EFFECTS OF LOCAL COOLING ON SKIN PERFUSION RESPONSE TO PRESSURE: IMPLICATIONS TO PRESSURE ULCER PREVENTION

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Pressure ulcers have long been an important healthcare issue in both acute and long-term care settings. Temperature is one of the extrinsic causative factors for this multi-factorial disease not yet fully explored. Previous animal studies revealed that skin cooling reduced the severity of ulceration compared to non-cooling. Cooling is also used widely in plastic surgery and organ transplants for tissue preservation. However, the underlying protective mechanism of local cooling remains unclear. Our study's objective was to measure the effect of cooling on tissue's response to pressure using skin perfusion response on human subjects. Reactive hyperemia is a normal protective physiological response occurring after vessel occlusion. Laser Doppler flowmetrey was used to measure cutaneous perfusion. We hypothesized that local cooling would reduce a rigid indenter induced post-ischemic reactive hyperemic response. Ten young healthy non-smokers were recruited into the study. A repeated measures design was used where all subjects were subjected to pressure with cooling to 25°C and pressure without cooling test sessions. Each test session contained five levels of pressure control: light contact (10 minutes), 60 mmHg (30 minutes), light contact (20 minutes), 150 mmHg (3 minutes), light contact (10 minutes). The cooling intervention was performed during the period of 60mmHg contact pressure. Our results showed a significantly attenuated peak perfusion response after 60mmHg

(p=0.019) but not after 150mmHg (p=0.241) of pressure for the cooling session compared to the non-cooling. This study suggests that local cooling may protect skin from the harmful effects of prolonged pressure in this young healthy population. The study protocol would be modified to investigate populations at risk of pressure ulcers.

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PREFACE

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1.0 INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Pressure ulcers have long been an important healthcare issue in acute and long term care settings (The National Pressure Ulcer Advisory Panel, 1989). It is especially significant in people with impaired mobility, including hospitalized elderly people (Allman, 1989; Amlung, Miller, & Bosley, 2001) and people with spinal cord injury (Stover & Fine, 1986). Researchers have investigated methods to prevent pressure ulcers. Since prolonged pressure is the main cause of pressure ulcers (Daniel, Priest, & Wheatley, 1981; Michael Kosiak, 1961), techniques such as pressure redistribution are widely used in pressure ulcers prevention (Brienza & Geyer, 2005; Jan & Brienza, 2006); and these devices are categorized in the Center for Medicaid and Medicare services for reimbursement (Jan & Brienza, 2006). Despite the technologies used, healthcare providers are educated and trained to reposition patients that are bed ridden at least every two hours for pressure redistribution (Panel for the Prediction and Prevention of Pressure Ulcers in Adults, 1992). Although prolonged pressure is regarded as the main causative factor of pressure ulcers, pathology of ulceration was not fully explored (Bouten, Oomens, Baaijens, & Bader, 2003). Many researches found that pressure ulcers resulted from tissue ischemia (Daniel, Priest, & Wheatley, 1981; Michael Kosiak, 1961). When skin perfusion is occluded, distribution of nutrition reduces and metabolic wastes accumulate. Tissue necrosis occurs as a result and finally

ulceration (Bouten, Oomens, Baaijens, & Bader, 2003). In other words, if the metabolic rate of the skin tissue could be reduced, tissue would better withstand ischemia and therefore lengthen the time period before tissue necrosis occurs.

The human metabolic rate increases 10% per degree Celsius (Ruch & Patton, 1965). It is believed that by decreasing the tissue temperature, the metabolic rate reduces and therefore slows down metabolic waste accumulation to preserve tissue viability (Kokate et al., 1995). Such cooling methods have been used in fields including plastic surgery to store the split skin grafts (Sterne, Titley, & Christie, 2000), and organ transplantation to maintain organ viability under ischemia (Riess, Camara, Kevin, An, & Stowe, 2004). The local cooling concept on tissue preservation under prolonged pressure was investigated through animal studies, and the results supported that local cooling on the skin could significantly prevent pressure ulcer development (Iaizzo, 2004; Iaizzo et al., 1995; Kokate et al., 1995). Increased temperature as one of the risk factors causing pressure ulcers is not fully explored (Kokate et al., 1995), and researchers found that skin temperature increases over time naturally after sitting on different types of wheelchair seat cushions (Cochran, 1985; Fisher, Szymke, Aptem, & Kosiak, 1978; Stewart, Palmieri, & Cochran, 1980). Given that skin temperature increases over time inevitably, it is important to investigate the effectiveness of local cooling on pressure ulcer prevention on human subjects.

1.2 OBJECTIVE AND HYPOTHESES

The objective of the study is to better understand the effectiveness of local cooling in preserving tissue viability under an ischemic event. In order to test our hypothesis non-invasively, parameters of the reactive hyperemic response were used. Reactive hyperemia is a normal

physiological response toward tissue ischemia (Bongard & Bounameaux, 1993). The magnitude and duration of the reactive hyperemic response is dependent on the severity of tissue ischemia and can be quantified by the following parameters: normalized peak skin blood flow (SBF), time to peak SBF, half life of SBF, and SBF area (refer to section 3.5 for parameter definitions) (Bongard & Bounameaux, 1993). These parameters were used in this study to non-invasively investigate the effectiveness of local cooling on sacral tissue viability under pressure.

In order to reach the goal of the study, eight hypotheses were tested: 1) Normalized peak SBF after 60mmHg pressure removal in subjects with cooling is smaller than that without cooling. 2) Time to peak SBF after 60mmHg pressure removal in subjects with cooling is longer than that without cooling. 3) Half life of the SBF after 60mmHg pressure removal in subjects with cooling is shorter than that without cooling. 4) SBF area after 60mmHg pressure removal in subjects with cooling is smaller than that without cooling. 5) Normalized peak SBF after 150mmHg pressure removal in subjects with cooling is smaller than that without cooling. 6) Time to peak SBF after 150mmHg pressure removal in subjects with cooling is longer than that without cooling. 7) Half life of the SBF after 150mmHg pressure removal in subjects with cooling is shorter than that without cooling. 8) SBF area after 150mmHg pressure removal in subjects with cooling is smaller than that without cooling.

1.3 SIGNIFICANCE

Although previous animal studies showed that local cooling could prevent pressure ulcer development, the mechanism of local cooling is not fully explored. In addition, effectiveness of local cooling on skin tissue preservation toward mechanical pressure has not been tested on

human subjects. In this study, by assessing changes in reactive hyperemic response after short duration non-damaging ischemia, we can better understand the effectiveness and mechanism of local cooling on soft tissues in healthy adults. In the future, we could apply the research protocol on people at risk of pressure ulcers.

2.0 REVIEW OF THE LITERATURE

2.1 PRESSURE ULCER BACKGROUND

2.1.1 Pressure Ulcer Definition

Pressure ulcers, also noted as decubitus ulcers (Michael Kosiak, 1961), bedsores, and pressure sores (The National Pressure Ulcer Advisory Panel, 1989), are "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" (National Pressure Ulcer Advisory Panel, 2007a). Pressure ulcers could occur at different parts of soft tissue. It is usually found in the area of a bony prominence (Amlung, Miller, & Bosley, 2001; Michael Kosiak, 1961; The National Pressure Ulcer Advisory Panel, 1989), such as sacrum, heel, ischial tuberosity, elbow, malleolus and greater trochanter (Bansal, Scott, Stewart, & Cockerell, 2005).

2.1.2 Pressure Ulcer Etiology

Researchers have long been investigating the etiology of pressure ulcers, and most findings suggested that sustained pressure and shear force are the main etiologic factors of this multifactor disease (Yarkony, 1994).

2.1.2.1 Sustained Pressure

Early studies revealed that pressure ulcers affected the population that are chair, bed-ridden, prosthesis or orthosis users, and the tissue breakdown spots were discovered at the area where the skin is exposed to prolonged pressure (M Kosiak, 1959). Landis (1930) recommended that the capillary perfusion pressure of 32mmHg was the threshold of such tissue damage. Rather than this specific threshold of pressure causing skin breakdown, studies thereafter suggested an inverse relationship between magnitude and duration of pressure exposure (Daniel, Priest, & Wheatley, 1981; Michael Kosiak, 1961; Reswick & Rogers, 1976) in ulceration. In other words, the smaller the loading, the longer time it takes for tissue damage to occur.

Although sustained pressure was well acknowledged as the key factor causing pressure ulcers, the underlying pathology and pathway remained unclear. Several different mechanisms could account for the effect of prolonged pressure on ulcer development, including localized ischemia, lymphatic impairment, mechanical cell deformation, and reperfusion injury. The term "ischemic ulcer" was used in the early pressure ulcer studies (M Kosiak, 1959; Michael Kosiak, 1961). It was believed that prolonged pressure on the skin disturbed the capillary circulation (Daniel, Priest, & Wheatley, 1981; Dinsdale, 1974; Michael Kosiak, 1961), and caused tissue anoxia by reducing blood perfusion to the cellular units and eliminating the exchange rate of metabolites (Daniel, Priest, & Wheatley, 1981; Hussain, 1953). Accompanied pathological changes include cellular infiltration, degeneration, extravasation, and interstitial capillary hemorrhage (M Kosiak, 1959). As a result, epithelium thickness, myositis and fibrous replacement occur and finally tissue necrosis (Michael Kosiak, 1961).

Regardless of capillary blood flow occlusion, mechanical loading on tissue could also cause interstitial fluid flow and lymphatic drainage impairments (Reddy, Cochran, & Krouskop,

1981). The skin consists of connective tissue fibers, and the spaces between these fibers are filled with interstitial fluids and ground substances (Reddy, Cochran, & Krouskop, 1981). The lymphatic system is responsible for transporting excess interstitial fluid from body tissues. Prolonged pressure could directly cause disruption on the lymphatic circulation(Reddy, Cochran, & Krouskop, 1981), and tissue ischemia could affect the lymphatic smooth muscles. Researchers believed that such slow viscous flow of the interstitial fluids was one of the mechanism responsible for pressure ulcer development (Reddy, Cochran, & Krouskop, 1981).

Other than blockage of capillary and lymphatic circulatory system, researchers discovered that cell deformation could be one of the mechanisms causing pressure ulcers. Studies *in-vitro* found that cell damage could occur directly under compressive forces in single and groups of muscle cells (Bouten, Knight, Lee, & Bader, 2001; Breuls, Bouten, Oomens, Bader, & Baaijens, 2003). Cell membrane buckling, and enlarged cell diameter was revealed under 20% compressive strain (Bouten, Knight, Lee, & Bader, 2001), and a large proportion of cells died within four hours after compression (Breuls, Bouten, Oomens, Bader, & Baaijens, 2003). The relationship between the compressive strain level and percentage of cell death agreed with the inverse relationship of load magnitude and duration before pressure ulcers occur (Breuls, Bouten, Oomens, Bader, & Baaijens, 2003). The results suggested that pressure ulcers could develop directly from cell deformation.

Another mechanism causing pressure ulcers is ischemia-reperfusion injury (Herrman, Knapp, Donofrio, & Salcido, 1999; Peirce, Skalak, & Rodeheaver, 2000). It is a cellular injury caused by blood perfusion to a previous ischemic tissue (Pretto Jr., 1991). Instead of the occurrence of reactive hyperemia after occlusion, “no-reflow phenomenon” was discovered in these injured tissues.(Levick, 2003) The reperfusion after ischemia “produces levels of oxygen-

derived free radicals that exceed the capacity of constitutive free radical scavenging mechanisms, thus causing a cytotoxic effect in the tissue (Pretto Jr., 1991)". Ischemia-reperfusion injury affects different organs, including skin, brain, heart, and liver. Pressure ulcer research using an animal model revealed that tissue under ischemia-reperfusion cycles developed larger tissue necrosis area (Peirce, Skalak, & Rodeheaver, 2000), and decreased functional capillary density (Tsuji, ICHIOKA, SEKIYA, & NAKATSUKA, 2005) compared to those under ischemia alone.

2.1.2.2 Shear force

In spite of sustained pressure, friction and shear force contribute to pressure ulcer development as well. Friction, or frictional force, is "the resistance to motion in a parallel direction relative to the common boundary of two surfaces" (National Pressure Ulcer Advisory Panel, 2007b). It is generated through the contact movement across the skin and the support surface (Reuler & Cooney, 1981), such as dragging the patient across the bed sheets. Friction at the surface of skin could cause epidermis lesion and increase the risk of pressure ulcer formation (Dinsdale, 1974). It is also believed that the friction at the skin surface leads to shear forces in the underlying tissue layers (Dinsdale, 1974). Shear force, is "the force per unit area exerted parallel to the plane of interest" (National Pressure Ulcer Advisory Panel, 2007b), and it could cause shear strain, which is "distortion or deformation of tissue" (National Pressure Ulcer Advisory Panel, 2007b). Shear force could occur naturally in clinics through recumbent position (i.e. semi-recumbent patient with head raised) (Reuler & Cooney, 1981). In this position, the force transmits easily to the sacrum and underlying deep fascia . Previous studies indicated that the hazard of shear on pressure ulcer formation was the easy collapse of cutaneous blood vessels (Reichel, 1958). Animal studies using combinations of pressure and shear forces found that with a sufficient amount of shear force, the magnitude of pressure required to occlude blood vessels

was about half with the presence of the shear force (Bennett, Kavner, Lee, & Trainor, 1979; Dinsdale, 1974). Besides, with the application of shear, the cut-off pressure on skin oxygen tension is significantly lower compared to that with pressure alone (Goossens, Zegers, Hoek van Dijke, & Snijders, 1994). Other than the tissue ischemic injury, shear force was found to be hazardous to skin and body wall tissues, and the onset of damage increased with the magnitude of shear force (Goldstein & Sanders, 1998). Shear force also decreases the fibrinolytic activity and tends to cause tissue necrosis (Bader, Barnhill, & Ryan, 1986). Although shear force is a factor of pressure ulcer formation, the studies described before also claimed that shear force induces pressure ulcer with the combination of pressure rather than shear force alone (Bennett, Kavner, Lee, & Trainor, 1979).

2.1.2.3 Ulcer Development

Since pressure ulcer formation is attributed to various mechanism and etiologic factors, there are two different pathways that explain the ulcer development (Bouten, Oomens, Baaijens, & Bader, 2003). Pressure ulcers could start to form in either deep tissue or superficial layers based on different surface loading (Bouten, Oomens, Baaijens, & Bader, 2003). Superficial ulcers start with “maceration and detachment of the superficial skin layers”, while ulcers develop from deep tissues (e.g. skeletal muscles) start with tissue ischemia (Daniel, Priest, & Wheatley, 1981). Furthermore, tissue necrosis that develops from deep layers progresses faster than that from superficial layers, since it is not easy to detect (Bouten, Oomens, Baaijens, & Bader, 2003).

2.1.3 Pressure Ulcer Risk Factors

Pressure ulcers often occur in elderly people (Allman, 1989; Amlung, Miller, & Bosley, 2001; Horn et al., 2004), people with spinal cord injury (Byrne & Salzberg, 1996; Y. Chen, DeVivo, & Jackson, 2005), other neurological deficit, or degenerative processes (Bergstrom, 2005). The common characteristics of these populations at risk are limited movements (Allman, 1989), impaired sensation and/or altered skin condition (Bennett, Kavner, Lee, & Trainor, 1979). Numerous factors contribute to the risk of pressure ulcer formation, including but not limited to previous medical condition (Allman, 1989; Byrne & Salzberg, 1996), physiological, nutrition (Horn et al., 2002), functional status (Schnelle et al., 1997), and cognition (Horn et al., 2002). A conceptual scheme produced by Braden and Bergstrom (2000) suggested that two major factors are associated with pressure ulcers risk. One is the amount and duration of pressure exposure and the other is the tissue tolerance toward pressure.

