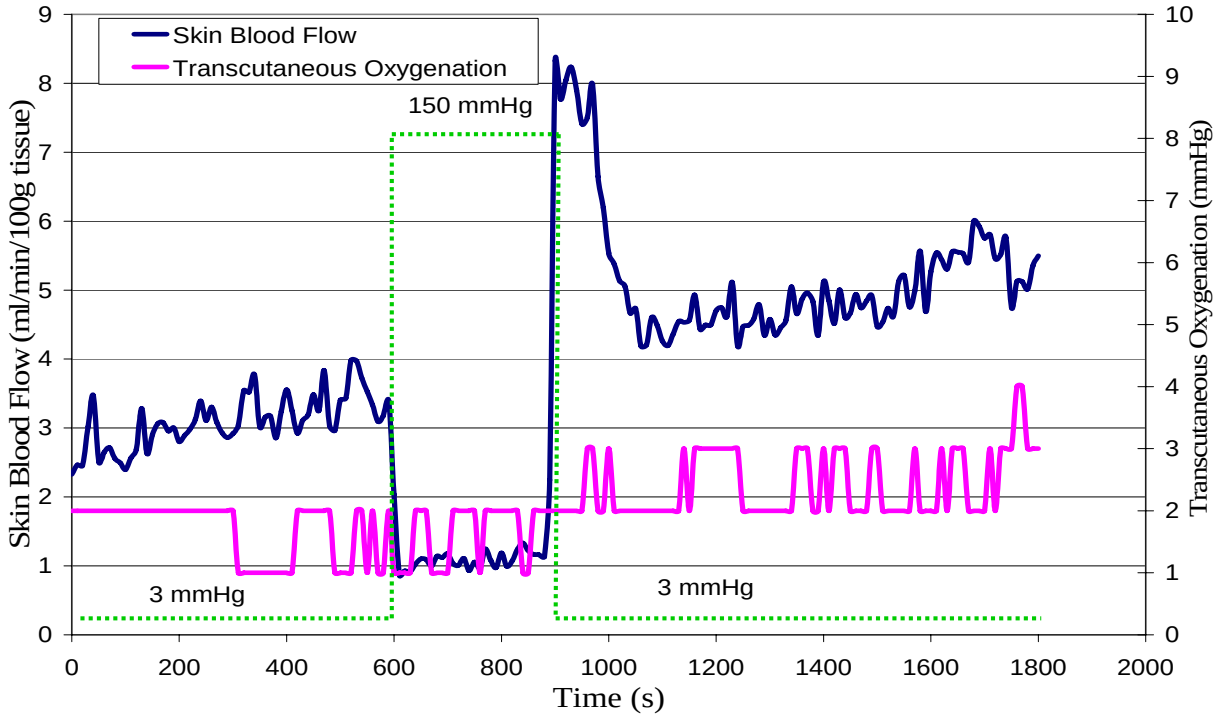

Figure 43. Subject 4, skin blood flow and $TcPO_2$ during hyperemic response at 37 °C

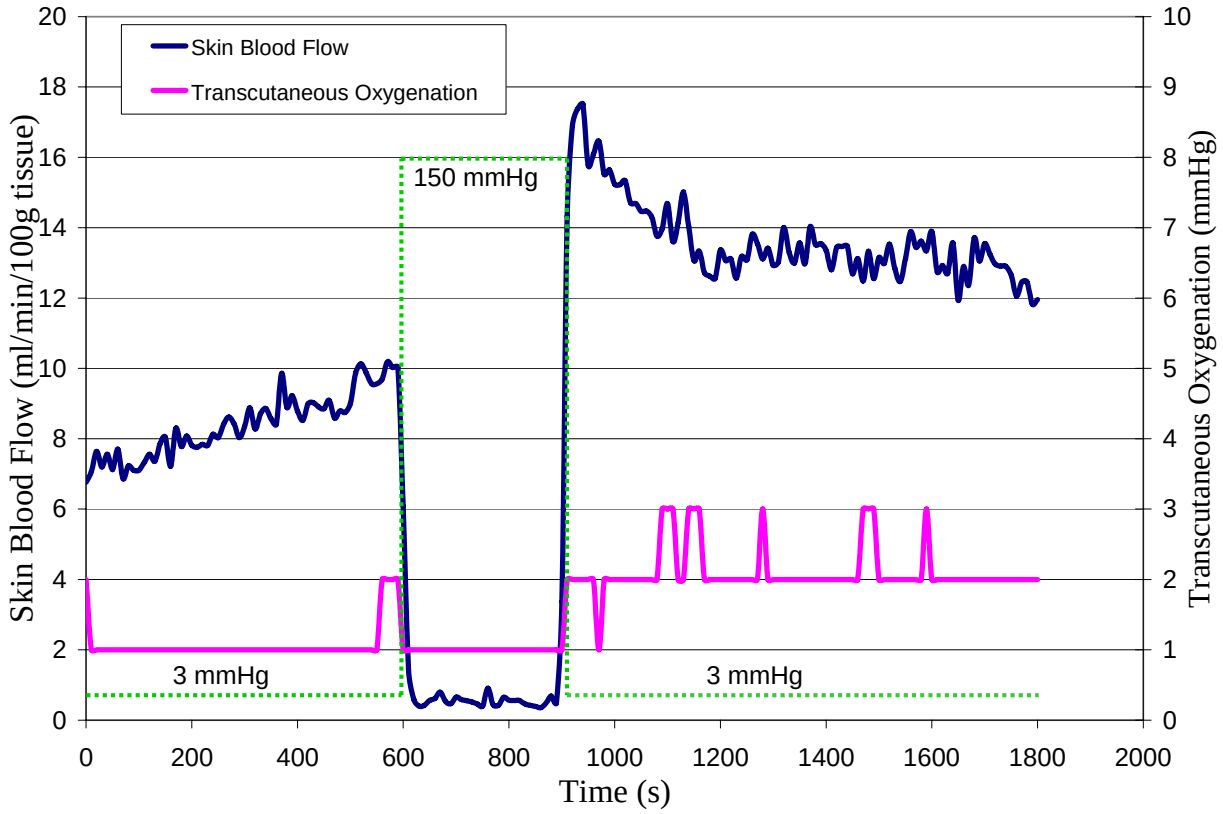

Figure 44. Subject 5, skin blood flow and $TcPO_2$ during hyperemic response at 37 °C

A nonparametric test of correlation, Spearman's rho, was performed on each subject's right sacrum skin blood flow and transcutaneous oxygenation. Correlation coefficients were obtained and a two tailed t-test was performed for significance; results are depicted in Table 11. The average correlation coefficient between the five subjects was 0.626.

Table 11. 37°C hyperemic response correlation coefficients

Subject	Correlation Coefficient	Significance Level
1	.677	.01
2	.305	.01
3	.818	.01
4	.516	.01
5	.813	.01
Average	.626	--

To characterize the skin blood flow's response to occluding pressure the following variables have been calculated. They are:

Resting Skin Blood Flow- The average skin blood flow value (ml/min/100g tissue) before indentation calculated by averaging the 200 seconds before indentation.

Peak Skin Blood Flow- The average value of skin blood flow (ml/min/100g tissue) following occlusion calculated by averaging the 200 seconds following occlusion.

Percent Difference- Percent difference between peak skin blood flow and resting skin blood flow.

Time to Peak- The amount of time (s) following occlusion needed to reach the maximum skin blood flow value.

The values of these variables are depicted in Table 12.

Table 12. 37°C hyperemic response skin blood flow

	1	2	3	4	5	Average
Resting Skin Blood Flow	3.90	3.59	8.40	3.32	9.16	5.67
Peak Skin Blood Flow	5.83	5.81	20.15	5.91	15.24	10.59
Percent Difference	49.47	61.86	139.87	78.06	66.38	86.61
Time to Peak	10	10	80	0	40	28

All subjects displayed a peak blood flow response following release of occlusion pressure. This peak is significantly different than the resting skin blood flow value, using a Wilcoxon Signed Ranks Test, ($p<0.05$). Blood flow tended to decrease throughout the post occlusion time period; however, blood flow did not return to baseline levels. Therefore, the time to peak is typically low because of the transient increase following release of pressure (average of 28 seconds).

Similar variables were analyzed to determined transcutaneous oxygenation's response during the occluding pressure protocol. They are:

Resting $TcPO_2$- The average value of $TcPO_2$ (mmHg) before indentation calculated by averaging the 200 seconds before indentation.

Peak $TcPO_2$- The average value of $TcPO_2$ (mmHg) following occlusion calculated by averaging the 200 seconds following occlusion.

Percent Difference- Percent difference between peak transcutaneous oxygenation and resting transcutaneous oxygenation

Time to Peak- - The amount of time (s) following occlusion needed to reach the maximum $TcPO_2$ value

The values of these variables are depicted in Table 13.

Table 13. 37°C hyperemic response transcutaneous oxygenation

	1	**2**	**3**	**4**	**5**	**Average**
Resting $TcPO_2$	1	2.19	2	1.52	1.19	1.58
Peak $TcPO_2$	3.38	2.67	23.33	2.14	2.10	6.72
Percent Difference	238.10	21.77	1066.67	40.98	76.07	325.57
Time to Peak	80	770	120	860	190	404

Subjects' transcutaneous oxygenation values displayed unique characteristics following release of occluding pressure. All subjects displayed very low pre occlusion values for $TcPO_2$. All subjects showed a significant increase in transcutaneous oxygenation following removal of occlusion pressure using the Wilcoxon Signed Ranks Test ($p<0.05$). Subject 1 displayed a transient increase in $TcPO_2$ with a return to or slightly above baseline. Subject 2 displayed elevated $TcPO_2$ valued following the entire post occlusion period. Subject 3 displayed a very high in magnitude, transient increase in $TcPO_2$ which eventually decreased to near baseline levels. Subject 4 displayed low pre occlusion $TcPO_2$ values, and occluding pressure had no effect on $TcPO_2$ levels. Following removal of occlusion, Subject 4 displayed slightly elevated $TcPO_2$ values throughout the entire post occlusion period. Subject 5 displayed very low pre occlusion values with slightly elevated post occlusion values throughout the entire testing period. Each

subjects transcutaneous oxygenation values over each anatomical site was plotted against time(Figures 45-49).