2.1.3.1 Amount and duration of pressure exposure

Based on the conceptual scheme, decreased mobility, activity, and sensory perception are related to the amount and duration of pressure exposure (B. J. Braden & Bergstrom, 2000). Mobility level had long been used as one of the catagories in the risk assessment scale (B. J. Braden & Bergstrom, 1989; Norton, Mclaren, & Exton-smith, 1975) and is highly related to pressure ulcer formation. Exton-Smith and Sherwin (1961) found that decreased spontaneous movement is interrelated to development of pressure ulcer. Another study observing movements on people with Parkinson's disease revealed that the people with pressure ulcers had a significantly reduced movement size and frequency (Nicholson, Leeman, Dobbs, Denham, &

Dobbs, 1988). In addition, study on bed or chair-bounded population found that people who are unable to reposition had higher incidence of pressure ulcer formation (Allman, 1997).

Activity level as another pressure ulcer risk predictor refers to the ability to get out of bed and weight shifting during walking or sitting (Bergstrom, 2005). People that are bed or chair bounded and require assistance with their daily activity are more prone to developing pressure ulcers (Berlowitz & Wilking, 1989; Brandeis, Ooi, Hossain, Morris, & Lipsitz, 1994). Sensory perception has also been used in the risk assessment scale for years, and was related to pressure ulcer development as well (B. Braden & Bergstrom, 1988). People with impaired sensation such as people with spinal cord injury or stroke may have deficits in detecting the uncomfortable area that are loaded with pressure (Bergstrom, 2005).

2.1.3.2 Tissue tolerance toward pressure

Tissue tolerance toward pressure was defined as "the ability to withstand the effects of pressure without developing a pressure ulcer" (Bergstrom, 2005). Both intrinsic and extrinsic factors contribute to tissue tolerance.

(a) Intrinsic factors

The intrinsic factor is the skin condition within the individual (Bergstrom, 2005), it includes nutritional status, and aging of the skin. Nutrition has been categorized in the risk assessment scale (B. Braden & Bergstrom, 1988) and malnutrition could increase risk of ulcer development (Byrne & Salzberg, 1996; Horn et al., 2002). Some revealed that dietary intake is associated with ulcer formation (Berlowitz & Wilking, 1989), while some supported hypoalbuminemia is the predictor of pressure ulcers (Byrne & Salzberg, 1996). Other indicators of nutritional factor in pressure ulcer formation include decreased body weight, lowered body

mass index, and reduced lymphocyte count (Allman, 1997; Horn et al., 2002). Of all indicators, researchers could not distinguish any one from the others as an independent factor related to increased pressure ulcer risk.

Aging of the skin is another intrinsic factor of pressure ulcer development. The aged skin is usually dry, wrinkled (Witkowski & Parish, 2000), lack of elasticity and subcutaneous fat (Garcia & Thomas, 2006) and thinner in epidermis, dermis and dermoepidermal junction (Witkowski & Parish, 2000). These structural changes increase the risk of aged skin in tissue breakdown caused by pressure, shear, and frictional forces.

(b) Extrinsic factors

The extrinsic factors that cause pressure ulcers accompanied with pressure are increased shear (refer to section 2.1.2.2), moisture and skin temperature. Moisture is categorized in several pressure ulcer risk assessment scales, including both Braden and Norton scales; and it is characterized by the existence of sweating or incontinences (B. J. Braden & Bergstrom, 1989; Norton, Mclaren, & Exton-smith, 1975). The presence of urinary or fecal incontinence is likely to increase the vulnerability of the skin (Allman, 1997; Ersser, Getliffe, Voegeli, & Regan, 2005; Horn et al., 2002; Horn et al., 2004). However the causal links among skin moisture, incontinece and pressure ulcer development remained unclear (Ersser, Getliffe, Voegeli, & Regan, 2005). Studies believed that constant wetness of the skin resulted in maceration (Ersser, Getliffe, Voegeli, & Regan, 2005), which reduced the stiffness of the skin and increased the vulnerability of tissue breakdown caused by fictional forces (Fader et al., 2003; Nach, Close, Yeung, & Ganse, 1981). Other then hydration, increase in pH value and bacterial infection caused by incontinence may also contribute to ulceration (Ersser, Getliffe, Voegeli, & Regan, 2005). On the contrary,

excessive dry skin may increase the risk of pressure ulcer development as well (Tsai & Maibach, 1999), since dry stratum corneum decreased the skin barrier with crack and fissures (Ersser, Getliffe, Voegeli, & Regan, 2005). However the underlying mechanism remained unclear.

Increased skin temperature is one of the least explored extrinsic risk factors causing pressure ulcers. Increased skin temperature is found to be related to higher risk of pressure ulcer development (Iaizzo et al., 1995; Kokate et al., 1995; Lachenbruch, 2005). While lying or sitting on a support surface, body heat is naturally trapped via convection between the support surface and the skin (Lachenbruch, 2005; Sae-Sia, Wipke-Tevis, & Williams, 2005), which results in elevated skin temperature. The skin tends to transfer heat between body and environment and between deep and periphery tissues (Rowell, 1974). When the temperature at skin surface is higher than that at deep tissue, heat is transferred from the peripheral to deep tissue rather than released to the surface (Rowell, 1974). The increased skin temperature elevates metabolic and oxygen demand of the tissue by 6% to 13% per Celsius (Ruch & Patton, 1965), which reduces the ability of skin to withstand breakdown (Kokate et al., 1995). In addition, elevated skin temperature may increase skin moisture through sweating, which might make the tissue more vulnerable to breakdown under frictional forces as described before (Lachenbruch, 2005). Previous animal studies have demonstrated that increased pressure ulcer risk was correlated to elevated skin temperature (Iaizzo et al., 1995; Kokate et al., 1995). Kokate et al. (1995) applied 100mmHg of pressure on the skin of the swine back for five hours with four different degrees of temperature (i.e. 25, 35, 40 and 45°C). They performed histological examination and visual assessment of skin integrity at the 7th day after pressure application. No soft tissue damage arose at the site where skin was cooled to 25°C (Kokate et al., 1995). The skin blood flow monitored throughout the 28 days after pressure application was compared to the control sites as

well, and there was no change at site with 25 and 35ºC, whereas a significant decrease of blood flow occurred at site with 45ºC. The results indicated that there is no underlying muscle and circulation damage at the sites with temperature application of 25 and 35ºC.

Further study adopted the same protocol and tested the skin under four different pressure application times (i.e. 2, 5 and 10 hours) (Iaizzo et al., 1995). The histological data obtained at the 7th day after application showed normal skin layers at the sites with 25ºC regardless of the duration of application time, whereas moderate muscle damage was found at sites with 35ºC and above. The tissue damage found at sites with temperature 35ºC and above worsened progressively with increased duration of pressure application, while no such phenomenon occurred at sites with 25ºC. The research team also measured the skin temperature and blood flow on the first and 7^{th} day after the pressure application (Iaizzo et al., 1995). The skin temperature and blood flow measured after application indicated the severity of tissue injury. The most severely injured sites had lower temperatures and skin perfusion. The skin blood flow at the 45ºC sites was even not detectable on the 7^{th} day after application. Based on the previous study results that skin temperature at 25ºC provided a protective mechanism of the tissue from damaging caused by sustained pressure, the research team further performed the same testing protocol on the skin under four different temperatures between 25 and 35ºC (i.e. 25, 27, 30 and 32ºC) as the second part of the study (Iaizzo et al., 1995). No significant difference of skin damage was found between the four different temperatures, but a stepwise trend was presented. In addition, the histological data gained from various tissue layers indicated that deep tissue had a greater sensitivity toward injury caused from increased temperature. In conclusion, local cooling at temperature under 30ºC could reduce the severity of soft tissue damage under

sustained mechanical pressure, and these study results all indicated that local skin cooling might be beneficial to preserve tissue viability in deep tissues.

2.2 IMPAIRED THERMOREGULATORY FUNCTION IN THE ELDERLY

Elderly people are one of the high-risk populations of pressure ulcers, recent surveys showed that people over 70 years old is the predominant group with pressure ulcers (>50%) (Amlung, Miller, & Bosley, 2001). Many factors contribute to the high risk of aged skin in developing pressure ulcers, including limited mobility, activity, cognition level (Garcia & Thomas, 2006), and altered skin condition through normal aging. Other then structural changes discussed before, differences related to reduced thermoregulation are the main interests of this study. Such aging related structural changes include loss of capillary loops in dermal papillae, decreased vascularity in reticular dermis and thinned vessel walls (Witkowski & Parish, 2000); these might cause the attenuated cutaneous blood perfusion (Garcia & Thomas, 2006) and impaired thermoregulatory function of the aged skins (Grassi et al., 2003; Scremin & Kenney, 2004; Thompson, Holowatz, & Kenney, 2005). In general, cutaneous vasodilation is induced under heat stress while vasoconstriction occurs under cold stress. The cutaneous circulation modulates the thermoregulatory function through both neural and local mechanisms (D. L. Kellogg, Jr., 2006). The neuronal response toward temperature changes is mediated via sympathetic nervous system (Holowatz et al., 2003; Johnson, Yen, Zhao, & Kosiba, 2005; D. L. Kellogg et al., 1995; Scremin & Kenney, 2004). This reflex control contains two branches: the active vasodilation and noradrenergic vasoconstriction (Holowatz et al., 2003; Johnson, Yen, Zhao, & Kosiba, 2005; D. L. Kellogg et al., 1995; Scremin & Kenney, 2004).

2.2.1 Vasodilation Under Heat Stress

The active vasodilator system is largely (80-95%) responsible for the increase in skin blood flow under heat stress (D. L. Kellogg, Jr., 2006). Previous studies found that an active vasodilation system is mediated through a cholinergic cotransmitter system (Hokfelt, Johansson, Ljungdahl, Lundberg, & Schultzberg, 1980), which involves release of acetylcholine and possibly corelease of vasoactive intestinal peptide (D. L. Kellogg et al., 1995). In addition, nitric oxide (NO) also plays a significant role in active vasodilation. Studies found that acetylcholine-mediated NO production affects the early response in heat stress (Shibasaki, Wilson, Cui, & Crandall, 2002); besides, a functional nitric oxide synthase is required for a full expression of active vasodilation (D. L. Kellogg, Crandall, Chrarkoudian, & Johnson, 1998; Shastry, Dietz, Halliwill, Reed, & Joyner, 1998). The local vascular response toward temperature changes has its own mechanism, and the majority of the response is independent of the adrenergic system (Pergola, Kellogg, Johnson, Kosiba, & Solomon, 1993). Local heating of the skin includes two independent mechanisms: neural response and local NO generation (Christopher T. Minson, Berry, & Joyner, 2001). Studies found that the local vascular response toward heating is biphasic, starting with a brisk vasodilation and then a prolonged plateau phase (D. L. Kellogg, Jr., 2006). The axon reflex is responsible for the initial rise of the skin blood flow (Christopher T. Minson, Berry, & Joyner, 2001), while local endothelial NO production contributes to the secondary plateau vasodilation (D. L. Kellogg, Liu, Kosiba, & O'Donnell, 1999; Christopher T. Minson, Berry, & Joyner, 2001).

In aged skin, studies revealed attenuated vasodilation under heat stress. This is true with both whole body and local heating (Grassi et al., 2003; Holowatz et al., 2003; Kenney et al., 1997; C. T. Minson, Holowatz, Wong, Kenney, & Wilkins, 2002; Pierzga, Frymoyer, & Kenney,

2003). With normal aging, the attenuated skin blood flow response is attributed to decreased active vasodilation (Kenney et al., 1997). This may be due to diminished release or response toward the cholinergic cotransmitters; and the acetylcholine-mediated NO might be critical to the response in the elderly as well (Holowatz et al., 2003). In addition, such decreased skin blood flow response might also be attributed to diminished area corresponding to the vasodilated microvessels and reduced cutaneous vascular conductance of these vasodilated areas (Pierzga, Frymoyer, & Kenney, 2003). For local vasodilation response, researchers revealed that aged skin had significantly diminished in both phases (C. T. Minson, Holowatz, Wong, Kenney, & Wilkins, 2002). This indicated that normal aging has effect on both axon reflex and No-dependent vasodilation mechanisms. The exact mechanism within the attenuated response remained unclear; however researchers suggested the following possibilities: Normal aging 1) has influences on the nerves that control the axon reflex or release of neurotransmitters, and 2) decreased local NO generation (C. T. Minson, Holowatz, Wong, Kenney, & Wilkins, 2002). The diminished vasodilation in the elderly population may be vital and cause the tissue to be more prone to damage (C. T. Minson, Holowatz, Wong, Kenney, & Wilkins, 2002).

2.2.2 Vasoconstriction Under Cold Stress

Researchers first discovered that active vasoconstriction works mainly through release of norepinephrine (NE) on postjunctional α_1 and α_2 receptors (Flavahan, 1991; Johnson, Yen, Zhao, & Kosiba, 2005; D. L. Kellogg, Jr., 2006). Studies later on revealed that there is a nonnoradrenergic mechanism involved in the active vasoconstriction; although the exact neurotransmitters are unknown, this mechanism works through β-adrenergic receptors, which is likely to adjust the vasomotion by providing a minor vasodilation (Stephens, Aoki, Kosiba, &

Johnson, 2001). Thompson and Kenney's study (2004) on young healthy subjects proved that only 40% of vasoconstriction is attributed to activation of α and β receptors under hypothermia; while other unknown cotransmitters are responsible for the rest of vasoconstriction. Stephens et al. (2001; , 2004) revealed neuropeptide Y (NPY) as a cotransmitter with a major effect on vasoconstriction through the active NPY Y_1 receptor. Unlike local vasodilation mechanism, local vasoconstriction depends on intact noradrenergic vasoconstriction (Johnson, Yen, Zhao, & Kosiba, 2005; Pergola, Kellogg, Johnson, Kosiba, & Solomon, 1993). The skin response toward local cooling contains two phases: initial and following prolonged phase (Johnson, Yen, Zhao, & Kosiba, 2005). The initial phase contains a noradrenergic vasoconstriction and nonneurogenic vasodilator (Johnson, 2007). NE and/or other noradrenergic cotransmitters play a significant role in the initial vasoconstriction (Pergola, Johnson, Kellogg, & Kosiba, 1996), and it requires both intact sensory and sympathetic functions (Johnson, Yen, Zhao, & Kosiba, 2005). The underlying vasodilator response is nonadrenergic and depends on the cooling rate (Yamazaki et al., 2006). However, the mechanism and origin of such phenomenon remains unclear (Johnson, 2007). The following prolonged vasoconstriction response toward local cooling is also mediated through nonneurogenic mechanism, and researchers found that Rho-Rho kinase system are attributed to 60% of this (Thompson-Torgerson, Holowatz, Flavahan, & Kenney, 2007).

Attenuated vasoconstriction response has been discovered in the elderly people as well. Researchers found that decreased non-noradrenaline mediated vasoconstriction in aged skin during whole body cooling (Thompson & Kenney, 2004). For local cooling, even though the vasoconstriction response toward local cooling alone is preserved in aged skin, studies revealed diminished noradrenaline mediated post-junctional response in aged skin (Thompson, Holowatz, & Kenney, 2005).

2.3 CURRENT STRATEGY USED IN SUPPORT SURFACE TECHNOLOGY

A support surface is "a specialized device for pressure redistribution designed for management of tissue loads, micro-climate, and/or other therapeutic functions (i.e. any mattresses, integrated bed system, mattress replacement, overlay, or seat cushion, or seat cushion overlay)" (National Pressure Ulcer Advisory Panel, 2007b). All currently available support surface devices reimbursed by Medicare program are designed for the purpose of pressure redistribution (U.S. Agency for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001). These products are categorized into different groups based on the materials they are made of and the features they provided (Brienza & Geyer, 2005). Researchers have tried to compare the effectiveness of these products in pressure ulcer prevention; however the results are mixed and suggest that "it is impossible to determine the most effective surface for either prevention or treatment" (Cullum, McInnes, Bell-Syer, & Legood, 2004). There is no literature on any support surface devices that focuses mainly on skin cooling, however the microclimate control feature of each currently available products will be discussed in following paragraphs.