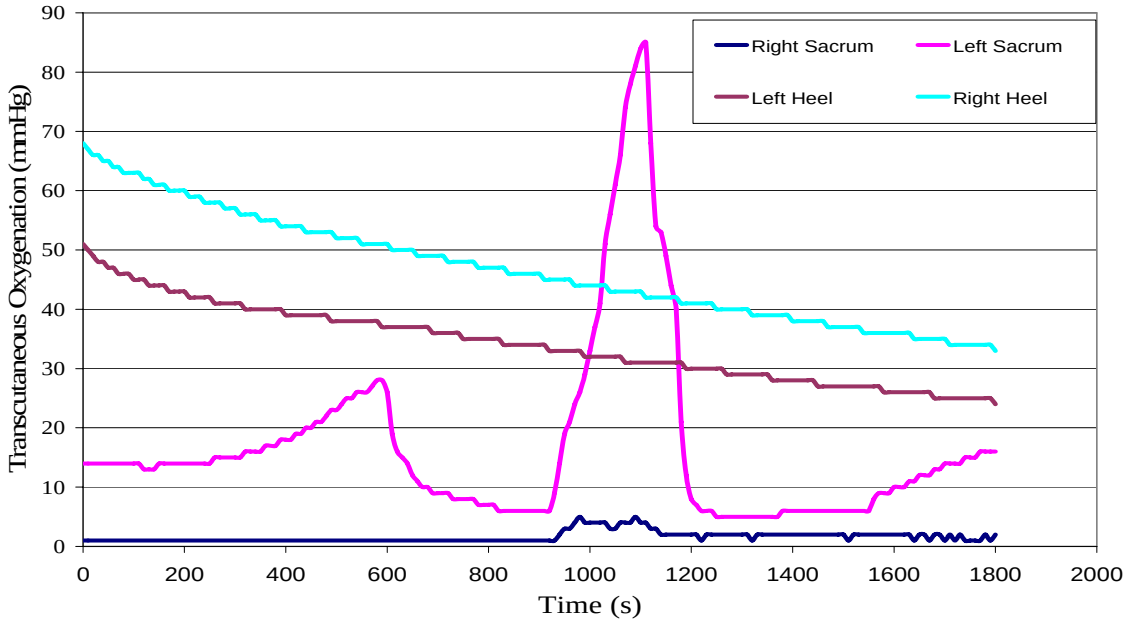

Figure 45. Subject 1, $TcPO_2$ during hyperemic response at 37 °C

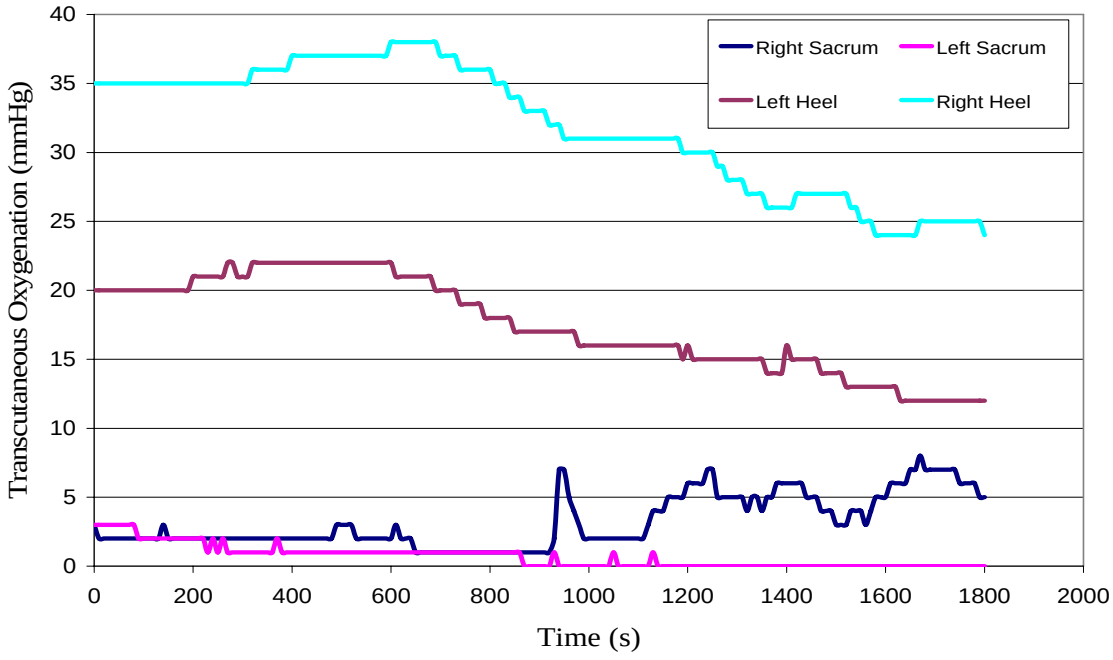

Figure 46. Subject 2, $TcPO_2$ during hyperemic response at 37 °C

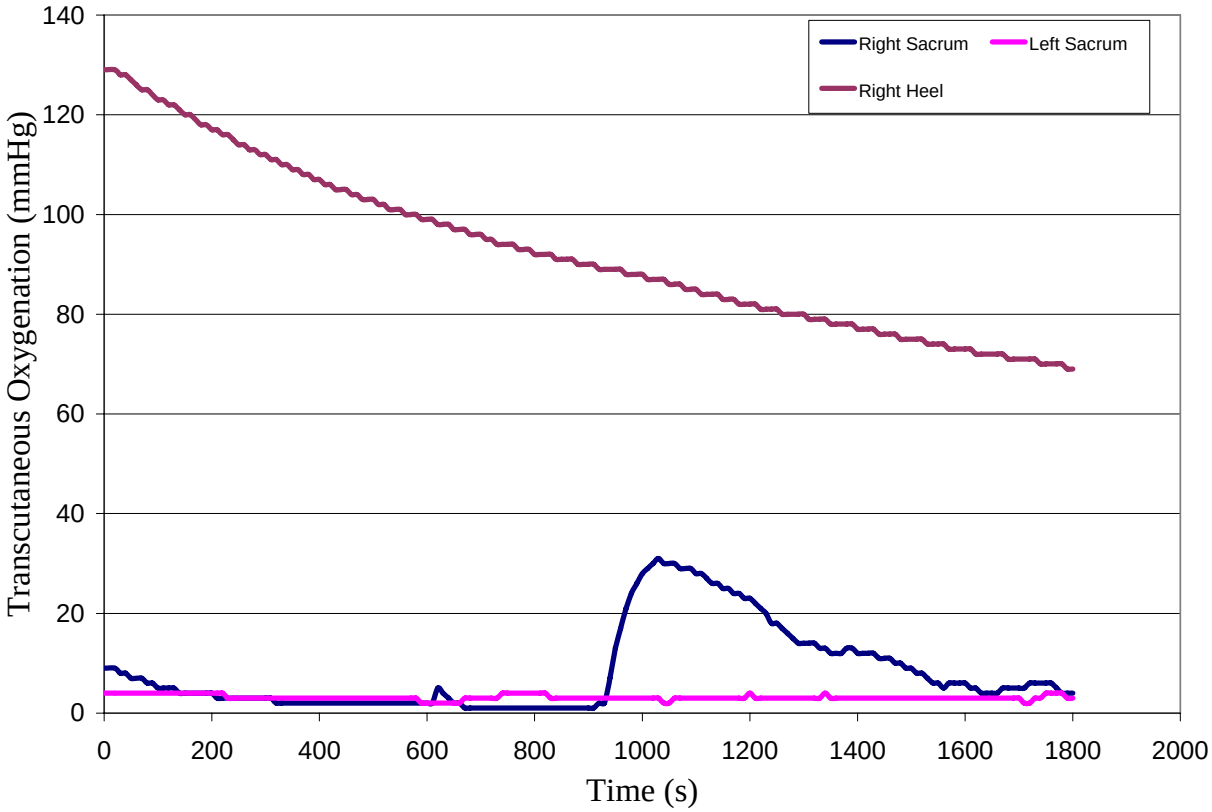

Figure 47. Subject 3, $TcPO_2$ during hyperemic response at 37 °C

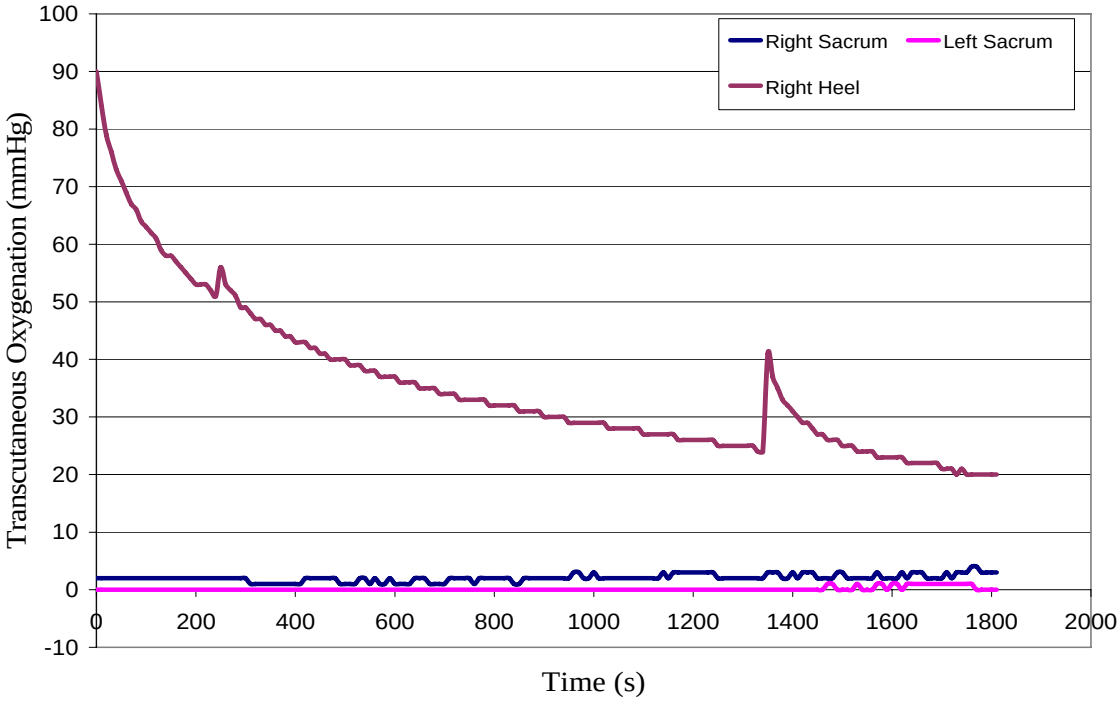

Figure 48. Subject 4, $TcPO_2$ during hyperemic response at 37 °C

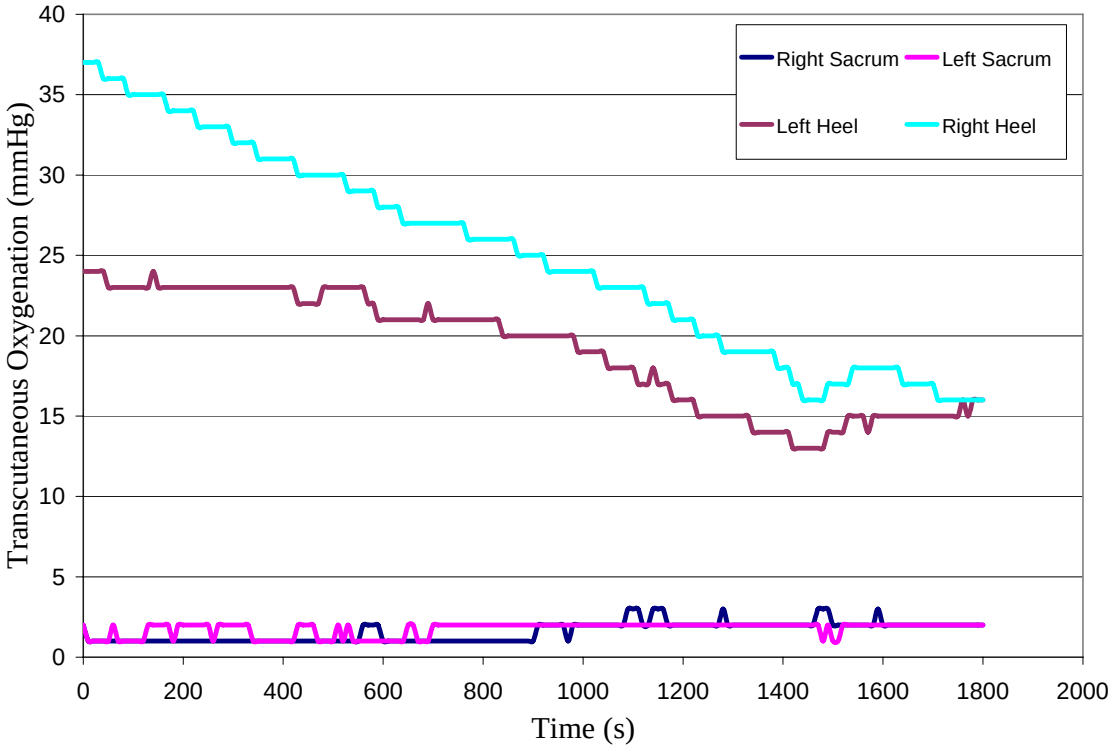

Figure 49. Subject 5, $TcPO_2$ during hyperemic response at 37 °C

Wavelet analysis was performed on the skin blood flow data for each subject. The data was broken up into two segments, pre occlusion skin blood flow and post occlusion skin blood flow. The first two minutes of pre occlusion skin blood was eliminated from analysis to eliminate any edge effects. The last two minutes of post occlusion skin blood flow was also eliminated to eliminate any edge effects. The power was normalized and averaged for all five subjects, the results of which are depicted in Figure 50. A two-tailed test of significance was performed on post and pre occlusion values. No significant increase or decrease in power was detected (p=.05).

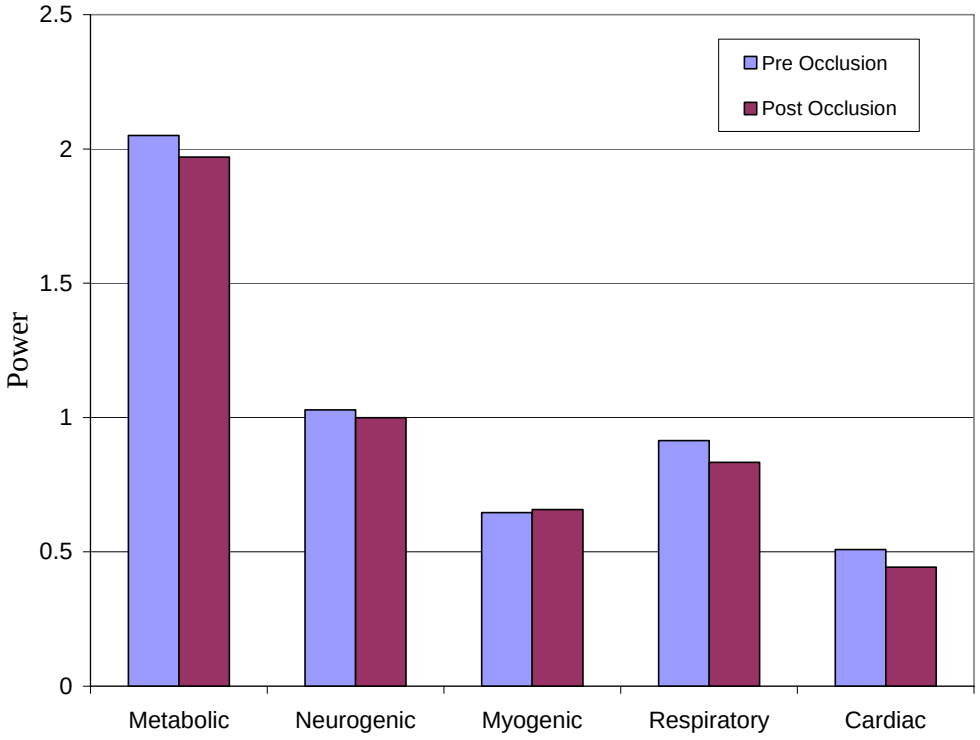

Figure 50. Normalized power comparison of the five characteristic frequency bands

5.0 DISCUSSION

5.1 ORTHOSTATIC STIMULATION

The results of orthostatic stimulation suggest that both anatomical location and heat have major implications in the outcome of skin blood flow and transcutaneous oxygenation following orthostasis. The results of our study show that skin blood flow at 44 °C over the right sacrum *increases* on average 96.86 percent following change in posture from prone to sitting. Conversely, skin blood flow at 37 °C over the right sacrum *decreases* on average 38.12 percent following change in posture from prone to sitting. This suggests that heating the skin to higher temperatures completely eliminates the vasoconstrictive response typically seen following orthostatic stimulation, while slightly lower, but still heated temperatures are not sufficient to overcome the postural vasoconstriction response. The effects on postural change on transcutaneous oxygenation are mixed, depending largely on anatomical site. At 44 °C $TcPO_2$ values over the right and left sacrum *increased* following orthostasis on average by 76.16% and 116.24% respectively. However, at 44 °C $TcPO_2$ values over the right and left heels *decreased* following orthostasis on average by 9.87% and 13.27% respectively. $TcPO_2$ values at 37 °C provided no information, with orthostasis resulting in no effect on values.

Glabrous skin, such as the heel, is known to have numerous arteriovenous shunts. These shunts are controlled mainly by the sympathetic nervous system. Although the exact mechanism

of posture induced vasoconstriction is controversial, these results suggest that the sympathetic nervous system plays a vital role in posture induced vasoconstriction.

Heating the skin to 44 °C completely eliminated the postural vasoconstriction response on skin blood flow following orthostasis, in all subjects. Under unheated conditions, skin blood flow is expected to decrease following orthostasis, which is supported in numerous studies.[101,103,106]

Under heated conditions, skin blood flow's response to orthostasis has been shown to be unpredictable. Using LDF under slightly heated conditions of 33 °C, Hassan reported a normal response to orthostasis in subjects over the dorsum of the foot, and a non-significant reduction in skin blood flow over the plantar surface of the big toe following orthostasis.[171] At 35 °C Rendell reported no significant difference in blood flow following orthostasis on skin sites with and without arteriovenous shunts. At 44 °C Rendell reported a large, significant increase in blood flow following orthostasis at skin sites with and without arteriovenous shunts.[104] Ubbink reported increased skin blood flow following orthostasis using LDF at 36 °C over the pulp of the great toe.[107] Under heated conditions of 44 °C we found that skin blood flow over the right sacrum increased, on average 96.86 percent, following orthostasis. However at 37 °C we found that skin blood flow over the right sacrum decreased, on average 38.12 percent, following orthostasis.

The differences between our study and prior studies in skin blood flow following orthostasis most likely are a result of methodological differences. Our results are from direct heating over the sacrum, a site known to have very few arteriovenous shunts, and following an orthostatic stimulation of having the subject change positions from a prone to a seated position. Other studies have used different heating methods, on different sites, and different methods of

orthostatic stimulation. Hassan used an indirect heating method. He used an electric warming blanket placed around the trunk of the subjects to induce a skin temperature increase to 33 °C over the plantar surface of the big toe and the dorsum of the foot.[171] The dorsum of the foot is also known to have few arteriovenous shunts, and he observed decreased skin blood flow upon dependency. On the plantar surface of the big toe, a site known for many arteriovenous shunts, Hassan reported small, non-significant increases in skin blood flow following orthostasis. Hassan induced dependency by passively lowering the foot 50 cm below the heart.[171] Rendell also induced dependency by passively lowering the arm or leg. Rendell measured areas that are known to have large and small amounts of arteriovenous shunts.[104] Ubbink measured skin blood flow on the bulb of the great toe, inducing dependency by changing posture from supine to sitting.[107]

The temperature used, the site of testing, and the method to induce dependency all may play a role in the results following orthostatic simulation. The results of our study suggest that temperature and location play a critical role in the response to orthostatic stimulation. Although we did not test the effects that different means of inducing dependency have on the results, Vissing has reported that method of inducing dependency has many effects on the orthostatic stimulation.[109]

Perhaps another methodological difference in our study, compared to others, is the lack of allowance to steady state levels before testing began. One of the aims of our study was to simultaneously measure both skin blood flow and transcutaneous oxygenation and to calculate their correlation. Previous studies on orthostatic stimulation have allowed variables of interested to reach steady state level, usually by allowing 20 minutes, before beginning actual baseline and intervention phases. Because we took simultaneous measures, and skin blood flow has been

reported to reach a steady state after 50 minutes of heating and transcutaneous oxygenation after 20 minutes of heating, we allowed no time for the response to reach steady state levels. On that end we found that skin blood flow and transcutaneous oxygenation have correlation coefficients of 0.423 and 0.661 at 37 and 44 °C, respectively, throughout the entire orthostatic stimulation test. Skin blood flow appears to better correlated with $TcPO_2$ at higher temperatures. This is somewhat paradoxical because $TcPO_2$ dependents largely on arterial pO_2 at higher temperatures and is more dependent on local skin blood flow at lower temperature. However, the values of $TcPO_2$ we obtained at lower temperatures (37 °C) had a continual downward trend that orthostasis made no difference in, thus $TcPO_2$ was not correlated well with skin blood flow. Perhaps if $TcPO_2$ levels stabilized prior to testing, then $TcPO_2$ values at lower temperature would be better correlated to skin blood flow.

Wavelet analysis was also performed on the skin blood flow signal at 37 and 44 °C. The power from this time-frequency analysis is reported in the literature.[41,42,85] This study did not produce any significant changes in pre and post orthostatic stimulation in any of the five characteristic frequency bands. This could be due to the very low sample size, and/or the multiple, interrelated control mechanisms that may be in play with both heating and orthostatic stimulation. At unheated temperatures, wavelet analysis may prove to be very powerful in detecting time-frequency changes in those undergoing an orthostatic stimulation test.

The results of our study and the results of previous studies suggest that anatomical sites (those with and without arteriovenous shunts), the temperature and method of heating used, and the method to invoke dependency can greatly influence the results of the testing. Postural changes are often used clinically to test the integrity of microvascular constriction mechanisms in leg ischemia, especially in the case of peripheral vascular occlusive disease.[107] However, the

results could change if different methods of inducing the posture, amount of heat, and testing location are varied. With more research into this field a standard procedure could be developed which would take into account the anatomical location of the testing site, methods of inducing dependency, and the level of heat to be used.

5.2 HYPEREMIC RESPONSE

Skin blood flow during the hyperemic response at 44 °C shows a gradual increase following occlusion. Past studies have shown at 44 °C no increase in skin blood flow is observed following occlusion.[21] However, in those studies skin blood flow is allowed to reach steady state levels before occlusion. In our study, we aimed to correlate skin blood flow and $TcPO_2$ and we allowed no time for the response to reach steady state levels before testing began.

One subject, subject 1, showed a typical hyperemic response, characterized by a transient increase in skin blood flow with a gradual decrease to baseline levels. However, the other 4 subjects displayed an upward trend of skin blood flow throughout the entire post occlusion phase.

At 37 °C a more typical hyperemic response is found. There is a transient increase in skin blood flow following occlusion; however, post occlusion blood levels after the transient increase in skin blood flow remained elevated above pre occlusion skin blood flow values. These results suggests that at 44 °C vasoconstriction in inhibited, which is supported by the literature.[22]

Transcutaneous oxygenation values at 44 °C following occlusion are unclear. In two subjects $TcPO_2$ values continue to increase throughout the post occlusion phase. In the other three subjects $TcPO_2$ values do in fact show a typical hyperemic response, characterized by a transient increase in $TcPO_2$ levels, with a gradual decline to baseline levels. In these three subjects skin blood flow values are continuing to rise while $TcPO_2$ values are returning to baseline levels. At 37 °C, release of occluding pressure has a definite effect on $TcPO_2$. One subject displayed a transient increase in $TcPO_2$ levels followed by a return to baseline. All other subjects displayed an increase in $TcPO_2$ following release of occluding pressure with subsequent $TcPO_2$ values decreased, but above baseline levels.

The effects that the hyperemic response has on transcutaneous oxygenation are unclear. However, upon examination of the contra-lateral sacrum more understanding can be established. At 44 °C release of occluding pressure causes no substantial change in $TcPO_2$ values on the right sacrum compared to the left sacrum. This suggests that vasoconstriction mechanisms are inhibited by the heat. $TcPO_2$ values over the left and right sacrum follow nearly identical oscillatory patterns throughout testing. However, at 37 °C left and right sacrum $TcPO_2$ values do not follow the same pattern following release of occluding pressure. This suggests that vasoconstriction mechanisms are not inhibited by the lower heat.

These results differ from other researchers. Ubbink measured $TcPO_2$ levels during reactive hyperemia at 37 and 44 °C. $TcPO_2$ levels at both temperatures reached baseline levels following the removal of occluding pressure.[21] In Ewald's study at temperatures between 35-37 °C a reactive hyperemic response of $TcPO_2$ was observed, with subsequent return to baseline levels. At temperatures higher than 37 °C Ewald reported $TcPO_2$ values to return to baseline following removal of occluding pressure.[22]

The results obtained in our study had very different methodologies than those of Ubbink and Ewald. Ubbink, measuring $TcPO_2$ on the dorsal surface of the foot, induced occlusion by a pneumatic cuff around the ankle for 3 minutes.[21] Ewald, measuring $TcPO_2$ on the anterior forearm, induced occlusion by a pneumatic cuff for 4 minutes.[22] There is evidence that direct compression (as in our experiment) and indirect compression (via a pneumatic cuff) have very different effects on the hyperemic response. Direct compression results in a significantly greater hyperemic magnitude and duration when compared to proximal cuff compression.[123] Direct compression may result in uneven pressure distribution (edge effects) which could affect skin blood flow. A rounded indenter head fixed to an armature with multiple degrees of freedom was utilized in trying to achieve equal pressure distribution to minimize any edge effects. In addition, our occlusion period differed from Ubbinks's and Ewald's. In our study we applied an occluding pressure for 5 minutes, while Ubbink and Ewald used 3 and 4 minutes, respectively. Tee reported that the hyperemic response is dependent on the duration of the occluding pressure.[124] In addition, we did not allow skin blood flow and $TcPO_2$ to reach steady state levels before testing began, in order to obtain correlation coefficients. Skin blood flow and transcutaneous oxygenation over the right sacrum have correlation coefficients of 0.626 and 0.6526 at 37 and 44 °C, respectively. $TcPO_2$ at lower temperatures depends more on skin blood flow, while at higher temperatures $TcPO_2$ is correlated with arterial oxygenation. Perhaps if we allowed skin blood flow and $TcPO_2$ levels to reach steady before testing then $TcPO_2$ would be correlated more with skin blood flow at lower temperatures.

Wavelet analysis was performed on the skin blood flow data at 37 and 44 °C. However there was no significant difference in any of the five characteristic frequency bands when comparing pre and post occlusion values. Wavelet analysis has been shown to be very useful in

the study of skin blood flow.[41,42,85] However, the sample size in our study was very small, and the interrelated control mechanisms that may be at work throughout the testing (heating and pressure) may have limited its ability in analysis of the skin blood flow data.