Pressure is the force per unit area applied on the body by a support surface (Brienza & Geyer, 2005; National Pressure Ulcer Advisory Panel, 2007b). Theoretically, the more contact area of the body with the support surface, the smaller the average pressure that is distributed to each skin unit. The principle of the pressure redistribution support surface relies on the ability of immersion and envelopment (Brienza & Geyer, 2005). Immersion is the "depth of penetration (sinking) into a support surface" (National Pressure Ulcer Advisory Panel, 2007b), and envelopment is "the ability to conform, so to fit or mold around irregularities in the body" (National Pressure Ulcer Advisory Panel, 2007b). Several devices serve the function of pressure

redistribution, and they could be categorized as follows based on their components and features, including foam, fluid-filled, air-fluidized, low-air-loss and alternating pressure.

2.3.1 Foam

Foam products are low-tech surfaces (Cullum, McInnes, Bell-Syer, & Legood, 2004) commonly seen in hospitals since they are inexpensive and require low maintenance (Priebe, Martin, Wuermser, Castillo, & McFarlin, 2003). Foam products usually consist of foam layers with various densities or combined with other materials such as gel or fluid-filled bladders (Brienza & Geyer, 2005). Some foam cushions are contoured to provide better pressure distribution to the skin, since precontouring increases immersion, envelopment, and contact area (Brienza & Geyer, 2005). The foam products "conform in proportion to the applied weight" (National Pressure Ulcer Advisory Panel, 2007b), and its features differ slightly based on the types of foam it is made of. The elastic foam tends to return to its original shape while viscoelastic foam softens at higher temperatures (Brienza & Geyer, 2005). The common disadvantage of foam products are the tendency to increase skin temperature (Stewart, Palmieri, & Cochran, 1980) and bottom out (in about three years) (Brienza & Geyer, 2005).

2.3.2 Fluid-filled

This specific term is not frequently found in the support surface literatures. However, it was defined as products that "consist of small or large chambers filled with air, water, or other viscous fluid materials, such as silicon elastomer, silicon, or polyvinyl" (Brienza & Geyer, 2005). These products are considered to be support surfaces that provide a "high degree of

immersion"; and both ROHO (air-flotation) and Jay2 (viscous fluid-filled) are filed in this category (Brienza & Geyer, 2005). Maintenance and monitoring are required for the effectiveness of these products, such as inflation level of ROHO and position of viscous material in Jay2 (Brienza & Geyer, 2005). Changes in skin temperature depend highly on the material construct of the support surface products; materials with high specific heat such as water could decrease the skin temperature more efficiently than others (Brienza & Geyer, 2005).

2.3.3 Air-fluidized

Air-fluidized is a feature of support surfaces defined as providing "pressure redistribution via fluid-like medium created by forcing air through beads as characterized by immersion and envelopment" (National Pressure Ulcer Advisory Panel, 2007b). The air-fluidized products contain a tank filled with solid particles, such as glass (Brienza & Geyer, 2005) or silicone-coated microsphere beads (U.S. Agency for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001). Air drawn from the room into the system is pressurized through the beads, which causes the solid particles to behave like fluid (Jan & Brienza, 2006; U.S. Agency for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001). Air-fluidized products are believed to provide the highest degree of immersion compared with other products (Brienza & Geyer, 2005). Active air temperature control is available in these devices (Jan & Brienza, 2006), and the air is usually warmed up to 28-35°C (Brienza & Geyer, 2005). Pros and cons of this feature depend on the requirements of the person using the product; it might be a disadvantage based on previous literature review on pressure ulcer formation since it could dehydrate the skin (Brienza & Geyer, 2005; U.S. Agency

for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001) and increase the metabolic demands through elevated skin temperature (Brienza & Geyer, 2005).

2.3.4 Low-air-loss

Low-air-loss is a feature of support surface defined as "providing a flow of air to assist in managing the heat and humidity (microclimate) of the skin" (National Pressure Ulcer Advisory Panel, 2007b). Theses products consist of air pillows grouped in zones to distribute weight evenly over each zone (U.S. Agency for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001). Air and vapor are permeable through the pore of the surface to control the humidity of the skin in contact with the device (Brienza & Geyer, 2005; National Pressure Ulcer Advisory Panel, 2007b; U.S. Agency for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001). Active temperature control (heating or cooling) is available in these products as well (Jan & Brienza, 2006).

2.3.5 Alternating Pressure

Alternating pressure by definition is "a feature of support surface that provides pressure redistribution via cyclic changes in loading and unloading as characterized by frequency, duration, amplitude, and rate of change parameters" (National Pressure Ulcer Advisory Panel, 2007b). Support surfaces with this feature distribute the pressure at different sites of the skin alternately (Brienza & Geyer, 2005). Kosiak (1961) first proved that alternating pressure provides a protective effect on ischemic ulcer development, and studies thereafter revealed that alternating pressure could increase skin blood flow on people with spinal cord injury (Jan &

Brienza, 2006). Microclimate control of these products depend on the materials of the cover; and further investigation of alteration cycle and patterns are required for optimal use of these products (Brienza & Geyer, 2005).

2.3.6 Microclimate Control

Most currently available products reimbursed by Medicaid and Medicare feature pressure redistribution; however, the ability of these products to control the microclimate has not been included as a criterion. Several studies investigated the microclimate control feature of these products. On the skin temperature aspect, studies showed that skin temperature changes on non-powered products depend on the heat sink quality of the device material (Stewart, Palmieri, & Cochran, 1980). Skin temperature increased with the use of rubber (Fisher, Szymke, Aptem, & Kosiak, 1978) and foam (Fisher, Szymke, Aptem, & Kosiak, 1978; Seymour & Lacefield, 1985; Stewart, Palmieri, & Cochran, 1980) types of cushion for merely 30 minutes; while skin temperature was lowered with the use of gel and water floatation types of cushion for up to two hours (Cochran, 1985). Although skin temperature was lowered with the use of gel and water floatation products, skin temperature showed a trend of increase in these devices (Cochran, 1985; Fisher, Szymke, Aptem, & Kosiak, 1978). In powered devices, both air-fluidized and low-air-loss products provide active temperature control. As mentioned before, air is usually warmed up to 28-35°C (Brienza & Geyer, 2005) in air-fluidized products and low-air-loss devices provide options of heating and cooling of the circulated air (Jan & Brienza, 2006).

On the humidity aspects, humidity control of the currently available products depend highly on the cover materials (Brienza & Geyer, 2005). In non-powered devices, relative

humidity in gel, water flotation and Roho types of cushions increase more than the foam products (Cochran, 1985; Stewart, Palmieri, & Cochran, 1980); this might be caused by the water-impermeable covers. In powered devices, as mentioned before, air-fluidized surfaces might cause dehydration of the skin due to the fluid loss caused by high temperature (U.S. Agency for Healthcare Research and Quality Center for Practice and Technology Assessment, 2001).

2.4 EMERGING STRATEGY TO PREVENT PRESSURE ULCERS: SKIN COOLING

Since previous studies (Brienza & Geyer, 2005; Iaizzo et al., 1995; Knox, 1999; Kokate et al., 1995; Sae-Sia, Wipke-Tevis, & Williams, 2005) indicated that skin local cooling might be beneficial to pressure ulcer prevention, effectiveness and mechanism of local cooling on tissue viability preservation is discussed as follows. Several mechanisms explain its protective effect on tissue preservation, including decrease in (1) metabolism, oxygen consumption, (2) ischemic-reperfusion injury and (3) tissue inflammation response.

Decreased metabolism and oxygen consumption is the most often discussed effect of hypothermia on tissue preservation, including heart (Q. Chen et al., 2002; Chitwood, Sink, Hill, Wechsler, & Sabiston, 1979; Riess, Camara, Kevin, An, & Stowe, 2004), brain (Rosomoff & Holaday, 1954), and skeletal muscles (Segal & Faulkner, 1985; Seiyama, Kosaka, Maeda, & Shiga, 1996). The alteration in metabolism under hypothermia remained unclear, and researchers found that it could be due to the physiological alteration of the tissue at the cellular level: membrane stabilization (Seiyama, Kosaka, Maeda, & Shiga, 1996) or mitochondrial

energy balance (Riess, Camara, Kevin, An, & Stowe, 2004). Membrane stabilization is a main factor explaining mammalian cell survival under hypothermia (Seiyama, Kosaka, Maeda, & Shiga, 1996). It is indicated by the "ion gradients across the plasma membrane" (Seiyama, Kosaka, Maeda, & Shiga, 1996). Under hypothermia, ion overload is prevented therefore membrane stabilization is maintained and so is the mitochondrial energy balance (Riess, Camara, Kevin, An, & Stowe, 2004).

Ischemia-reperfusion injury is another etiological factor of pressure ulcer development that could be prevented through hypothermia as well. Despite the mechanism of cellular changes described before, research on rat livers also proved that hypothermia decreased hepatocellular damage (Choi et al., 2005). Histological investigation showed that liver necrosis after 24-hours of reperfusion was >75% under normothermia, whereas necrosis occurred <50% under hypothermia. The same protective effect was also confirmed in skeletal muscle study, where 10-20% more of tissue viability and decreased muscle edema were found in tissue under hypothermia (Mowlavi et al., 2003). The clinical application of using hypothermia to protect injury caused by ischemia or ischemic-reperfusion was widely used, including free flap plastic surgery, organ transplant, and cardiac surgery (Riess, Camara, Kevin, An, & Stowe, 2004).

Other than the protective mechanism toward tissue ischemia, studies found that local cooling preserves tissue viability through reducing inflammation, which is the early stage of tissue damage (Armstrong, Sangalang, Jolley, Maben, & Kimbriel, 2005). The study on local cooling on the foot after exercise suggested that local cooling could be a prophylactic tool in preventing tissue breakdown on the diabetic foot (Armstrong, Sangalang, Jolley, Maben, & Kimbriel, 2005). In addition to this study, cryotherpy on acute injury has been widely adopted. Ice is used as one of the methods that could reduce swelling, inflammation, bleeding (Jurkovich,

Pitt, Curreri, & Granger, 1988) and tissue damage resulting from hypoxia (Tisherman, Rodriguez, & Safar, 1999).

2.5 INDIRECT METHOD TO MEASURE SKIN TISSUE RESPONSE TO COOLING

The purpose of this study was to investigate skin tissue response to local cooling under prolonged pressure on young healthy human subjects. Instead of histological assessment used in animal studies, non-invasive techniques were considered. Several non-invasive techniques used to measure microvascular functions in pressure ulcer studies and risk assessments, including transcutaneous oximetry ($TcPO_2$), near-infrared spectroscopy, thermometer and laser Doppler flowmetry (LDF) were taken into consideration (Knight, Taylor, Polliack, & Bader, 2001).

2.5.1 Transcutaneous oximetry

$TcPO_2$ is a reliable method for tissue viability assessment by the detection of arterial oxygen tension (PO_2) (Jan & Brienza, 2006; Newson & Rolfe, 1982). Blood gas contains information on gas exchanges between vessels and tissues, and estimation of arterial oxygen tension could provide information on tissue oxygenation (Togawa, Tamura, & Öberg, 1997a) and microcirculation (Jan & Brienza, 2006). Measurement of $TcPO_2$ takes places at the skin surface. In general, skin is not permeable to gas, and the permeability could be increased with elevated skin temperature (Togawa, Tamura, & Öberg, 1997a). In previous studies, skin temperature was required to be heated up to 40-45°C to gain the accurate arterial oxygen tension (Bongard &

Bounameaux, 1993; Jan & Brienza, 2006). Since cooling is a major intervention of this study, this measurement tool was excluded.

2.5.2 Near-infrared spectroscopy

Near-infrared spectroscopy, also noted as reflectance spectroscopy, is a useful non-invasive tool to detect tissue viability, and it was investigated in several studies on its ability to assess tissue viability during surgery (Stranc, Sowa, Abdulrauf, & Mantsch, 1998; Thorniley, Sinclair, Barnett, Shurey, & Green, 1998). The near-infrared spectroscopy is performed by emitting a white light source toward the skin (Hagisawa, Ferguson-Pell, Cardi, & Miller, 1994). The light scattered back contains information, including deoxyhemoglobin, oxyhemoglobin, and water (Stranc, Sowa, Abdulrauf, & Mantsch, 1998). The near-infrared spectroscopy has lower validity and reproducibility as compared with both laser Doppler flowmetry and tissue oxygenation oximetry. In addition, a previous study had concerns about the reliability of skin viability assessment in the sacrum area (Keller, Schuurman, & Werken, 2006), which was the target area of our study. This measurement tool was excluded.

2.5.3 Thermometer

Measurement of skin temperature was used to detect the reactive hyperemic response, and occurrence of increased temperature indicated increased perfusion response. Change in skin temperature is one of the predictors of pressure ulcers. At stage I, both the increase and decrease in skin temperature indicated a potential skin integrity problem. Sprigle et al. found that 62% of erythmatic sites had increased temperature, while 23% had decreased temperature (Sprigle,

Linden, Mckenna, Davis, & Riordan, 2001). Study from Sae-Sia also supported this view by assessing the relationship between the Braden scale and skin temperature. The higher the sacral skin temperature, the higher the risk of developing a pressure ulcer (Sae-Sia, Wipke-Tevis, & Williams, 2005). Since skin temperature control was our main intervention, this measurement tool was excluded as well.

2.5.4 Laser Doppler flowmetry

Laser Doppler fluxmetry can monitor skin microcirculation (Schubert & Fagrell, 1991a) and the perfusion response to pressure-induced ischemia by "utilizing the Doppler shift of laser light as the information carrier" (Togawa, Tamura, & Öberg, 1997b). In addition, it is a reliable non-invasive tool to evaluate the risk of pressure ulcers (Herrman, Knapp, Donofrio, & Salcido, 1999; Schubert & Fagrell, 1991a). The measurement is performed by projecting a laser beam to the skin, then the scattered light was collected through the optical fibers (Togawa, Tamura, & Öberg, 1997b). Schuber and Fagrell (1991a) investigated tissue viability through the measurement of the reactive hyperemic response.

Reactive hyperemia is a normal physiological response of increased perfusion after a period of tissue ischemia (Levick, 2003). It occurs in various tissues including the skin, heart, liver etc. This response was adopted to assess tissue viability in previous studies (Hagisawa, Ferguson-Pell, Cardi, & Miller, 1994; Schubert & Fagrell, 1991a; Sprigle, LInden, & Riordan, 2002). Several parameters were discussed in these studies; and these parameters could provide information on tissue metabolism and vasomotion. The parameters will be discussed in detail in section 3.6.2.

3.0 METHOD

3.1 RESEARCH DESIGN

This study was a repeated measures design. The experiment contained two test sessions, one had local cooling intervention and the other did not. Each participant went through both test sessions. The order of the two experimental sessions was randomized.

3.2 PARTICIPANTS

The research protocol of this study was approved by the Institutional Review Board of the University of Pittsburgh (IRB # 0611134) to test on young healthy subjects. Ten young adults (five female and five male) ranging in age from 20 to 40 were recruited in this study from the campus area of the University of Pittsburgh. Since this is a pilot study to investigate the effectiveness of local cooling on human subjects, and the outcome measure is the skin perfusion response, people with any disease that might influence the vasomotion were excluded. The participants were all free of any cardiovascular, pulmonary diseases or diabetes. They were not smokers and did not take any medications that affect their cardiovascular functions. Any caffeinated food or drinks were prohibited twelve hours prior to the experiment. To ensure

participants have similar body composition, the body fat percentage of the subjects was <35% in female and <25% in male.

3.3 INSTRUMENTATION

3.3.1 Skin Fold Caliper

Harpenden skinfold caliper (Creative Health, Ann arbor, MI) was used in this study to measure skinfold thickness and to estimate body composition of the participants, since body composition might influence the effect of cooling on the skin and the underlying tissues (Otte, Merrick, Ingersoll, & Cordova, 2002). The 4-site system was selected, and the measurement took place at four sites including biceps, triceps, subscapular, and suprailiac over the right side of the body. Each site was measured twice, and the value was the average of the two. If the difference between two measurements was more than 1 mm, the measurement was repeated at that site (British Indicators, 1998).