6.0 CONCLUSIONS, LIMITATIONS, FUTURE DIRECTIONS

The results of our orthostatic stimulation study suggest that both location of testing and temperature effect how the microvasculature responds to orthostatic stimulation. However, our results were obtained using a very small sample size, in a healthy population. Future studies should examine how temperature, anatomical location, and method of inducing dependency affect the results of orthostatic stimulation in a larger sample size and different population bases, such as those with spinal cord injury. Spinal cord injury patients may spend the majority of their day in an upright, orthostatic stimulated position while in a wheelchair. Because of the effects this position has on skin blood flow and $TcPO_2$, future work should examine how external pressure affects skin blood flow and transcutaneous oxygenation in the upright position. This will allow for more accurate assessment of how pressure, and ultimately support surfaces affect the microvasculature of individuals with spinal cord injury.

Skin blood flow during the hyperemic response suggests that at 44 °C the vasoconstriction mechanisms is inhibited, thus eliminating the normal return to baseline levels. At 37 °C however, the vasoconstriction mechanisms are not fully inhibited. At 44 °C removal of occluding pressure does not appear to affect $TcPO_2$ levels when comparing to the contra-lateral side. However, at 37 °C there is a definite effect on $TcPO_2$ levels. This study was limited by its small sample size. In addition there were possibilities for edge effects by the computer controlled indenter which would allow for unequal pressure distribution. The indenter head was

rounded and fixed to an armature with multiple degrees of freedom in order to minimize any edge effects; however, the possibility cannot be ruled out that there were indeed edge effects. In addition, there was no allowance of steady state levels to be reached for skin blood flow and transcutaneous oxygenation. Therefore, the data was still trending at the time of application of stimulus (orthostatic stimulation or occluding pressure). Thus, any effects that the stimulus had on skin blood flow and transcutaneous oxygenation levels may not have been directly caused by the stimulus. Data analysis was also limited by the allowance of near zero or erroneous data points in the data analysis. $TcPO_2$ values, especially at lower temperatures, displayed very low values, which in data analysis may have skewed the results.

Future work in this field could be applied to spinal cord injury patients, since the hyperemic response appears in denervated tissue. Simultaneous measures of skin blood flow and $TcPO_2$ could yield significant information on blood flow and oxygenation control mechanisms in this population.

BIBLIOGRAPHY

1. Patterson, R. P., Cranmer, H. H., Fisher, S. V., & Engel, R. R. (1993). The impaired response of spinal cord injured individuals to repeated surface pressure loads. *Arch Phys Med Rehabil, 74*(9), 947-953.

2. Schubert, V., Schubert, P. A., Breit, G., & Intaglietta, M. (1995). Analysis of arterial flowmotion in spinal cord injured and elderly subjects in an area at risk for the development of pressure sores. *Paraplegia, 33*(7), 387-397.

3. Rodriguez, G. P., & Garber, S. L. (1994). Prospective study of pressure ulcer risk in spinal cord injury patients. *Paraplegia, 32*(3), 150-158.

4. Salzberg, C. A., Byrne, D. W., Cayten, C. G., Kabir, R., van Niewerburgh, P., Viehbeck, M., et al. (1998). Predicting and preventing pressure ulcers in adults with paralysis. *Adv Wound Care, 11*(5), 237-246.

5. National Spinal Cord Injury Statistical Center. (2006). *Spinal Cord Injury: Facts and Figures at a Glance*. Birmingham, AL: University of Alabama.

6. Byrne, D. W., & Salzberg, C. A. (1996). Major risk factors for pressure ulcers in the spinal cord disabled: a literature review. *Spinal Cord, 34*(5), 255-263.

7. Consortium for Spinal Cord Medicine. (2000). *Pressure Ulcer Prevention and Treatment Following Spinal Cord Injury: A Clinical Practice Guideline for Health Care Professionals*. Washington, DC: Paralyzed Veterans of America.

8. Teasell, R. W., Arnold, J. M., Krassioukov, A., & Delaney, G. A. (2000). Cardiovascular consequences of loss of supraspinal control of the sympathetic nervous system after spinal cord injury. *Arch Phys Med Rehabil, 81*(4), 506-516.

9. Bader, D. L., & Gant, C. A. (1988). Changes in transcutaneous oxygen tension as a result of prolonged pressures at the sacrum. *Clin Phys Physiol Meas, 9*(1), 33-40.

10. Shepherd, A. P., & Oberg, P. A. (1990). *Laser-Doppler Blood Flowmetry*. Boston: Kluwer Academic Publishers.

11. Mawson, A. R., Siddiqui, F. H., Connolly, B. J., Sharp, C. J., Summer, W. R., & Biundo, J. J., Jr. (1993). Sacral transcutaneous oxygen tension levels in the spinal cord injured: risk factors for pressure ulcers? *Arch Phys Med Rehabil, 74*(7), 745-751.

12. Ballard, J. L., Eke, C. C., Bunt, T. J., & Killeen, J. D. (1995). A prospective evaluation of transcutaneous oxygen measurements in the management of diabetic foot problems. *J Vasc Surg, 22*(4), 485-490; discussion 490-482.

13. Liu, M. H., Grimm, D. R., Teodorescu, V., Kronowitz, S. J., & Bauman, W. A. (1999). Transcutaneous oxygen tension in subjects with tetraplegia with and without pressure ulcers: a preliminary report. *J Rehabil Res Dev, 36*(3), 202-206

14. Xakellis, G. C., Frantz, R. A., Arteaga, M., & Meletiou, S. (1991). A comparison of changes in the transcutaneous oxygen tension and capillary blood flow in the skin with increasing compressive weights. *Am J Phys Med Rehabil, 70*(4), 172-177.

15. Andreozzi, G. M., Riggio, F., Butto, G., Barresi, M., Leone, A., Pennisi, G., et al. (1995). Transcutaneous PCO2 level as an index of tissue resistance to ischemia. *Angiology, 46*(12), 1097-1102.

16. Caspary, L. A., Creutzig, A., & Alexander, K. (1996). Orthostatic vasoconstrictor response in patients with occlusive arterial disease assessed by laser Doppler flux and transcutaneous oximetry. *Angiology, 47*(2), 165-173.

17. Colin, D., & Saumet, J. L. (1996). Influence of external pressure on transcutaneous oxygen tension and laser Doppler flowmetry on sacral skin. *Clin Physiol, 16*(1), 61-72.

18. Fromy, B., Legrand, M. S., Abraham, P., Leftheriotis, G., Cales, P., & Saumet, J. L. (1997). Effects of positive pressure on both femoral venous and arterial blood velocities and the cutaneous microcirculation of the forefoot. *Cardiovasc Res, 36*(3), 372-376.

19. Schubert, V. (2000). The influence of local heating on skin microcirculation in pressure ulcers, monitored by a combined laser Doppler and transcutaneous oxygen tension probe. *Clin Physiol, 20*(6), 413-421.

20. Minson, C. T., Berry, L. T., & Joyner, M. J. (2001). Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *J Appl Physiol, 91*(4), 1619-1626.

21. Ubbink, D. T., Jacobs, M. J., & Slaaf, D. W. (1995). Can transcutaneous oximetry detect nutritive perfusion disturbances in patients with lower limb ischemia? *Microvasc Res, 49*(3), 315-324.

22. Ewald, U., Huch, A., Huch, R., & Rooth, G. (1987). Skin reactive hyperemia recorded by a combined TcPO2 and laser Doppler sensor. *Advances in Experimental Medicine & Biology, 220*, 231-234.

23. Intaglietta, M. (1991). Arteriolar vasomotion: implications for tissue ischemia. *Blood Vessels*, 28(Suppl 1), 1-7.

24. Colantuoni, A., Bertuglia, S., & Intaglietta, M. (1994). Microvascular vasomotion: origin of laser Doppler flux motion. *Int J Microcirc Clin Exp*, 14, 151-158.

25. Lossius, K., & Eriksen, M. (1995). Spontaneous flow waves detected by laser Doppler in human skin. *Microvasc Res*, 50, 94-104.

26. Bertuglia, S., Colantuoni, A., Arnold, M., & Witte, H. (1996). Dynamic coherence analysis of vasomotion and flow motion in skeletal muscle microcirculation. *Microvasc Res*, 52, 235-244.

27. Ursino, M., & Fabbri, F. (1992). Role of the myogenic mechanism in the genesis of microvascular oscillations (vasomotion); analysis with a mathematical model. *Microvasc Res*, 52, 235-244.

28. Achakri, H., Racev, A., Stergiopulos, N., & Meister, J.J. (1994). A theoretical investigation of low frequency diameter oscillations of muscular arteries. *Ann Biomed Eng*, 22, 253-263.

29. Bracic, M., & Stefanovska, A. (1998). Wavelet-based analysis of human blood-flow dynamics. *Bull Math Biol*, 60, 919-935.

30. Stergiopulos, N., Porret, C.A., De Brouwer, S., & Meister, J.J. (1998). Arterial vasomotion: effect of flow and evidence of nonlinear dynamics. *Am J Physiol*, 274, H1858-H1864.

31. Schubert, R., & Mulvany, M.J. (1999). The myogenic response: established facts and attractive hypothesis. *Clin Sci*, 96, 313-326.

32. Butler, P.J., Weinbaum, S., Chien, S., & Lemons, D.E. (2000). Endothelium-dependent, shear-induced vasodilation is rate-sensitive. *Microcirculation,* 7, 53-65.