3.3.2 Thermoelectric Cooling System

A thermoelectric cooling system (TE Technology, Traverse City, MI) was used in the study to control the skin temperature. It includes four parts: customized thermistor (modification of thermistor model MP2444), thermoelectric cooling (TE) module (CH-38-1.0-0.8), temperature controller (TC-36-25 RS232), and heat radiator.

The thermistor, TE module, and heat radiator are attached with conductive glue (TSE3941, Toshiba, Japan) and thermal grease. The relative position of the three components to skin was: skin→ thermistor→ cold side of TE module→ heat side of TE module→ heat radiator (Figure 1). To allow for better and safer contact of the cooling system to the skin, the customized thermistor was made by inserting the commercial thermistor into a copper plate. Thermal grease was used at the contact area between the copper plate and the commercial thermistor.

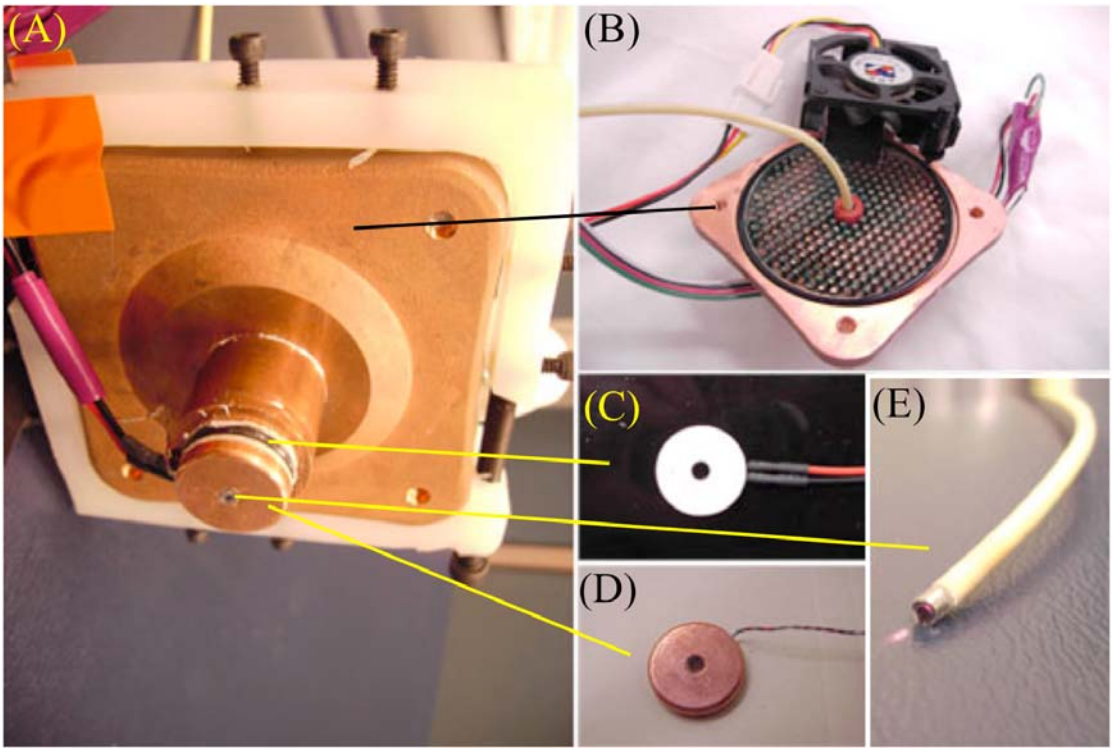

Figure 1. (A)Thermoelectric cooling system, (B) heat radiator, (C) TE cooler, (D) customized thermistor, and (E) Laser Doppler probe

The TE module is a solid-state component that transfers heat actively between its two sides: the cold and heat side (Figure 2). The Peltier effect is the active heat transportation

mechanism of the TE module; heat is transferred from cold side to heat side when it is powered. The copper radiator mounted at the heat side of the TE module served as the heat sink, and a mini fan was attached at the radiate side to take away more heat during the cooling procedure (Figure 3).

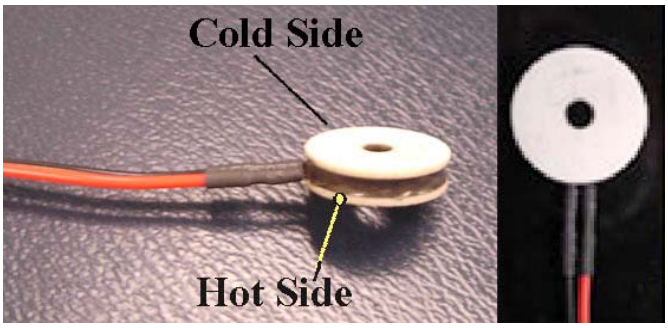

Figure 2. Thermoelectric module

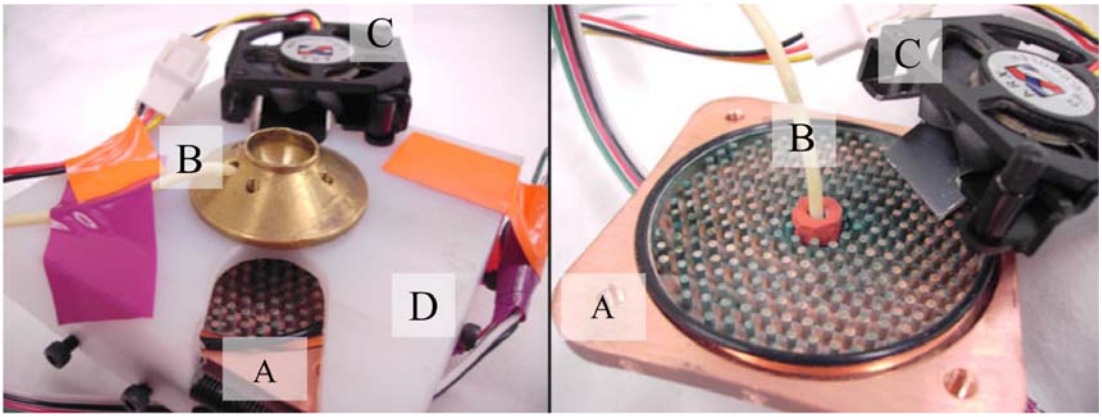

Figure 3. Copper heat radiator and mini fan with (left) and without (right) radiator holder. (A) copper radiator, (B) LDF probe, (C) mini fan, and (D) copper radiator holder

The temperature controller is a commercial electric board that executes the command from the computer to the TE module (Figure 4). The computer control interface was created with LabVIEW program (version 7.3, National Instrument, Austin, TX), and the control of both

temperature and pressure application were integrated on the same page (Figure 5). Proportional-integral-derivative (PID) controller was used to maintain skin temperature during the cooling procedure. Skin temperature read by the thermistor was compared to the target value within the controller system (i.e. error); based on the error, and the set parameters (i.e. proportional bandwidth: 1°C, derivative gain: 10 repeats/ min, interval gain: 10 cycles/ min), the magnitude of power provided to the TE module was adjusted to control the temperature at target value. The skin temperature along with applied pressure and blood perfusion were presented in real-time on the computer for easy monitoring (Figure 5).

The total cooling area of the skin was 3.8 cm^2. A hole of 2mm diameter was in the center of the thermistor, TE module and heat radiator for the LDF probe to measure skin perfusion simultaneously with pressure and temperature control.

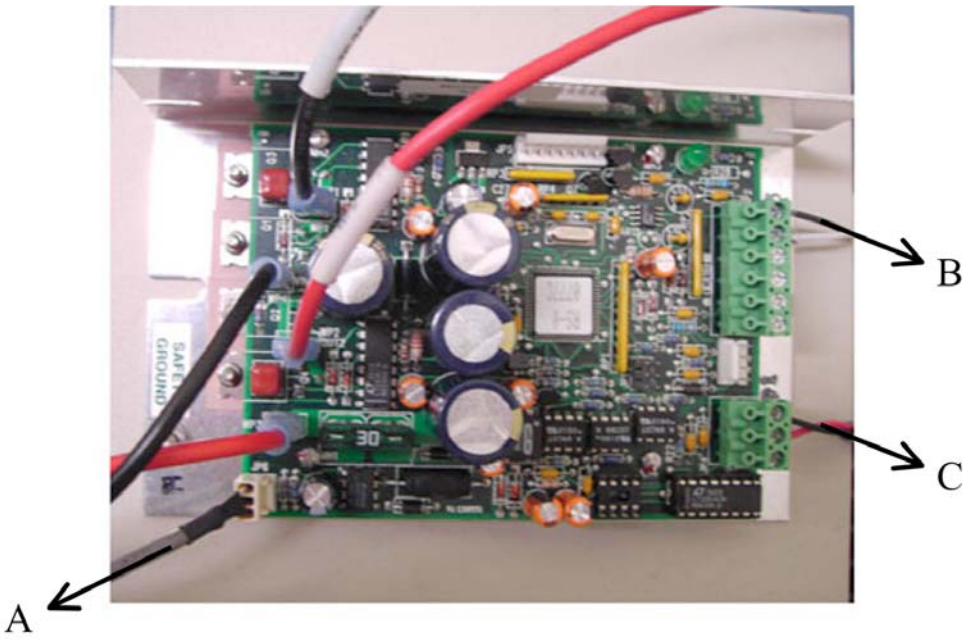

Figure 4. Temperature controller. (A) connect to TE module, (B) connect to thermistor, (C) connect to computer

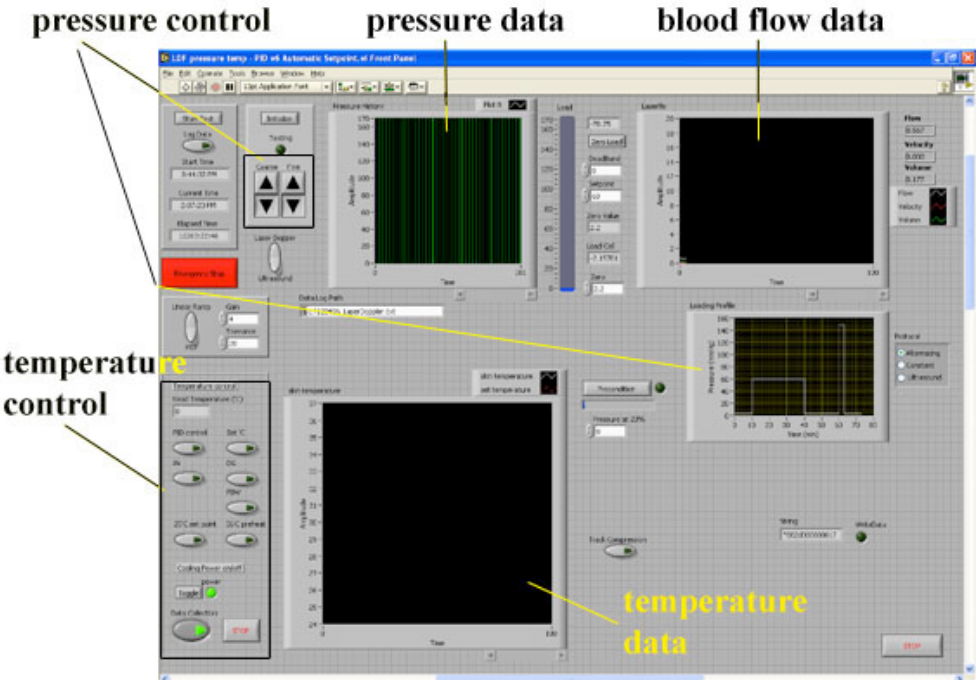

Figure 5. Layout of pressure and temperature control interface

3.3.3 Laser Doppler Flowmetry

Laserflo Blood Perfusion Monitor 2 (Vasamedics, Eden Prairie, MN) and the Softip pencil probe (P-435) were used in the study (Figure 6). It is an FDA-approved device that provides noninvasive measurements of skin blood flow at a depth of about 1 mm via laser and fiber optics technology. The softip pencil probe was placed at the center of the cooling system at the tip of indenter head to obtain skin perfusion data while the integrated system controlled pressure and temperature at the same time. The skin perfusion data was also presented in real-time for monitoring.

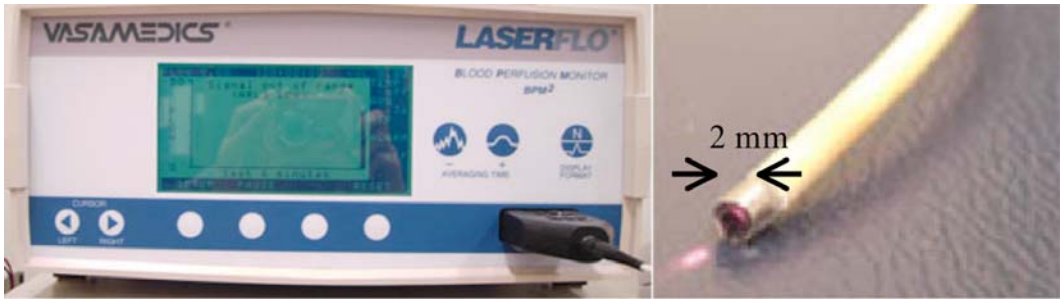

Figure 6. Laser Doppler flowmetry system. (Left) Laserflo Blood Perfusion Monitor 2, (Right) Softip pencil probe

3.3.4 Computer-Controlled Indenter

The customized computer controlled indenter in this study was used to control the magnitude of pressure applied on the skin. It contained four parts: the motor, stage, load cell and joint for TE cooling system (Figure 7). The load cell (LC703, Omegadyne Inc., Sunbury, OH) with ±10lb. capacity was located at the bottom of the stage. It read the force applied on the skin through the deformation of the strain gauge within the load cell. The stage (MTR-13-E, National Aperture Inc., Salem, NH) controlled by the motor on the top brought the indenter toward and away from the skin. When the indenter was applied on the skin, the value read from the load cell was converted into "mmHg" by the LabVIEW program based on the area of pressure application in the LabVIEW program. The PID controller was used in pressure control as well. The converted load value was compared with the target value, based on the difference between the two, the controller decided the amount and speed of the stage movement through the motor. A lever at the top of the system counter-weighted the indenter to provide 150 mmHg of applied pressure based on the deadweight of the indenter for better pressure control with the subjects' respiratory

movement. A schematic figure of the loading system (Figure 7) provided explanation of the mechanism of the computer-controlled indenter.

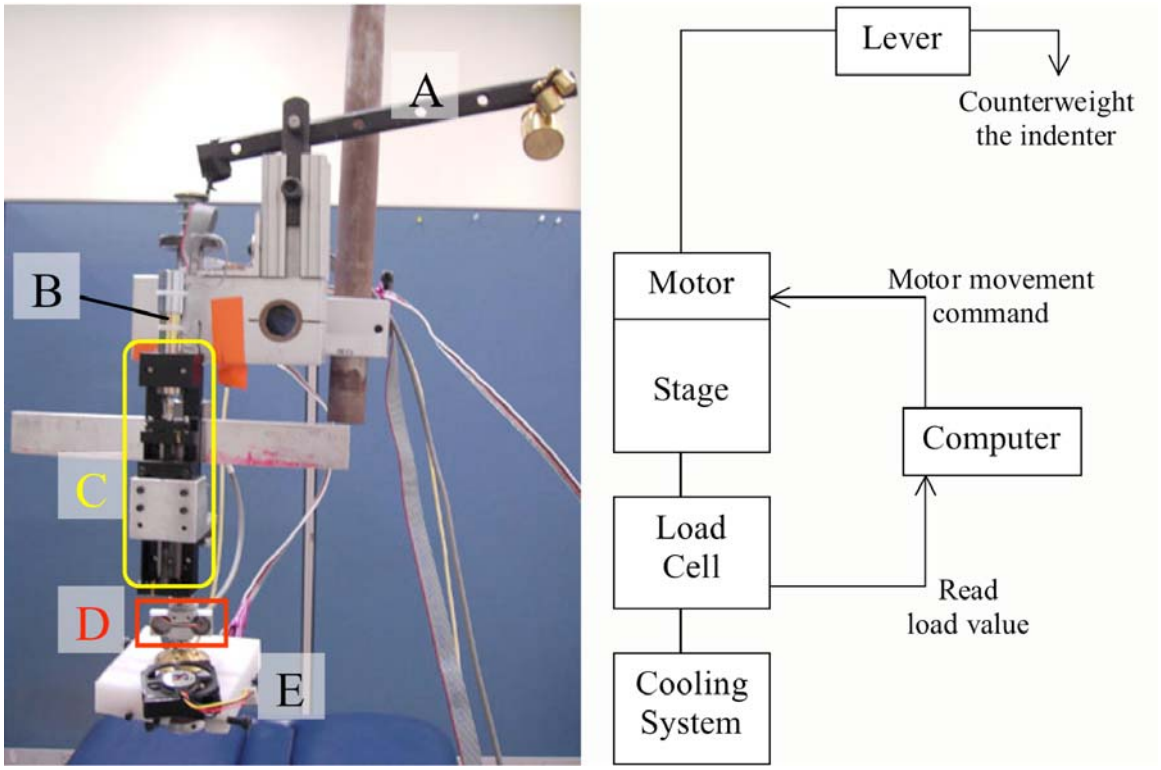

Figure 7. Left side: picture of computer controlled indenter (A) lever, (B) motor, (C) stage, (D) load cell, and (E) cooling system; Right side: schematic figure of the loading system.

3.4 RESEARCH PROCEDURES

This research procedure contained two parts: subject screening and experiment sessions. An initial telephone screening was made to determine the eligibility of participants based on their age, self-reported medical and smoke history, and medications taken. Informed consent was

obtained before any screening or experiments were done on the subjects. A face-to-face screening was then performed to obtain demographic data and health information, including self-reported height, weight, and body fat percentage measured by the investigator. The subject then proceeded to the experimental sessions once they were successful in meeting the recruitment criteria.

There were two test sessions (73 minutes each) in the experimental procedure: the cooling session, and non-cooling session; with a 30 minute washout period between the two test sessions. The ten participants were randomized into two different orders of the experiment: five (3 male, 2 female) underwent non-cooling session first, while the other five (2 male, 3 female) underwent cooling session first. Throughout the experimental procedure, the subject lay prone on a mat table and the test was performed on his/her skin at the right sacrum with the use of the integrated indenter and LDF probe (Figure 8).

The two test sessions were almost identical except for the existence of the cooling intervention. Skin perfusion and temperature were measured continuously through the test session, and there were five different phases of pressure control on the skin: 3mmHg for 10 minutes, 60mmHg for 30 minutes, 3mmHg for 20 minutes, 150mmHg for 3 minutes, and 3mmHg for 10 minutes. Pressure of 3mmHg is light contact of the indenter head and skin, 60mmHg simulates the pressure of sitting on a cushion, and 150mmHg is a pressure that occludes the skin blood flow. The cooling test session included the local cooling intervention of 25°C (77°F) over the 30 minute time period accompanied with 60mmHg of pressure control. Figure 9 is a schematic representation of the pressure and temperature control throughout the experiment.

Blood pressure and pulse rate were measured before and after each test session to ensure subject safety. The subjects were also closely monitored by the investigator throughout the experiment. An additional standard thermistor (YSI 700, WU-08415-27, YSI Inc., Yellow Springs, OH) was taped to the left sacrum for adjacent skin temperature.

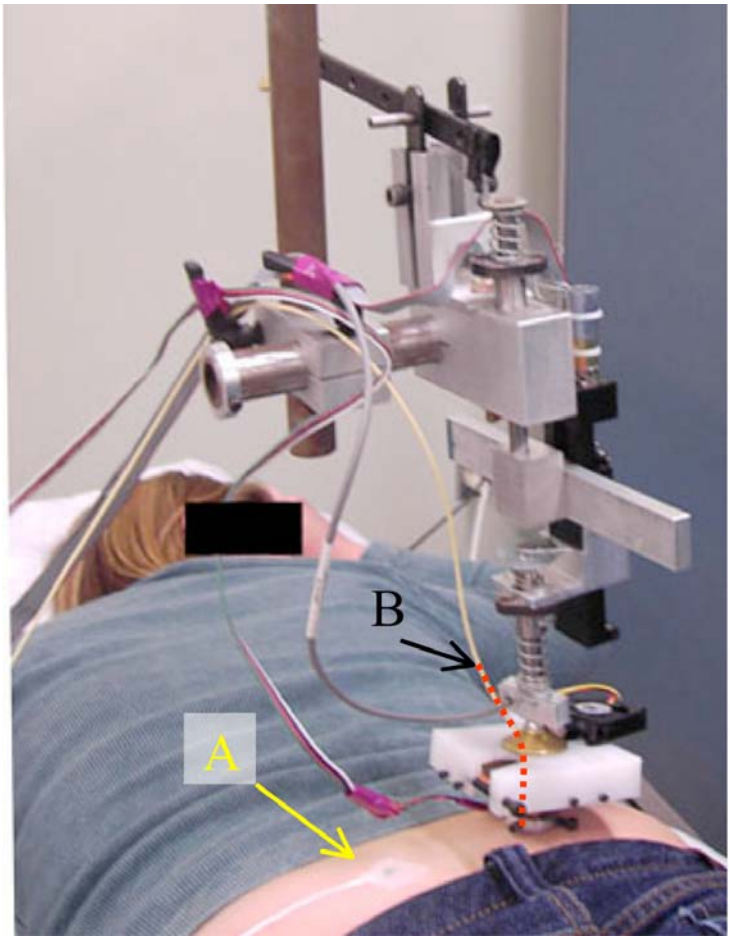

Figure 8. Experimental setting, (A) arrow points to the standard thermistor probe for adjacent temperature measurement, and (B) laser Doppler probe, and the dash line represents the placement of probe within the cooling system

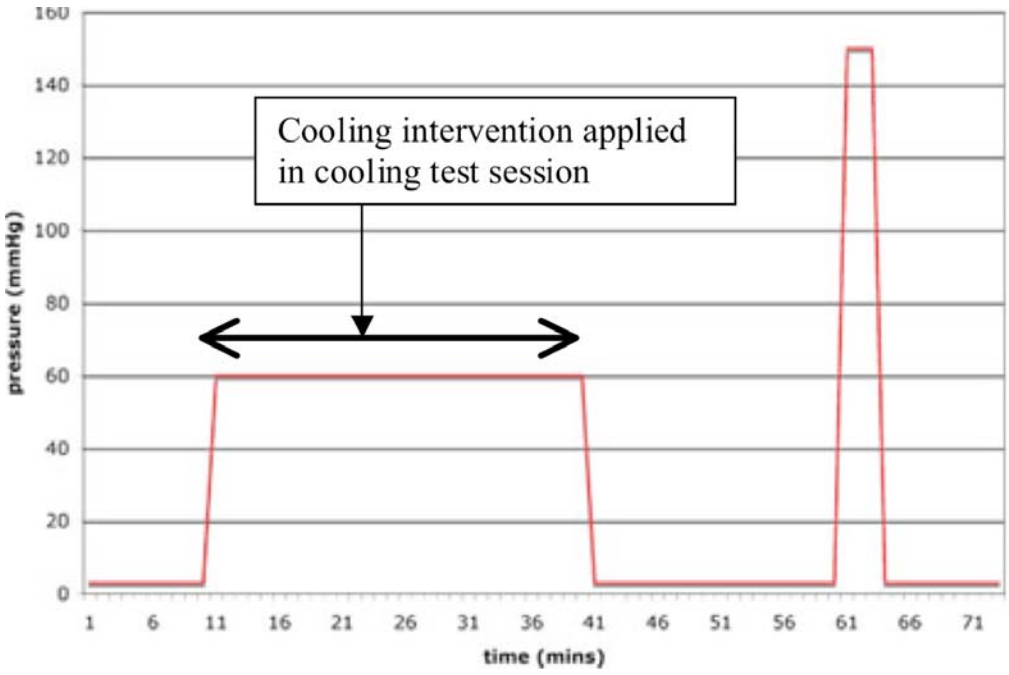

Figure 9. Schematic representation of pressure and temperature control

3.5 DATA COLLECTION

Self-reported demographic data (height/weight), and a measured body fat percentage were obtained prior to the experiment for eligibility of subjects. Body mass index (BMI) was calculated based on the height and weight of subject using the equation (Centers for Disease Control and Prevention):

$$BMI = \text{weight (kg)} / [\text{height (m)}]^2$$

Body fat percentage was calculated based on the 4-site system equations (British Indicators, 1998):

$$\text{Body density (BD)} = C - [M (\text{Log}_{10} \text{ sum of all four skinfolds})]$$

$$\text{Fat \%} = [(4.95/BD) - 4.5] \times 100$$

Experimental data including pressure applied on skin, skin temperature, and skin perfusion were collected through the LabVIEW program at a rate of 20Hz. The applied pressure and skin temperature data were averaged every 30 seconds to provide an overall view of changes over the test session. The skin perfusion signal went through the fourth-order Butterworth low-pass filter with cutoff frequency of 0.15 Hz (to eliminate the effect of respiration and heart rate) (Assous, Humeau, Tartas, Abraham, & L'Huillier, 2006; Carolan-Rees, Tweddel, Naka, & Griffith, 2002). Four parameters of the reactive hyperemic response were investigated and they were selected based on the filtered blood perfusion signal after the relief of 60 & 150 mmHg of pressure.

Figure 10 is the filtered skin perfusion signal of subject S01 in non-cooling session. The reactive hyperemic response could be characterized by following: “A” is the baseline of skin perfusion, “B” is the peak perfusion after pressure relief, “C” is the duration from pressure relief to the peak, “D” is the duration between peak and the halfway value toward baseline, and “E” is the total perfusion area after pressure relief.

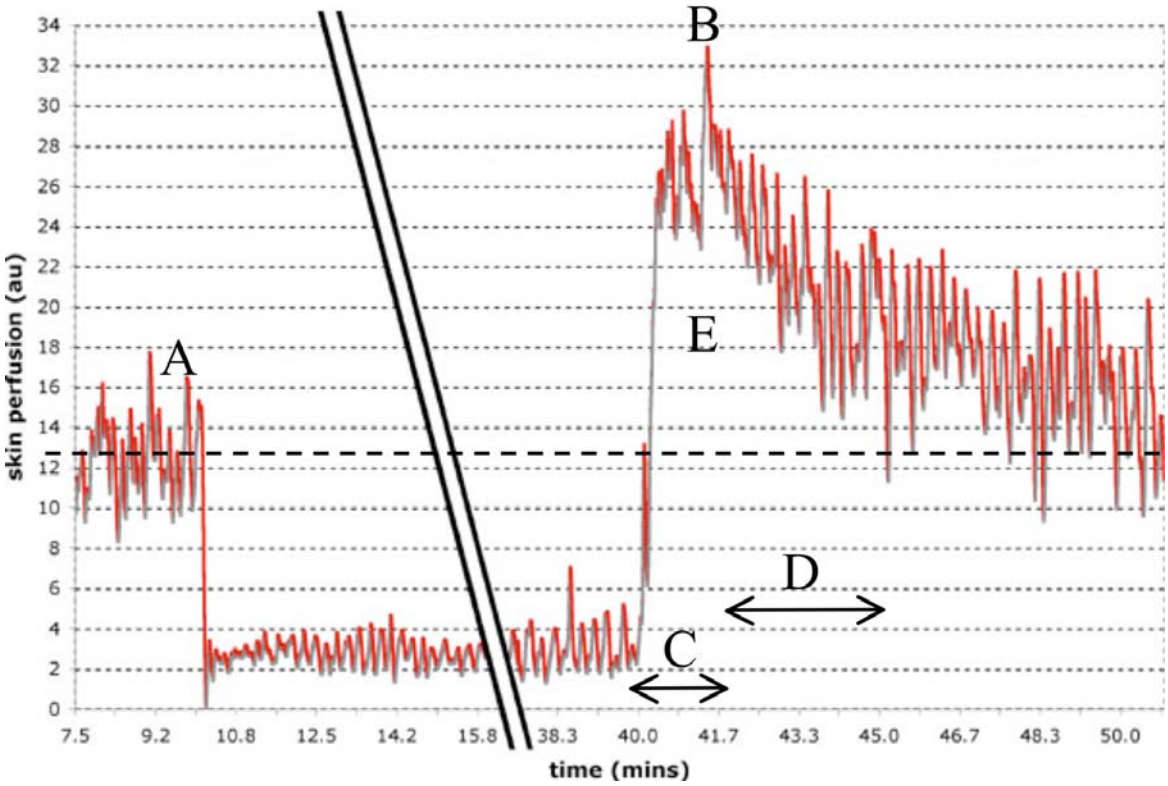

Figure 10. Filtered skin perfusion signal of subject S01 in non-cooling

The 11 parameters (with abbreviation and definition) analyzed in the perfusion response are listed below based on the perfusion characteristics described above. Since the perfusion response occurred after both 60 & 150mmHg pressure relief, parameters 2- 6 were defined for relief of 60mmhg and 7- 11 were for relief of 150mmHg:

1. bSBF (baseline SBF): the averaged value of skin blood flow data collected five minutes right before the 60mmHg pressure application
2. pSBF60 (peak SBF after 60mmHg pressure): the spike in SBF right after the 60mmHg pressure relief

3. Nor-pSBF60 (normalized peak SBF after 60mmHg pressure, ((pSBF60-bSBF)/bSBF)*100%): the spike in SBF right after the 60mmHg pressure relief normalized by bSBF
4. tpSBF60 (time to peak SBF after 60mmHg pressure): the time period from 60mmHg pressure relief to pSBF60
5. half60 (half life of peak SBF after 60mmHg pressure): the time between pSBF60 till SBF dropped halfway to baseline SBF
6. aSBF60 (perfusion area after 60mmHg pressure): the area between the peak and the baseline curve of SBF
7. pSBF150 (peak SBF after 150mmHg pressure): the spike in SBF right after the 150mmH pressure relief
8. Nor-pSBF150 (normalized peak SBF after 150mmHg pressure, ((pSBF150-bSBF)/bSBF)*100%): the spike in SBF right after the 150mmHg pressure relief normalized by bSBF
9. tpSBF150 (time to peak SBF after 150mmHg pressure): the time period from 150mmHg pressure relief to pSBF150
10. half150 (half life of peak SBF after 150mmHg pressure): the time elapsed between pSBF150 till SBF dropped halfway to baseline SBF
11. aSBF150 (perfusion area after 150mmHg pressure): the area between the peak and the baseline curve of SBF

Related skin temperature, i.e. skin temperature at baseline and peak SBF were defined below to provide a comprehensive investigation of the tissue response toward cooling and prolonged pressure:

1. bTemp (baseline skin temperatue): the averaged value of skin temperature data collected five minutes right before the 60mmHg pressure application
2. pTemp60 (skin temperature at peak SBF after 60mmHg pressure): the skin temperature measured at the spike in SBF right after the 60mmH pressure relief
3. Nor-pTemp60 (normalized skin temperature at peak SBF after 60mmHg pressure, ((pTemp60-bTemp)/bTemp)*100%): the skin temperature at peak SBF right after 60mmHg normalized by bTemp
4. pTemp150 (skin temperature at peak SBF after 150mmHg pressure): the skin temperature measured at the spike in SBF right after the 150mmH pressure relief
5. Nor-pTemp150 (normalized skin temperature at peak SBF after 150mmHg pressure, ((pTemp150-bTemp)/bTemp)*100%): the skin temperature at peak SBF right after 150mmHg normalized by bTemp

3.6 DATA ANALYSIS

The data analysis of this study included two parts: (1) visualization of plotted figures of applied pressure and skin temperature averaged every 30 seconds; (2) statistical analysis of reactive hyperemic response parameters, and relative skin temperature parameters between two test sessions.

3.6.1 Visualization of Plotted Figures of Applied Pressure and Skin Temperature

Both applied pressure and skin temperature were plotted subject by subject on time domain for visualization purpose. No filtering was performed and the raw data was averaged within every 30 seconds (600 data points) for plotting.