33. Bouskela, E., & Grampp, W. (1992). Spontaneous vasomotion in hamster cheek pouch arterioles in varying experimental conditions. *Am J Physiol,* 262, H478-H485.

34. Fujii, K., Heistad, D.D., & Faraci, F.M. (1990). Ionic mechanisms in spontaneous vasomotion of the rat basilar artery in vivo. *J Physiol,* 430, 389-398.

35. Gustafsson, H. (1993). Vasomotion and underlying mechanisms in small arteries. *Acta Physiol Scand,* 614, 2-44.

36. Verbeuren, T.J., Vallex, M.O., Lavielle, G., & Bouskela, E. (1997). Activation of thromboxane receptors and the induction of vasomotion in the hamster cheek pouch microcirculation. *Br J Pharmacol,* 122, 859-866.

37. Meyer, J.U., Lindbom, L., & Intaglietta, M. (1987), Coordinated diameter oscillations at arteriolar bifurcations in skeletal muscle. *Am J Physiol,* 253, H568-H573.

38. Salerud, E.G., Tenland, T., Nilsson, G.E., & Oberg, P.A. (1983). Rhythmical variations in human skin blood flow. *Int J Microcirc Clin Exp,* 2, 91-102.

39. Wilkin, J.K. (1986). Periodic cutaneous blood flow during postocclusive reactive hyperemia. *Am J Physio,* 250, H765-H768.

40. Wilkin, J.K. (1988). Periodic cutaneous blood flow during aldehyde provoked hyperemia. *Microvas Res,* 35, 287-289.

41. Brienza, D.M., Geyer, M.J., & Jan, Y.K. (2005). A comparison of changes in rhythms of sacral skin blood flow in response to heating and indentation. *Arch Phys Med Rehabil,* 86, 1245-1251.

42. Geyer, M.J., Jan, Y.K., Brienza, D.M., & Boninger, M.L. (2004). Using wavelet analysis to characterize the thermoregulatory mechanisms of sacral skin blood flow. *J Rehabil Res Dev,* 41(6), 797-806.

43. Claydon, V.E., Steeves, J.D., & Krassioukov (2006). Orthostatic hypotension following spinal cord injury: understanding clinical pathophysiology. *Spinal Cord,* 44, 341-351.

44. Calaresu, F.R., & Yardley, C.P. (1988). Medulalary basal sympathetic tone. *Ann Rev Physiol,* 50, 511-524.

45. Osborn, J.W., Taylor, R.F., & Schramm, L.P. (1989). Determinants of arterial pressure after chronic spinal transaction in rats. *Am J Physiol,* 256, R666-R673.

46. Krum, H., *et al.* (1992). Risk factors for cardiovascular disease in chronic spinal cord injury patients. *Paraplegia,* 30, 381-388.

47. Devivo, M.J., Krause, J.S., & Lammertse, D.P. (1999). Recent trends in mortality and causes of death among persons with spinal cord injury. *Arch Phys Med Rehabil,* 80, 1411-1419.

48. Garshick, E., *et al.* (2005). A prospective assessment of mortality in chronic spinal cord injury. *Spinal Cord,* 43, 408-416.

49. Verberne, A.J., & Owens, N.C. (1998). Cortical modulation of the cardiovascular system. *Prog Neurobiol,* 54, 149-168.

50. Furlan, J.C., Rehlings, M.G., Shannon, P., Norenberg, M.D., & Krassioukov, A.V. (2003). Descending vasomotor pathways in humans: correlation between axonal preservation and cardiovascular dysfunction after spinal cord injury. *J Neurotrauma,* 20, 1351-1363.

51. Ditunno, J.F., Little, J.W., Tessler, A., & Burns, A.S. (2004) Spinal shock revisted: a four-phase model. *Spinal Cord,* 42, 383-395.

52. Atkinson, P.P., & Atkinson, J.L.D. (1996). Spinal shock. *Mayo Clin Proc,* 71, 384-389.

53. Mathias, C.J., Mallipeddia, R., & Bleasdale-Barr, K. (1999). Symptoms associated with orthostatic hypotension in pure autonomic failure and multiple system atrophy. *J Neurol,* 246, 893-898.

54. Mathias, C.J., & Frankel, H.L. (1983). Clinical manifestations of malfunctioning sympathetic mechanisms in tetraplegia. *J Auton Nerv Syst,* 7, 303-312.

55. Pressure ulcer stages revised by NPUAP (2007). http://www.npuap.org/pr2.htm

56. Bansal, C., Scott R., Stewart D., & Cockerell C.J. (2005). Decubitus ulcers: A review of the literature. *International Journal of Dermatology,* 44 (10), 805–810.

57. National Pressure Ulcer Advisory Panel Board of Directors, Cuddigan, J., Berlowitz, D. R., & Ayello, E. A. (2001). Pressure ulcers in America: prevalence, incidence, and implications for the future. An executive summary of the National Pressure Ulcer Advisory Panel monograph. *Advances in Skin & Wound Care*, 14(4), 208-215.

58. Defloor, T., & Schoonhoven, L. (2004). Inter-rater reliability of the EPUAP pressure ulcer classification system using photographs. *J Clin Nurs,* 13, 952-959.

59. Black, J.M., NPUAP. (2005). Moving toward consensus on deep tissue injury and pressure ulcer staging. *Advances in Skin & Wound Care,* 18(8). 415-421.

60. Crenshaw, R.P., & Vistnes, L.M. (1989). A decade of pressure sore research: 1977-1987. *J Rehabil Res Dev,* 26(1), 63-74.

61. Niezgoda, J.A., & Mendez-Eastman, S. (2006). The effective management of pressure ulcers. *Adv Skin Wound Care,* 19 (suppl 1), 3-15.

62. Dinsdale, S. (1974). Mechanical factors in the pathogenesis of ischemic skin ulcers in swine (dissertation). University of Minnesota.

63. Bennett, L., Kavner, D., Lee, B.K., & Trainor, F.A. (1979). Shear vs pressure as causative factors in skin blood flow occlusion. *Arch Phys Med Rehabil,* 60(7), 309-314.

64. Reuler, J. B., & Cooney, T. G. (1981). The pressure sore: pathophysiology and principles of management. *Annals of Internal Medicine,* 94(5), 661-666.

65. Jan, Y.K., & Brienza, D.M. (2006). Technology for pressure ulcer prevention. *Top Spinal Cord Inj Rehabil,* 11(4), 30-41.

66. Clark, F.A., Jackson, J.M., Scott, M.D., Carlson, M.E., & Atkins, M.S., Uhles-Tanaka, D., Rubayi, S. (2006). Data-based models of how pressure ulcers develop in daily-living contexts of adults with spinal cord injury. *Arch Phys Med Rehabil,* 87, 1516-1525.

67. Courtney, B.A., Ruppman, J.B., & Cooper, H.M. (2006). Save our skin: initiative cuts pressure ulcer incidence in half. *Nursing Management,* 37(4), 36-45.

68. Peterson N.C., & Bittman S. (1971) The epidemiology of pressure sores. *Scand J Plast Reconstr Surg Hand Surg,* 5, 62–66.

69. Morrison S. (1984). Monitoring decubitus ulcers: a monthly survey method. *Quart Rev Bull* , 10,112–117.

70. Guralnik J.M., Harris T.B., White L.R., et al. (1988). Occurrence and predictors of pressure ulcers in the national health and nutrition examination survey follow-up. *J Am Geriatr Soc,* 36, 807–812.

71. Thomas D.R. (2001). Issues and dilemmas in the prevention and treatment of pressure ulcers. *J Geronto,* 56(6), M328–M340.

72. Beckrich K., & Aronovitch, S.A. (1999). Hospital-acquired pressure ulcers: a comparison of costs in medical vs. surgical patients. *Nurs Econ,* 17, 263-271.

73. Zhan C., & Miller, M.R. (2003). Excess length of stay, charges, and mortality attributable to medical injuries during hospitalization. *JAMA,* 290, 1868-1874.

74. Pompeo, M. Q. (2001). The role of "wound burden" in determining the costs associated with wound care. *Ostomy Wound Management,* 47(3), 65-71.

75. Landis E.M. (1930). Micro-injection studies of capillary blood pressure in human skin. *Heart,* 15, 209-228.

76. Bader, D. L. (1990). The recovery characteristics of soft tissues following repeated loading. *J Rehabil Res Dev, 27*(2), 141-150.

77. Lima A., & Bakker, J. (2005). Noninvasive monitoring of peripheral perfusion. *Intensive Care Med,* 31, 1316-1326.

78. Fullerton, A., Stucker, M., Wilhelm, K. P., Wardell, K., Anderson, C., Fischer, T., et al. (2002). Guidelines for visualization of cutaneous blood flow by laser Doppler perfusion imaging. A report from the Standardization Group of the European Society of Contact Dermatitis based upon the HIRELADO European community project. Contact Dermatitis, 46(3), 129-140.

79. Schubert, V., & Heraud, J. (1994). The effects of pressure and shear on skin microcirculation in elderly stroke patients lying in supine or semi-recumbent positions. Age &Ageing, 23(5), 405-410.

80. Feldman, D. L., Sepka, R. S., & Klitzman, B. (1993). Tissue oxygenation and blood flow on specialized and conventional hospital beds. Annals of Plastic Surgery, 30(5), 441-444.

81. Lotric, M.B., Stefanovska, A., Stajer, D., & Urbancic-Rovan, V. (2000). Spectral components of heart rate variability determined by wavelet analysis. *Physiol Meas,* 21(4), 441-457.