3.6.2 Statistical Analysis of Skin Perfusion & Related Skin Temperature

The skin perfusion underwent a low pass filter first, and the perfusion parameters were selected visually, based on the filtered signals. A descriptive analysis was made to determine which statistical analysis to use to compare the parameters between test sessions upon selecting all parameters listed in section 3.5 in all subjects and both test sessions,. Paired *t*-test was used for normally distributed data while Wilcoxon signed rank test was used for data that was not normally distributed. Software SPSS13 for Mac (SPSS Inc., Chicago, IL) was used for all statistical analysis and a p value of <0.05 was recognized as significant.

4.0 RESULTS

4.1 PARTICIPANTS

The demographic data for the subject is listed in Table 1. Ten young healthy subjects (five female and five male) were recruited. Age of the subject ranged from 20-40 years old (average = 28.31 years old). The body fat percentage was <25% in male subjects (average = 18.71%), and <35% in female subjects (average = 29.39%). The subjects' blood pressures were all within the normal range.

4.2 APPLIED PRESSURE

The pressure applied on skin was consistent across all subjects. Figure 11 showed applied pressure averaged over all ten participants in both non-cooling and cooling test sessions.

Paired *t*-test (0-10 minutes: p= 0.432 >0.05, 60-63 minutes: p=0.872 >0.05) and Wilcoxon (10-40 minutes: p=0.959 >0.05, 40-60 minutes: p=0.878 >0.05, 63-73 minutes: p=0.285 >0.05) signed rank test showed no significant difference between the two test sessions in applied pressure. Applied pressures throughout the tests reached all target values except at 60-63 minutes in some subjects. Pressure at this period of time was slightly lower than target value of 150mmHg (mean= 146.91mmHg in non-cooling, and mean= 146.77mmHg in cooling).

Table 1. Subject demographic data

Subject ID	Age	Gender	Ethnicity	BMI*	Body Fat %	Blood Pressure
S01	35.43	Male	Caucasian	28	24.59	124/78
S02	26.91	Male	Caucasian	21.6	12.69	120/70
S04	27.12	Female	Asian	19.8	30.28	110/80
S07	32.94	Female	Latino	24	33.43	110/72
S08	23.31	Female	Asian	26.6	27.92	104/80
S09	24.59	Male	Caucasian	30.2	26.04	132/84
S10	30.09	Female	Caucasian	21.8	26.30	112/72
S11	32.67	Male	Caucasian	27	18.13	130/84
S13	23.85	Female	Asian	20.5	29.02	118/78
S15	26.16	Male	Caucasian	23	12.12	122/68
Mean	28.31	--	--	M 25.96 F 22.54	M 18.71 F 29.39	--
Standard deviation	4.23	--	--	M 3.57 F 2.78	M 6.49 F 2.69	--

*BMI = body mass index

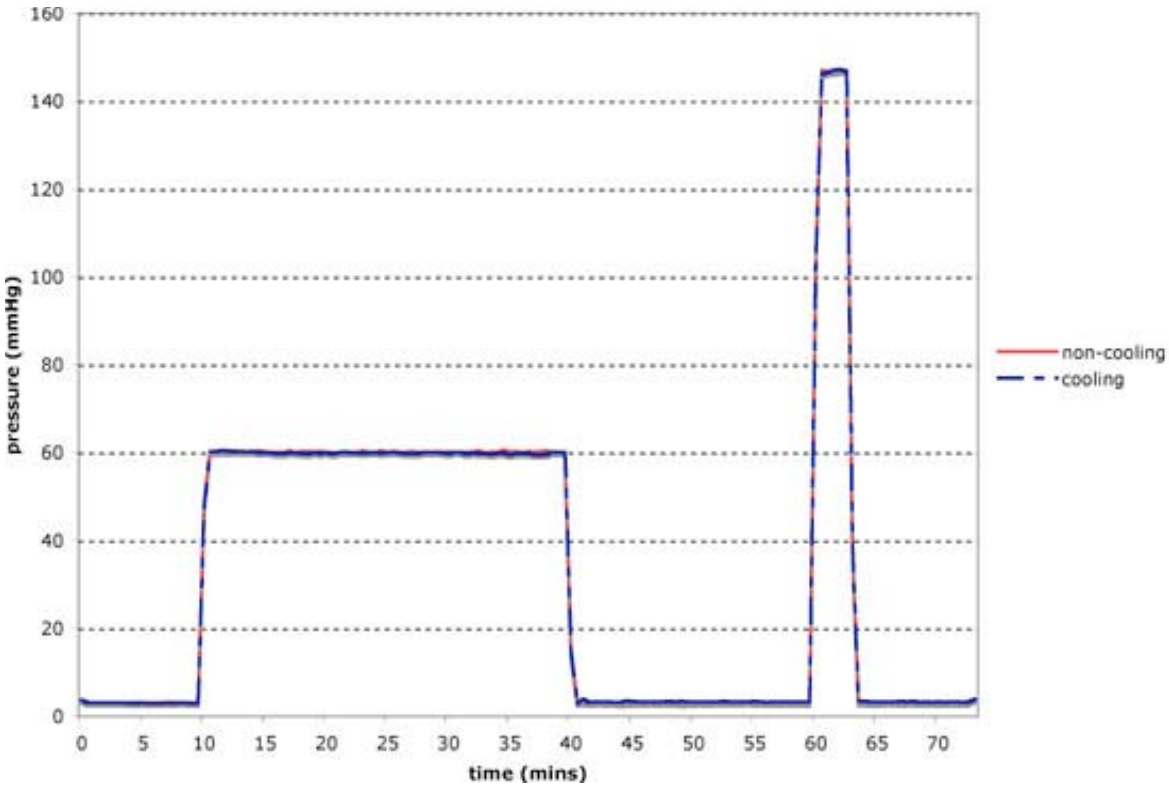

Figure 11. Applied pressure averaged among 10 subjects in non-cooling session

4.3 SKIN TEMPERATURE

The following graphs illustrate the skin temperature averaged among all ten subjects in non-cooling (Figure 12) and cooling (Figure 13) test sessions. The curve in the middle is the mean value and the curves below and above are minimum and maximum values for all ten subjects. The skin temperature varied among subjects including the baseline value, and the trend of temperature changed over the test session. The coefficient of variation of baseline temperature was 2.27% in non-cooling, and 1.41% in cooling session.

In non-cooling, the trend of temperature changes was the same in all subjects. Skin temperature decreased overtime during all time periods with 3mmHg of pressure: 0-10 minutes (decreased 0.8°C), 40-60 minutes (decreased 1.2°C), and 63-73 minutes (decreased 0.7°C). On the contrary, skin temperature increased overtime during the two periods with higher applied pressure, and the increase in temperature was smaller than 1°C: at 10-40 minutes (increased 0.63°C), and at 60-63 (increased 0.7°C).

Trend of temperature changes showed two patterns in cooling. Skin temperature in all subjects decreased gradually during the first 10 minutes (about 0.8°C), which was consistent with that in non-cooling. The temperature then dropped to 25°C within one minute and remained constant within ±0.02°C in all subjects. Skin rewarming occurred immediately after removal of local cooling. The skin temperature returned to a value about 1°C lower than the baseline initially during rewarming. In six subjects, the skin temperature then remained constant thereafter, while the other four subjects had a gradual decrease (about 0.5°C). In cooling session, skin temperature increased over time about 0.5°C during 150mmHg of pressure in all subjects; this was consistent with that in non-cooling.

Statistical analysis was performed to compare the skin temperature between two test sessions. The baseline skin temperature in both test sessions were normally distributed, and paired *t*-test showed no significant difference between the two (p=0.647 >0.05). Skin temperature at 60mmHg in non-cooling (mean=28.34°C) was significantly higher than that in cooling session (mean=25.01, p<0.001). Further comparison of skin temperature relative to skin perfusion are made in section 4.4.

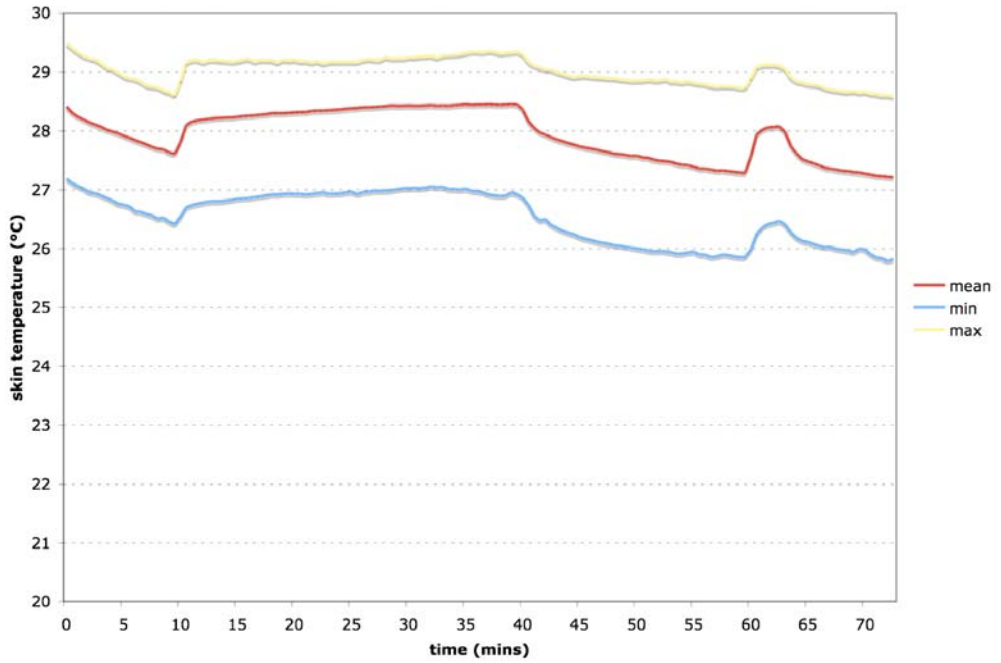

Figure 12. Skin temperature averaged among 10 subjects in non-cooling session

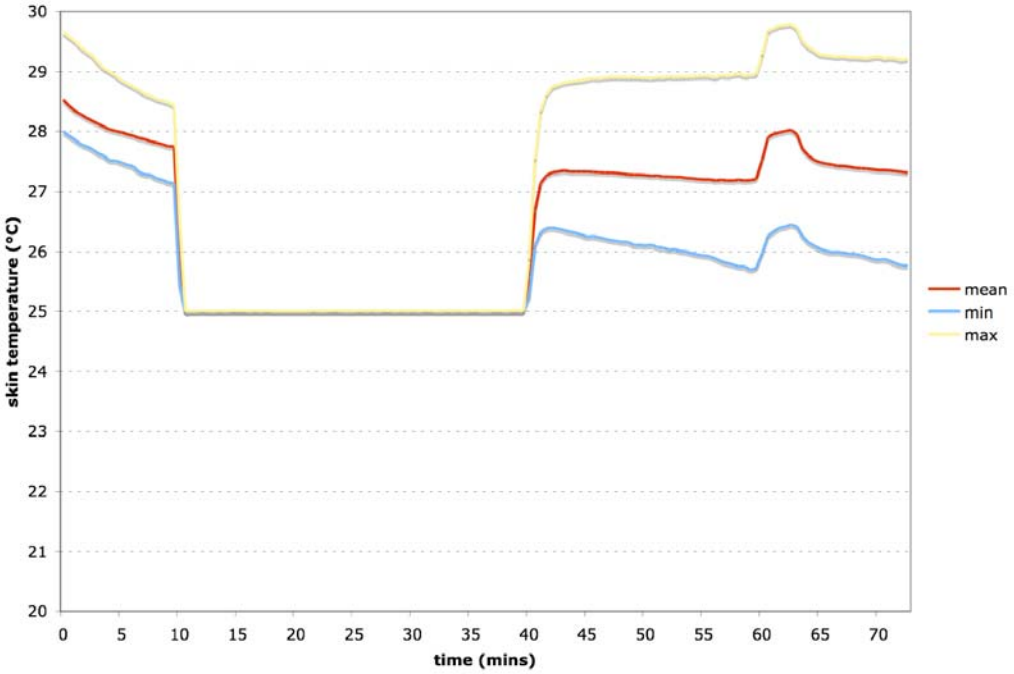

Figure 13. Skin temperature averaged among 10 subjects in cooling session

All skin temperatures used for plotting and statistical analysis was collected through the customized thermistor. A reference value measured with the standardized thermistor located at an adjacent site on the sacrum showed about 2-4°C of temperature difference (Table 2). Such temperature difference was consistent between cooling and non-cooling within the subject and the reference temperature remained constant throughout the test at the adjacent skin sites.

Table 2. Comparison between baseline skin temperature measured with customized thermistor and standard thermistor, and averaged ambient temperature measured with standard thermistor

Subject ID	Skin temperature measured by customized thermistor		Adjacent skin temperature		Averaged ambient temperature
	Cooling	Non-cooling	Cooling	Non-cooling	
S01	27.3	27.7	29.2	29.5	21.0
S02	27.7	28.2	32.3	32.6	20.3
S04	28.3	28.8	32.5	32.9	20.8
S07	27.7	26.6	29.1	30.3	20.9
S08	28.1	28.2	32.1	32.7	21.2
S09	27.9	27.6	30.0	30.0	20.5
S10	27.5	27.2	31.1	31.1	21.0
S11	28.0	28.2	31.9	32.3	21.3
S13	27.6	28.0	30.1	31.4	20.2
S15	28.6	27.3	32.5	32.0	20.7

4.4 SKIN PERFUSION

4.4.1 Skin Perfusion After 60mmHg

Figure 14 shows a typical reactive hyperemic response that occurred after 60mmHg relief during a non-cooling session. Such a response was less obvious in the cooling session in the same subject (Figure 15). Due to a limitation of Microsoft Excel, part of the skin perfusion signal during 60mmHg was clipped.

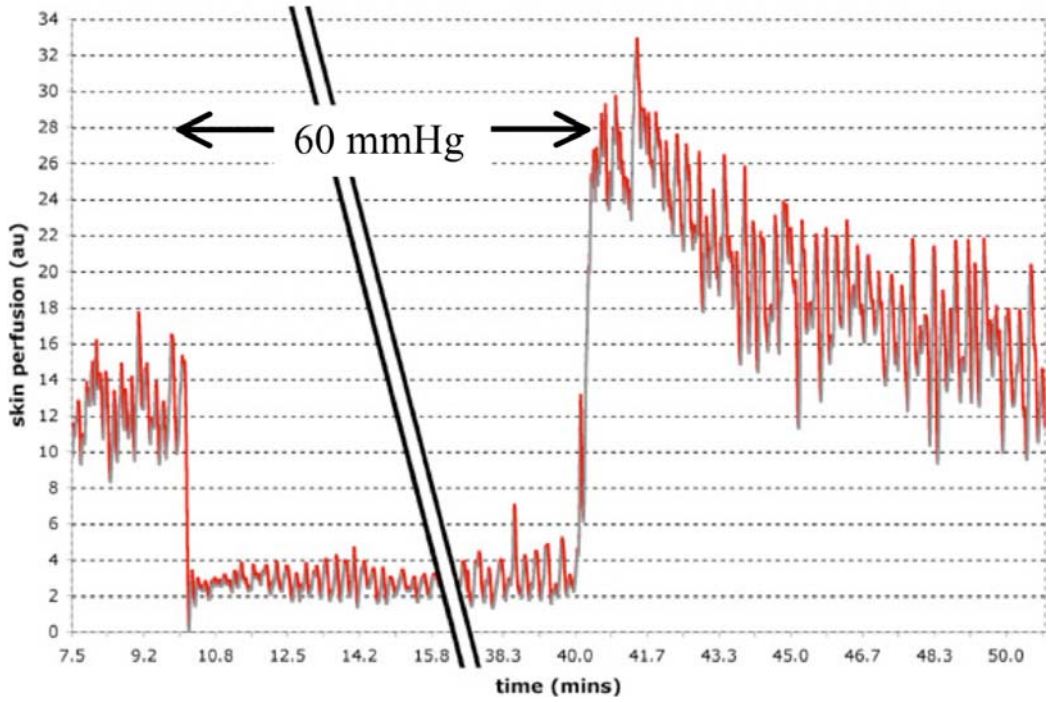

Figure 14. Skin perfusion at baseline, 60mmHg, and after 60mmHg removal in non-cooling session

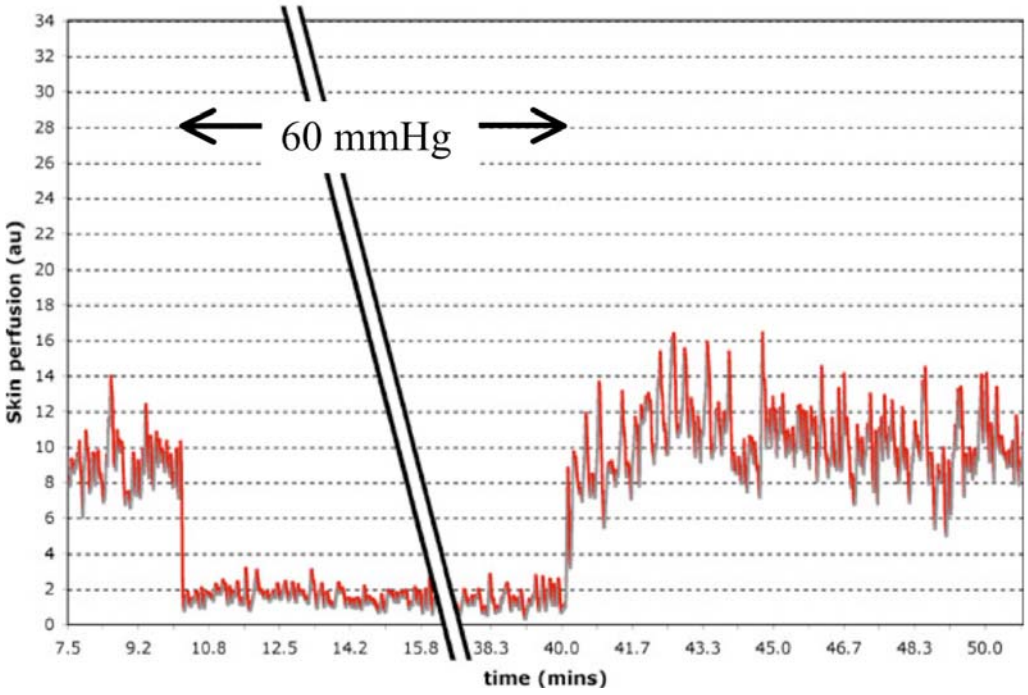

Figure 15. Skin perfusion at baseline, 60mmHg, and after 60mmHg removal in cooling session

Descriptive analysis of all skin perfusion and related skin temperature parameters compared in this study showed that most of them were normally distributed except aSBF60, half60, and tpSBF60. Peak SBF was normalized by its baseline value, since baseline SBF varied widely (coefficient of variation of the baseline SBF was 38.5% in non-cooling and 31.78% in cooling session).

Paired *t*-test showed that there was no significant difference between cooling and non-cooling in baseline skin blood flow and temperature (Figure 16). The normalized peak SBF in non-cooling session was significantly higher than that in cooling (p= 0.019 <0.05). Wilcoxon signed rank test showed normalized skin temperature at peak SBF after 60mmHg was significantly lower than non-cooling (Figure 17). Seven subjects showed a shorter half life in the cooling session, however there was no significant difference. There was also no significant difference between test sessions in time to peak SBF and SBF area.

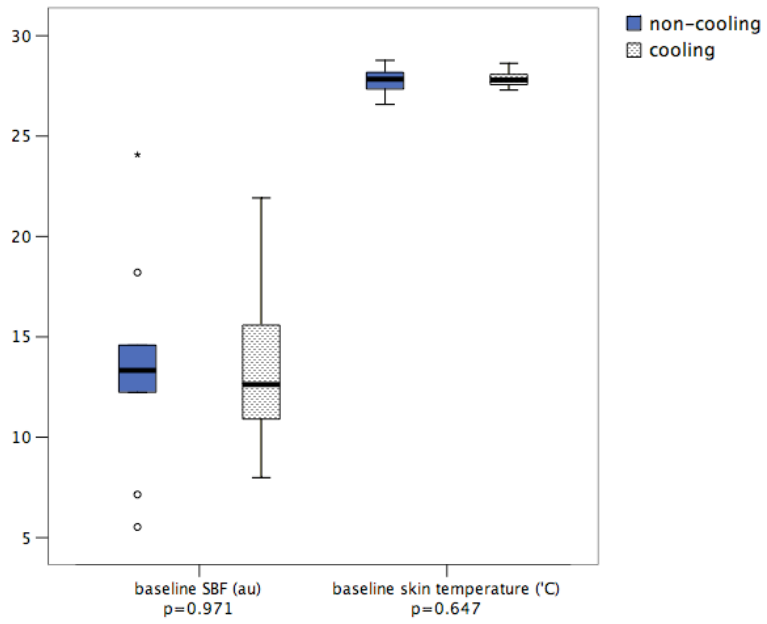

Figure 16. Comparison of baseline SBF and temperature between cooling and non-cooling

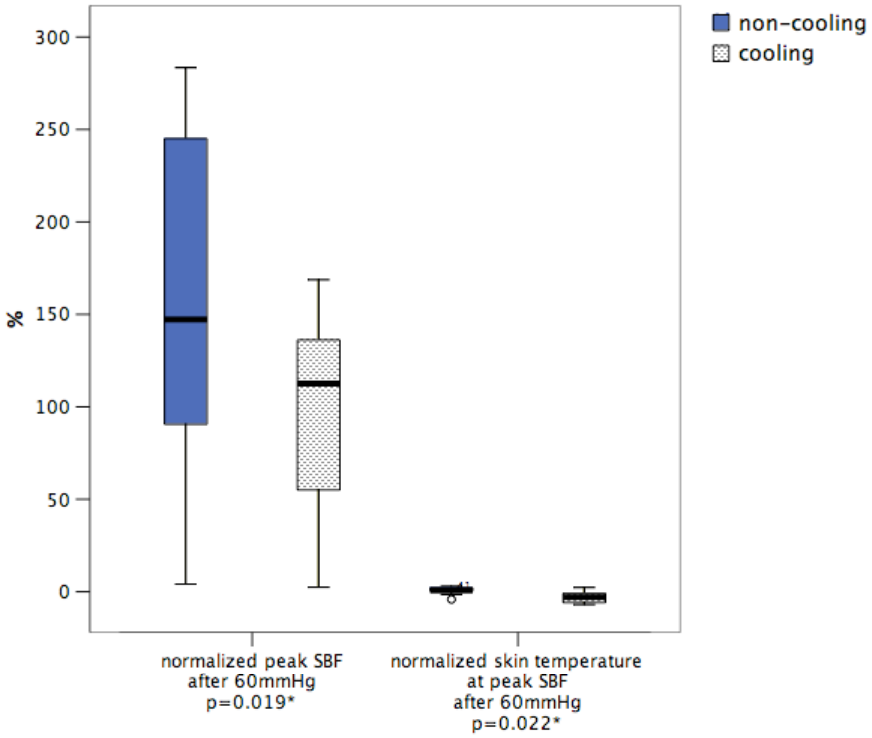

Figure 17. Comparison of normalized peak SBF and temperature between cooling and non-cooling

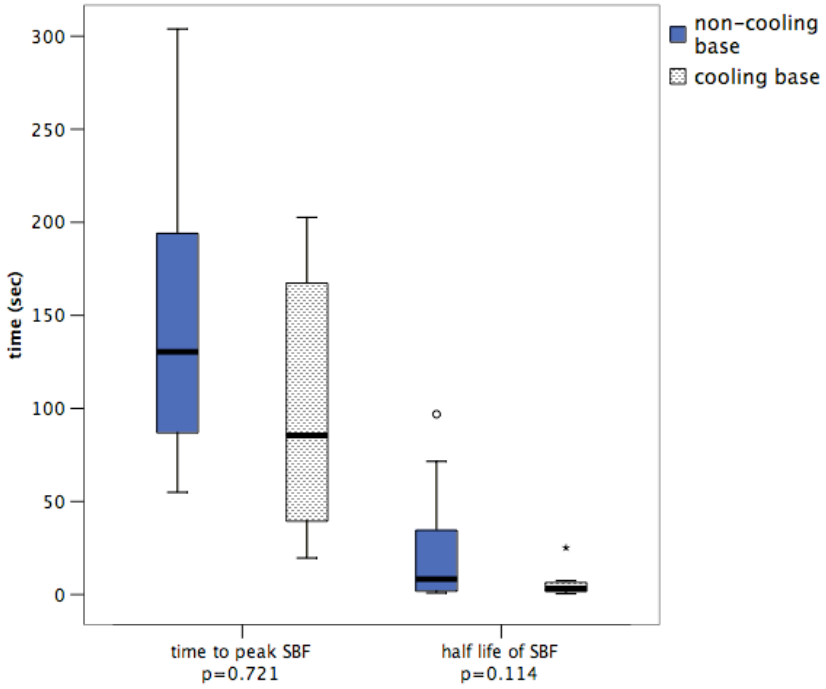

Figure 18. Comparison of time to peak SBF and half life of SBF between cooling and non-cooling

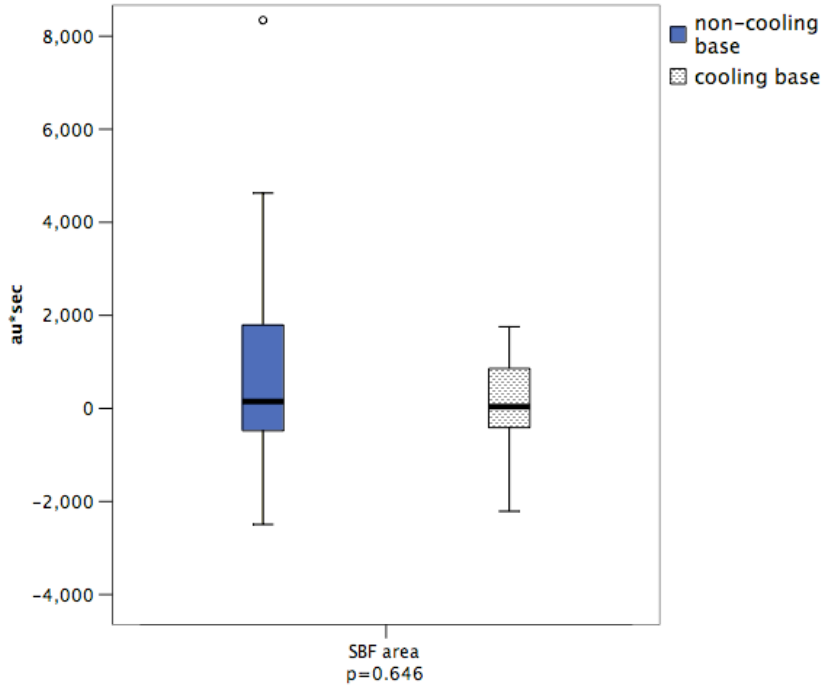

Figure 19. Comparison of SBF area between cooling and non-cooling

4.4.2 Skin Perfusion After 150mmHg

The reactive hyperemic response parameters were analyzed after relief of 150mmHg of pressure. Descriptive analysis of all skin perfusion and related skin blood flow parameters showed that only skin temperature at peak SBF was normally distributed, and the others were not normally distributed. Paired *t*-test showed the difference between the normalized skin temperatures at peak SBF in the two sessions (p= 0.078 >0.05) were closed to but not statistically significant. Based on the Wilcoxon signed rank test, six subjects had a higher normalized pSBF in non-cooling, and seven subjects had a larger SBF area in non-cooling (Table 3). However, there were no significant differences between the two sessions for these parameters.

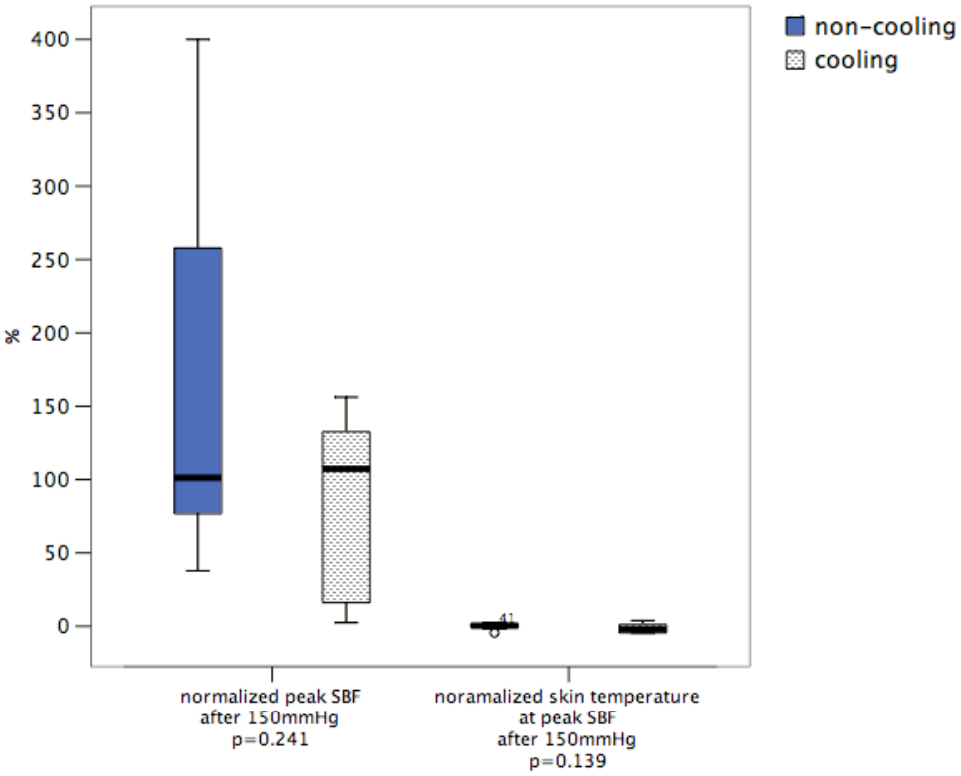

Figure 20. Comparison of normalized peak SBF and temperature between cooling and non-cooling

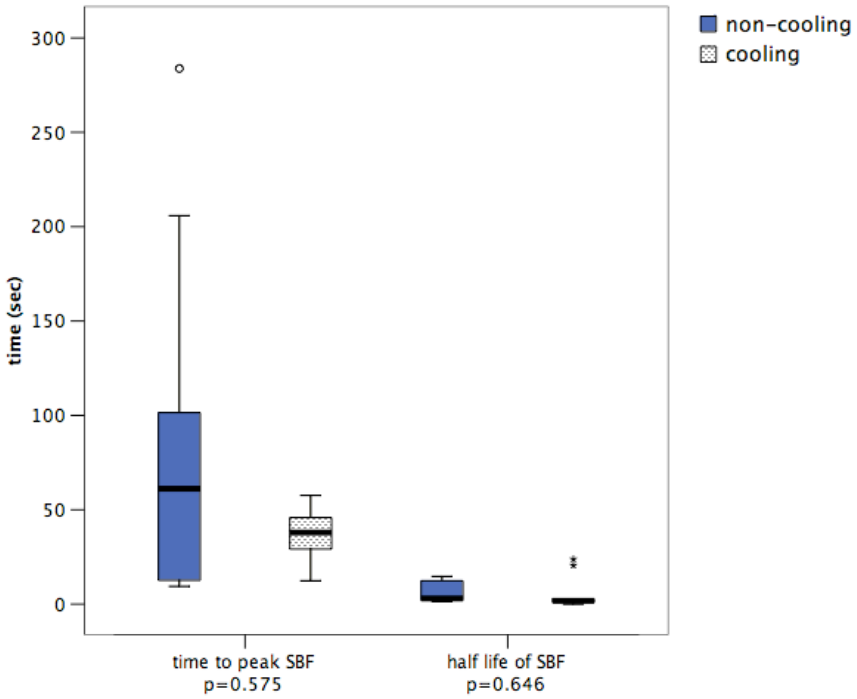

Figure 21. Comparison normalized peak SBF and temperature between cooling and non-cooling