82. Karlsson, S., Yu, J., & Akay, M. (2000). Time-frequency analysis of myoelectric signals during dynamic contractions: a comparative study. *IEEE Trans Biomed Eng,* 47(2), 228-238.

83. Stefanovska, A., & Bracic, M. (1999). Physics of the human cardiovascular system. *Contemp Phys,* 40(1), 31-55.

84. Stefanovska, A., Bracic, M., & Kvernmo, H.D. (1999). Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. *IEEE Trans Biomed Eng,* 46(10), 1230-1239.

85. Jan, Y.K., Brienza, D.M., & Geyer, M.J. (2005). Analysis of week-to-week variability in skin blood flow measurements using wavelet transforms. *Clin Physiol Funct Imaging,* 25, 253-262.

86. Li, Z., Leung, J.Y., Tam, E.W., & Mak, A.F. (2006). Wavelet analysis of skin blood oscillations in persons with spinal cord injury and able-bodied subjects. *Arch Phys Med Rehabil,* 87, 1207-1212.

87. Capovilla, J., VanCouwenberghe, C., & Miller, W. A. (2000). Noninvasive blood gas monitoring. *Critical Care Nursing Quarterly, 23*(2), 79-86.

88. Porter, J., Bonello, E., & Reynolds, F. (1998). Effects of epidural fentanyl on neonatal respiration. *Anesthesiology,* 89(1), 79-85.

89. Wutschert, R., & Bounameaux, H. (1997). Determination of amputation level in ischemic limbs: reappraisal of the measurement of $TcPO_2$. *Diabetes Care,* 20(8), 1215-1318.

90. Fife, C.E., Buyuckvakir, C., Otto, G.H., Sheffield, P.J., Warriner, R.A., Love, T.L., & Mader, J. (2002). The predictive value of transcutaneous oxygen tension measurement in diabetic lower extremity ulcers treated with hyperbaric oxygen therapy: a retrospective analysis of 1144 patients. *Wound Rep Reg,* 10, 198-207.

91. Ouriel, K. (2001). Peripheral arterial disease. *The Lancet,* 358, 1257-1264.

92. Khodabandehlou, T., Vimeux, M., & Devehat, C.L. (2003). Measurements of transcutaneous oxygen pressure and changes in blood rheology as markers of prognosis of critically ischemic limb in diabetes mellitus patients. *Lower Extremity Wounds,* 2(1), 13-18.

93. Melillo, E., Catapano, G., Dell'Omo, G., Iabichella, L., Berchiolli, R., Ferrari, M., & Pedrinelli, R. (1995). Transcutaneous oxygen and carbon dioxide measurement in peripheral vascular disease. *Vascular Surgery,* 29(4), 273-280.

94. Cina, C., Katsamouris, A., Megerman, J., Brewster, D.C., Strayhor, E.C., Robison, J.G., & Abbott, W.M. (1984). Utility of transcutaneous oxygen tension measurements in peripheral arterial occlusive disease. *J Vasc Surg,* 1, 362-371.

95. Pabderg, F.T., Back, T.L, Thompson, P.N., & Hobson, R.W. (1996). Transcutaneous oxygen ($TcPO_2$) estimates probability of healing in ischemic extremity. *Journal of Surgical Research,* 60, 365-369.

96. Hauser, C.J., & Shoemaker, W.C. (1983). Use of a Transcutaneous PO_2 regional perfusion index to quantify tissue perfusion in peripheral vascular disease. *Ann Surg,* 197(3), 337-343.

97. McPhail, I.R., Cooper, L.T., Hodge, D.O., Cabenela M.E., & Rooke, T.W. (2004). Transcutaneous partial pressure of oxygen after surgical wounds. *Vascular Medicine,* 9(2), 125-127.

98. Sangeorzan, B.J., Harrington, R.M., Wyss, C.R., Czerniecki, J.M., & Matsen, F.A. (1989). Circulatory and mechanical response of skin to loading. Journal of Orthopaedic Research, 425-431.

99. Kellogg, D.L., Liu, Y., Kosiba, I.F., & O'Donnell, D. (1999). Role of nitric oxide in the vascular effects of local warming of the skin in humans. *J Appl Physiol*, 86, 1185-1190.

100. Arnal, J.F., Dinh-Xuan, A.T., Pueyo, M., Darblade, B., & Rami, J. (1999). Endothelium-dervied nitric oxide and vascular physiology and pathology. *Cell Mol Life Sci*, 55, 1078-1087

101. Gaskell, P., & Burton, A.C. (1953). Local postural vasomotor reflexes arising from the limb veins. *CircuRes,* 1, 27-39.

102. Hassan, A., & Tooke, J.E. (1988). Mechanism of the postural vasoconstrictor response in the human foot. *Clin Sci,* 75, 201-206.

103. Mayrovitz, H.N. (1997). Posturally induced leg vasoconstrictive responses: relationship to standing duration, impedance and volume changes. *Clinical Physiology*, 18(4), 311-319.

104. Rendell, M.S., Giiter, M., Bamisedun, O., Davenport, K., & Schultz, R. (1992). The laser Doppler analysis of posturally induced changes in skin blood flow at elevated temperatures. *Clinical Physiology*, 12, 241-252.

105. Rayman, G., Hassan, A., & Tooke, J. (1986). Blood flow in the skin of the foot related to posture in diabetes mellitus as well as in young diabetic individuals. *Br Med J*, 11, 87-90.

106. Belcaro, G., Vaskedis, S., Rulo, A., & Nicolaides, A. (1989). Evaluation of skin blood flow and venoarteriolar response in patients with diabetes and peripheral vascular disease by laser Doppler flowmetry. *Angiology*, 40, 953-957.

107. Ubbink, D.T., Jacobs, M.J.H.M., Tangelder, G.J., Slaaf, D.W., & Reneman, R.S. (1991). Posturally induced microvascular constrction in patients with different stages of leg ischaemia: effect of local skin heating. *Clin Sci,* 81, 43-49.

108. Jacobsen, T.N., *et. al* (1993). Relative contributions of cardiopulmonary and sinoaortic baroreflexes in causing sympathetic activation in the human skeletal muscle circulation during orthostatic stress. *Circ Res,* 73, 367-378.

109. Vissing, S.F., Sechere, N.H., & Victor, R.G. (1997). Mechanisms of cutaneous vasoconstriction during upright posture. *Acta Physiol Scand*, 159, 131-138.

110. Henriksen, O., Skagen, K., Haxholdt, O., & Dyrberg, V. (1983). Contribution of local blood flow regulation mechanisms to the maintenance of arterial pressure in upright position during epidural blockade. *Acta Physiol Scand*, 118, 271-280.

111. Moy, S., Opfer-Gehrking, T.L., Proper, C.J., & Low, P.A. (1989). The venoarteriolar reflex in diabetic and other neuropathies. *Neurology,* 39, 1490-1492.

112. Richardson, D.R., & Shepherd, S. (1989). Separate effects of gravity and venous pressure on regional and capillary blood flows in the human finger. *Microcirc Endothelium Lymphatics*, 5, 417-433.

113. Zoltie, N., Young, C., Faris, I., & Tan E. (1989). The veno-arteriolar reflex in free skin flaps. *Clin Physiol*, 9, 183-188.

114. Davis, M.J., & Hill, M.A. (1999). Signaling mechanisms underlying the vascular myogenic reponse. *Physiol Rev*, 79, 387-423.

115. Fokow, B. (1995). Hypertensive structural changes in systemic precapillary resistance vessels: how important are they for in vivo haemodynamics? *J Hypertens*, 13, 1546-1559.

116. Hill, M.A., Zou, H., Potocnik, S.J., Meininger, G.A., & Davis, M.J. (2001). Invited reviews: arteriolar smooth muscle mechanotransduction: Ca^{2+} signaling pathways underlying myogenic reactivity. *J Appl Physio,* 91, 973-983.

117. Illman, A., Stiller, K., & Williams, M. (2000). The prevalence of orthostatic hypotension during physiotherapy treatment in patients with an acute spinal cord injury. *Spinal Cord,* 38, 741-746.

118. Kosiak, M. (1961). Etiology of decubitus ulcers. *Arch Phys Med Rehabil*, 42, 19-29.

119. Frantz, R.A., & Xakellis, G.C. (1989). Characteristics of skin blood flow over the trochanter under constant, prolonged pressure. *Am J Phys Med Rehabil*, 68(6), 272-276.

120. Kemp, M.G., Keithley, J.K., Smith, D.W., & Morreale, B. (1990). Factors that contribute to pressure sores in surgical patients. *Research in Nursing & Health*, 13, 293-301.

121. Bader, D.L, Barhill, R.L., & Ryan, T.J. (1986), Effects of externally applied skin surface forces on tissue vasculature. *Arch Phys Med Rehabil*, 67, 807-811.

122. Dinsdale, S.M. (1974). Decubitus ulcers: role of pressure and friction in causation. *Arch Phys Med Rehabil*, 55, 147-152.

123. Mayrovitz, H.N., Sims, N., & Dribin, L. (2003). Heel skin hyperaemia: direct compression versus vascular occlusion. *Clin Physiol Funct Imagining*, 23, 354-359.

124. Tee, G.B.Y., Rasool, A.H.G., Halim, A.S., & Rahman, A.R.A. (2004). Dependence of human forearm skin postocclusive reactive hyperemia on occlusion time. *Journal of Pharmacological and Toxicological Methods*, 50, 73-78.

125. Gidlof, A., Lewsis, D., & Hammersen, F. (1988). The effect of prolonged total ischemia on the ultrastrucutre of human skeletal muscle capillaries: a morphometric analysis. *International Journal of Microcirculation, Clinical and Experimental*, 7, 257-265.

126. Herrman, E., Knapp, C., Donofrio, J., & Salcido, R. (1999). Skin Perfusion Responses to Surface Pressure Induced Ischemia: Implication for the Developing Pressure Ulcer. *Journal of Rehabilitaion Research and Development*, *36*(2), 109-120.

127. Bayliss, W. (1902). On the local reactions of the arterial wall to changes of internal pressure. *J Physiol*, 28, 220-231.

128. Guyton, A., Hall, J. (1996). Textbook of medical physiology. 9th ed. Philadelphia: WB Saunders.

129. Xakellis, G.C., Frantz, R.A., Arteagra, M., & Meletiou, S. (1993). Dermal blood flow response to constant pressure in healthy older and younger subjects. *J Gerontol,* 48, M6-M9.

130. Patel, S., Knapp, C.F., Donofrio, J.C., & Salcido, R. (1999). Temperature effects on surface pressure-induced changes in rat skin perfusion: implications in pressure ulcer developments. *J Rehabil Res Dev,* 36. 189-201.

131. Mayrovitz, H.N., & Smith, J.R. (1999). Adaptive skin blood flow increases during hip-down lying in elderly women. *Adv Wound Care,* 15, 295-301.

132. Johnson, P.C., Burton, K.S., Henrich, H., & Henrich, U. (1976). Effect of occlusion duration on reactive hyperemia in sartorius muscle capillaries. *American Journal of Physiology,* 230(3), 715-719.

133. Lombard, J.H., & Duling, B.R. (1981). Multiple mechanisms of reactive hyperemia in arterioles of the hamster cheek pouch. *American Journal of Physiology,* 241(5), H748-H755.

134. Hagisawa, S., Ferguson-Pell, M., Cardi, M., & Miller, D. (1994). Assessment of skin blood content and oxygenation in spinal cord injured subjects during reactive hyperemia. *Journal of Rehabilitation Research*, 31(1), 1-14.

135. Bidart, Y., & Maury, M. (1973). The circulatory behavior in complete chronic paraplegia. *Paraplegia*, 11, 1-24.

136. Mahanty, S., Roemer, R.B., & Meisel, H. (1981) Thermal response of paraplegic skin to the application of localized pressure. *Arch Phys Med Rehabil,* 62, 608-611.

137. Schubert, V., & Fagrell, B. (1991). Postocclusive hyperemia response in the skin microcirculation of subjects with spinal cord injury. *Scand J Rehabil Med,*23, 33-40.

138. Hagisawa, S., Barbenel, J.C., & Kenedi, R.M. (1991). Influence of age on postischaemic reactive hyperaemia. *Clin Phs Physiol Meas,* 12(3), 227-237.

139. Richy, M.L., Richey, H.K. & Fenske, N.A. (1988). Aging-related skin changes: development and clinical meaning. *Geriatrics,* 43, 49-64.

140. Davis, M. J., Shi, X., & Sikes, P. J. (1992). Modulation of bat wing venule contraction by transmural pressure changes. *Am J Physiol*, 262, H625-H634.

141. Berczi, V., Greene, A. S., Dornyei, G. et al. (1992). Venous myogenic tone: studies in human and canine vessels. *Am J Physiol*, 263, H315-H320.

142. Kuo, L., Arko, F., Chilian, W. M., & Davis, M. J. (1993). Coronary venular responses to flow and pressure. *Circ Res*, 72, 607-615

143. Dornyei, G., Monos, E., Kaley, G., & Koller, A. (1996). Myogenic responses of isolated rat skeletal muscle venules: modulation by norepinephrine and endothelium. *Am J Physiol*, 271, H267-H272.

144. Sun, D., Messina, E.J., Kaley, G., & Koller, A. (1992). Characteristics and origin of myogenic response in isolated mesenteric arterioles. *Am J Physiol*, 263, H1486-H1491.

145. Johnson, P. C. (1989). The myogenic response in the microcirculation and its interaction with other control systems. *J Hypertens*, 7, S33-S39.

146. Zou, H., Ratz, P. H. & Hill, M. A. (1995). Role of myosin phosphorylation and [Ca^{2+}] in myogenic reactivity and arteriolar tone. *Am J Physiol*, 269, H1590-H1596.

147. Meininger, G. A. & Davis, M. J. (1992). Cellular mechanisms involved in the vascular myogenic response. *Am J Physiol*, 263, H647-H659.

148. Engelke, K.A., Halliwill, J.R., Proctor, D.N., Dietz, N.M., & Joyner, M.J. (1996). Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *J Appl Physiol*, 81, 1807-1814.

149. Harder, D. R. (1984). Pressure-dependent membrane depolarization in cat middle cerebral artery. *Circ Res*, 55, 197-202.

150. Harder, D. R., Smeda, J., & Lombard, J. (1985). Enhanced myogenic depolarization in hypertensive cerebral arterial muscle. *Circ Res*, 57, 319-322.

151. Knot, H. J., & Nelson, M. T. (1998). Regulation of arterial diameter and wall [Ca^{2+}] in cerebral arteries of rat by membrane potential and intravascular pressure. *J Physiol*, 508, 199-210.

152. Knot, H. J., & Nelson, M. T. (1995). Regulation of membrane potential and diameter by voltage-dependent K+ channels in rabbit myogenic cerebral arteries. *Am J Physiol*, 269, H348-H355.

153. Harder, D. R., Gilbert, R., & Lombard, J. H. (1987). Vascular muscle cell depolarization and activation in renal arteries on elevation of transmural pressure. *Am J Physiol*, 253, F778-F781.

154. Smeda, J. S., & Daniel, E. E. (1988). Elevations in arterial pressure induce the formation of spontaneous action potentials and alter neurotransmission in canine ileum arteries. *Circ Res*, 62, 1104-1110.

155. Wesselman, J. P., Schubert, R., VanBavel, E. D., Nilsson, H., & Mulvany, M. J. (1997). KCa-channel blockade prevents sustained pressure-induced depolarization in rat mesenteric small arteries. *Am J Physiol*, 272, H2241-H2249.

156. Davis, M. J., Donovitz, J. A., & Hood, J. D. (1992). Stretch-activated single-channel and whole cell currents in vascular smooth muscle cells. *Am J Physiol*, 262, C1083-C1088.

157. Setoguchi, M., Ohya, Y., Abe, I., & Fujishima, M. (1997). Stretch-activated whole-cell currents in smooth muscle cells from mesenteric resistance artery of guinea-pig. *J Physiol*, 501, 343-353.

158. Brayden, J. E., & Nelson, M. T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* 256, 532-535.

159. Knot, H. J., Standen, N. B., & Nelson, M. T. (1998). Ryanodine receptors regulate arterial diameter and wall [Ca^{2+}] in cerebral arteries of rat via Ca^{2+} dependent K+ channels. *J Physiol*, 508, 211-222.

160. Nelson, M. T., Conway, M. A., Knot, H. J., & Brayden, J. E. (1997). Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. *J Physiol*, 502, 259-264.

161. McCarron, J. G., Crichton, C. A., Langton, P. D., MacKenzie, A., & Smith, G. L. (1997). Myogenic contraction by modulation of voltage-dependent calcium currents in isolated rat cerebral arteries. *J Physiol*, 498, 371-379.

162. Meininger, G. A., Zawieja, D. C., Falcone, J. C., Hill, M. A., & Davey, J. P. (1991) Calcium measurement in isolated arterioles during myogenic and agonist stimulation. *Am J Physiol*, 261, H950-H959.

163. Masumoto, N., Nakayama, K., Oyabe, A. et al. (1997). Specific attenuation of the pressure-induced contraction of rat cerebral artery by herbimycin A. *Eur J Pharmacol*, 330, 55-63.

164. Abraham, P., Fromy, B., Merzeau, S., & Saumet, J.L. (2001). Dynamics of local pressure-induced cutaneous vasodilation in the human hand. *Microvascular Research*, 61, 122-129.

165. Ewald, U., Rooth, G., & Tuvemo, T. (1981). Postischameic hyperaemia studied with a transcutaneous oxygen electrode used at 33-37 °. *Scan J Clin Lab Invest*, 41, 641-645.

166. Mayrovitz, H. N., Regan, M. B., & Larsen, P. B. (1993). Effects of rhythmically alternating and static pressure support surfaces on skin microvascular perfusion. *Wounds*, 5, 47-55.

167. Jan, Y. K. (2004). A study on skin blood flow control mechanisms using wavelet analysis: implications for alternating pressure support surfaces. *Ph.D. Dissertation.* University of Pittsburgh, Pittsburgh, PA.

168. Strang, G., & Nguyen, T. (1997). Wavelets and Filter Banks. Wellesley, MA: Wellesley-Cambridge Press.

169. Grossmann, A., & Morlet, J. (1984). Decomposition of Hardy functions into square integrable wavelets of constant shape. SIAM Journal on Mathematical Analysis, 15(4), 723-736.

170. Hubbard, B. B. (1996). The World According to Wavelets. Wellesley, MA: A K Peters, Ltd.

171. Hassan, A.A., Rayman, G., & Tooke, J.E. (1986). Effect of indirect heating on the postural control of skin blood flow in the human foot. *Clin Sci*, 70, 577-582.