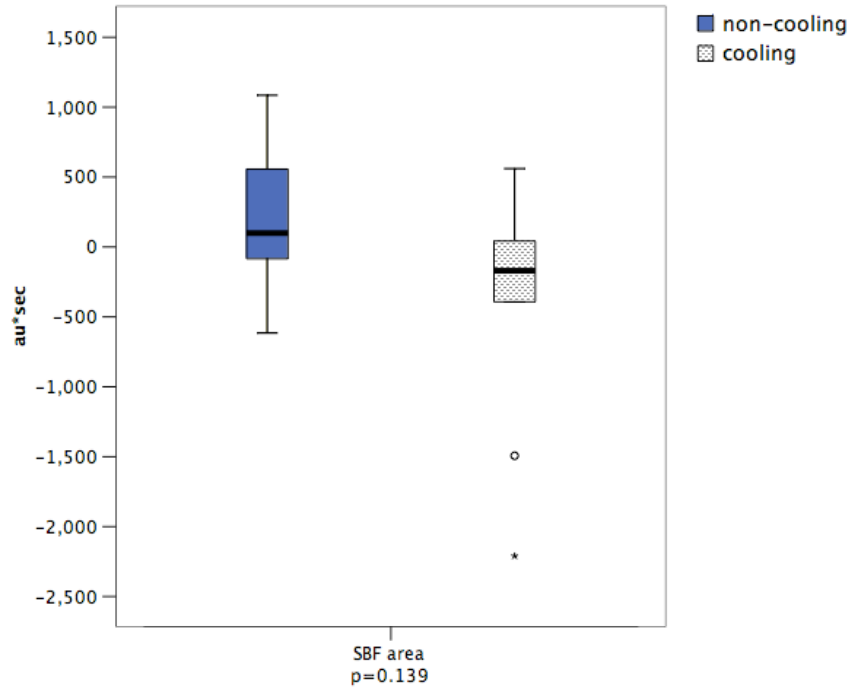

Figure 22. Comparison normalized peak SBF and temperature between cooling and non-cooling

Table 3. Ranks of perfusion and related temperature parameters after 150mmHg by using the Wilcoxon signed rank test, followed by explanation of each rank

		N	Mean Rank	Sum of Ranks
Normalized peak SBF after 150mmHg (cool) – Normalized peak SBF after 150mmHg	Negative Ranks	6(d)	6.50	39.00
	Positive Ranks	4(e)	4.00	16.00
	Total	10		
time to peak SBF after 150mmHg (cool) – time to peak SBF after 150mmHg	Negative Ranks	5(g)	6.60	33.00
	Positive Ranks	5(h)	4.40	22.00
	Total	10		
SBF area after 150mmHg (cool) – SBF area after 150mmHg	Negative Ranks	7(j)	6.00	42.00
	Positive Ranks	3(k)	4.33	13.00
	Total	10		
half life after 150 (cool) – half life after 150	Negative Ranks	5(m)	6.40	32.00
	Positive Ranks	5(n)	4.60	23.00
	Total	10		
Normalized temperature at peak SBF after 150mmHg (cool) – Normalized temperature at peak SBF after 150mmHg	Negative Ranks	6(p)	7.00	42.00
	Positive Ranks	4(q)	3.25	13.00
	Total	10		

d Normalized peak SBF after 150mmHg (cool) < Normalized peak SBF after 150mmHg
e Normalized peak SBF after 150mmHg (cool) > Normalized peak SBF after 150mmHg
g time to peak SBF after 150mmHg (cool) < time to peak SBF after 150mmHg
h time to peak SBF after 150mmHg (cool) > time to peak SBF after 150mmHg
j SBF area after 150mmHg (cool) < SBF area after 150mmHg
k SBF area after 150mmHg (cool) > SBF area after 150mmHg
m half life after 150 (cool) < half life after 150
n half life after 150 (cool) > half life after 150
p Normalized temperature at peak SBF after 150mmHg (cool) < Normalized temperature at peak SBF after 150mmHg
q Normalized temperature at peak SBF after 150mmHg (cool) > Normalized temperature at peak SBF after 150mmHg

5.0 DISCUSSION

5.1 PRESSURE CONTROL

The constant and precise applied pressure on the skin showed that the indenter could provide a consistent pressure control on all subjects for both test sessions. The primary variation of applied pressure noted at 150mmHg might be due to the instrument limitation of the computer-controlled indenter. The initial design of the indenter could not precisely control applied pressure at 150mmHg, and the range of such variation was wide (about 87.61-179.56mmHg) in our pilot experiment due to the respiratory movement of the subject. Modification of the indenter was made thereafter to provide more accurate pressure control by using deadweight of the indenter system to reach the pressure of 150mmHg. Based on the pressure application area, we calculated the exact weight required to reach pressure of 150mmHg. Since the weight of the indenter system was higher than required, the indenter was counter-weighted by a lever system added to the frame that held the indenter. The modified indenter provided a better control at 150mmHg of pressure, and the pressure variation during this period of time was due to the respiratory movement.

When the subject inhaled, pressure applied on the skin could reach no more than 150mmHg of pressure. However, when the subject exhaled, a delayed indenter movement of tracking the skin position resulted in a slightly lowered pressure value. The respiratory

movements and speed of the subjects varied. The parameters of PID control were adjusted for best simultaneous movement between the indenter and the subject respiration in most subjects.

5.2 SKIN TEMPERATURE

Normal skin temperature of healthy humans is about 32.5°C (Benedict & Parmenter, 1929). The value differed between body sites, and was reported to be 30-30.5°C at the buttocks (Benedict, Miles, & Johnson, 1919). The temperature recorded via our customized thermistor was about 2-3 degrees lower than the values reported previously. This might be due to the limitation of the customized thermister and the microclimate at the test site of the skin. Our customized thermistor covered a commercial thermistor with a copper plate to provide a comfortable contact surface to the skin. The ability of reading temperature of the thermistor with and without the copper plate was tested during the instrument configuration of the study. Both conditions: thermistor alone, and thermister with copper plate were tested by the investigator by holding them firmly between two fingertips to compare with the standard thermistor thermometer under the same condition. The difference was relatively small among the three. In the test sessions of this study, the major variation of skin temperature between the customized thermistor and standard one during the test was noted during the time periods of 3mmHg of pressure. This amount of pressure provided a light contact to the skin. Due to the respiratory movement and delayed indenter tracking of the skin position mentioned previously, some areas of the customized thermistor did not closely contact the skin at times. The ambient temperature (20.8±0.5°C), which was far lower than the skin temperature, might have caused a lower temperature reading. This may also explain the gradual elevated skin temperature at 60 and

150mmHg of applied pressure, where all areas of the thermistor were in close contact with the skin. The results also confirmed previous studies on skin temperature changes under similar localized pressure. Sae-Sia et al. (Sae-Sia, Wipke-Tevis, & Williams, 2007) measured sacral skin temperature in acute care hospitalized patients; they found the sacral skin temperature increased over two hours of clinically relevant pressure duration. In addition, Cochran's investigation on skin and cushion interface temperature revealed that skin temperature increased overtime, from duration of 30 minutes up to two hours (Cochran, 1985).

In the cooling session, the fast and consistent temperature control of 25°C at 60mmHg showed that the thermoelectric module system provided instant and precise skin temperature control. In addition, the statistically significant difference between the two sessions in temperature during the 60mmHg pressure period ensured the sufficiency of our cooling intervention in the cooling session. The rapid rewarming response noted right after cooling removal was faster than the results from previous studies. Jutte et al. (2001) found that human skin temperature returned from <10°C to ambient temperature of 25°C in 15 minutes and to baseline in more than one hour after 30 minutes of ice bag intervention. Researches on skin temperature response of cold gel pack application on dogs revealed that rewarming time after 30 minutes of cold application was more than two hours, and the slope of increased temperature was steep initially followed by a plateau after 30 minutes of rewarming (Akgun et al., 2004). Both studies cooled the skin down to lower than 25°C (about 8°C in human study, and 16°C in dog study), which was far below our target cooling value. The rewarming response in both studies showed a steep increase in the first 15 minutes and the changes in temperature were about 11°C in the human study, and 8°C in dog study. Since our cooling area is relatively small and our cooling intervention was very mild, it is reasonable that skin temperature returned to about

1.69°C within one minute after cooling removal. The rewarming stage reached a plateau in both studies before reaching the baseline value; however, this phenomenon was not observed in our study since the temperature reached baseline value within a very short time.

In non-cooling, the skin temperature decreased after pressure removal. This conflicted with a study done by Kemuriyama et al. They investigated temperature changes on rabbit ears during and after complete ischemia (300mmHg pressure) (Kemuriyama, Nitsuma, Yano, & Komeda, 1998). The skin temperature increased rapidly after pressure removal and they suggested that the increased temperature was related to the increased perfusion of the reactive hyperemic response. The localized pressure in our study was relatively mild and we did not occlude the skin blood flow completely in all subjects. In addition, due to the limitation of our instrumentation mentioned previously, instead of a rapid increase in skin temperature after pressure removal, a slight drop in skin temperature was noted in our study.

5.3 SKIN PERFUSION

The reactive hyperemic response was induced in this study to analyze the effect of local cooling on skin perfusion after prolonged pressure. The parameters in reactive hyperemia were selected through visualization of the filtered signal.

5.3.1 Skin Perfusion After 60mmHg

The reduced reactive hyperemic response in the cooling sessions found in this study was consistent with previous research studies. Olivecrona, Gotberg, Harnek, Van der Pals, & Erlinge

(2007) investigated the reactive hyperemic response on pigs after coronary artery ischemia under two different temperatures (mild hypothermia 34°C, and control 37°C). They found the peak flow in the cooling group significantly reduced by 43% compared to the controls. Peak SBF during reactive hyperemia was characterized as "how fast and extensively the vessels react to ischemia" (Hagisawa, Ferguson-Pell, Cardi, & Miller, 1994). Previous studies suggested that the peak SBF during reactive hyperemia was mediated by myogenic vasodilation and release of metabolic factors (Noble, Voegeli, & Clough, 2003). The myogenic mechanism, based on viscoelastic properties and stretch-dependent characteristics, is likely to be responsible for the reactive hyperemic response after a short period of occlusion. The metabolic mechanism, on the other hand, relies on the accumulation of vasodilator substances due to the increase in metabolic debt during ischemia (Wilkin, 1987), and it is likely to be the cause of the reactive hyperemic response after longer occlusion periods. The decrease in normalized peak SBF in the cooling session indicated an attenuated vasodilation during the early phase of reactive hyperemia. Since the reactive hyperemia in this session was induced after a relatively long time of occlusion, and the pressures applied on the skin in both test sessions were the same (same amount of mechanical force on the surrounding smooth muscles), the decrease of peak perfusion in cooling indicated a relatively lower metabolic requirement and reduced severity of tissue ischemia (Matsubara et al., 1998) under the prolonged pressure.

For temporal parameters including time to peak and half life, theses represent the occurrence of the vasomotion during the reactive hyperemia. In a previous study, differences in temporal parameters was recognized only in people taking vasodilators (Sprigle, LInden, & Riordan, 2002). The mechanism of vasodilators is to directly relax the smooth muscles surrounding the vessels. The lack of difference in the temporal parameters between the two

sessions indicated that local cooling did not significantly affect the myogenic vasomotion during reactive hyperemia.

Normalized skin temperature at peak was significantly lower in the cooling session. This was not surprising since skin temperature didn't return to the baseline value shortly after cooling removal (Jutte, Meerick, Ingersoll, & Edwards, 2001). Since temporal parameters showed that myogenic vasomotion was not significantly affected by local cooling during reactive hyperemia, the mild temperature difference between the two after pressure removal might mainly affect the reactive hyperemic response through metabolism. However, there is still a possibility that myogenic vasomotion has effect on the reactive hyperemia, and further signal analysis is required in the future to determine the percentage of these two mechanisms in the perfusion response.

The perfusion area represented the metabolic repayment after ischemia (Schubert & Fagrell, 1991b). Most perfusion area data in cooling were negative. The negative value of perfusion area conflicts with findings from previous studies (Schubert & Fagrell, 1991b). This might be due to the limitation of data calculation and insufficient reactive hyperemic response. As mentioned previously, some noise remained in the filtered signal. The noise could not be defined and removed from the signal and was calculated as part of the perfusion area. The insufficient reactive hyperemic response due to the mild occlusion made it difficult to define this parameter as well, since the reactive hyperemia signal was under the baseline value in some subjects.

Despite the metabolic debt and severity of ischemia under cooling, studies have also suggested that the reduced reperfusion response might also be beneficial in diminishing the

injury caused by ischemia-reperfusion (Olivecrona, Gotberg, Harnek, Van der Pals, & Erlinge, 2007).

5.3.2 Skin Perfusion After 150mmHg

The second pressure application in our test was used to compare the skin perfusion response 20 minutes after 60 mmHg pressure relief. The reactive hyperemic response was relatively more obvious after 150mmHg of pressure; the response was steep and rapid, and the spike in perfusion was sharp since the duration of ischemia was more severe and shorter. Normalized skin temperature during the reactive hyperemic response was not significantly different between the two test sessions. This was reasonable since the temperature returned to the baseline value 20 minutes after local cooling removal. The normalized peak SBF showed no significant difference as well; however, six out of ten subjects had lower normalized peak perfusion in the cooling session. This indicated that the effect of local cooling on tissue metabolism remained after 20 minutes of removal in these subjects, but the difference was small. The lower skin temperature after cooling removal may have still caused some minor metabolic changes during the 20 minutes in these subjects. This was also supported by the parameter of skin perfusion area. The skin perfusion area represents the metabolic repayment of tissue by skin perfusion (Schubert & Fagrell, 1991b). The skin perfusion areas of these six subjects were all larger in the non-cooling session as well. In other words, the effect of local cooling on tissue preservation could last in some subjects, but the result was not consistent with all subjects. Further analysis of skin perfusion after 150mmHg pressure should be made for a more comprehensive knowledge of the local cooling effect.

5.4 LIMITATIONS

There were two limitations found in this study, one was the measurement of skin temperature by using the customized thermistor (discussed in section 5.2), and the other was the selection and measurement of the skin perfusion parameters.

The selection of these parameters was difficult in some subjects, and this might be due to two reasons: (1) insufficient blood flow occlusion and (2) signal noise from subject movements. Schubert & Fagrell (Schubert & Fagrell, 1991b) found that magnitude of mechanical pressure required to occlude the skin blood flow varied in healthy subjects at the sacral area, and the average value they measured on healthy subjects (259±97mmHg) was higher than the values (60 and 150mmHg) that we used in this study. Other studies using 150mmHg to induce response had a longer duration of pressure application over the skin (at least 5 (Hagisawa, Ferguson-Pell, Cardi, & Miller, 1994) or 10 minutes (Sprigle, LInden, & Riordan, 2002)) compared to this study (3 minutes). The pressure value selected in our study during the first occlusion was not sufficient to induce the reactive hyperemic response in some subjects, and the reactive hyperemia parameters were very difficult to define.

Signal noises from subject movement were inevitable. The test session was long and healthy people have a tendency to move when feeling uncomfortable. Figure 23 is the skin perfusion signal of subject S15 and includes noise from body movement.. Point “A” is undoubtedly the peak of skin perfusion since it is on the trend of perfusion changes; whereas points “B” “C” and “D” are the noise rather than peak perfusion. When it was hard to decide which point represented the peak, the averaged raw data over 2.5 minutes intervals were used for peak point selection.

The low-pass filter used in this study had a cutoff frequency of 0.15Hz. Based on previous study results of the perfusion signal, this cutoff frequency mainly contains the effect of metabolic response (Assous, Humeau, Tartas, Abraham, & L'Huillier, 2006; Carolan-Rees, Tweddel, Naka, & Griffith, 2002). Advanced signal processing tool such as wavelet analysis is required in the future to unmask the underlying mechanism of the perfusion response under pressure and local cooling.

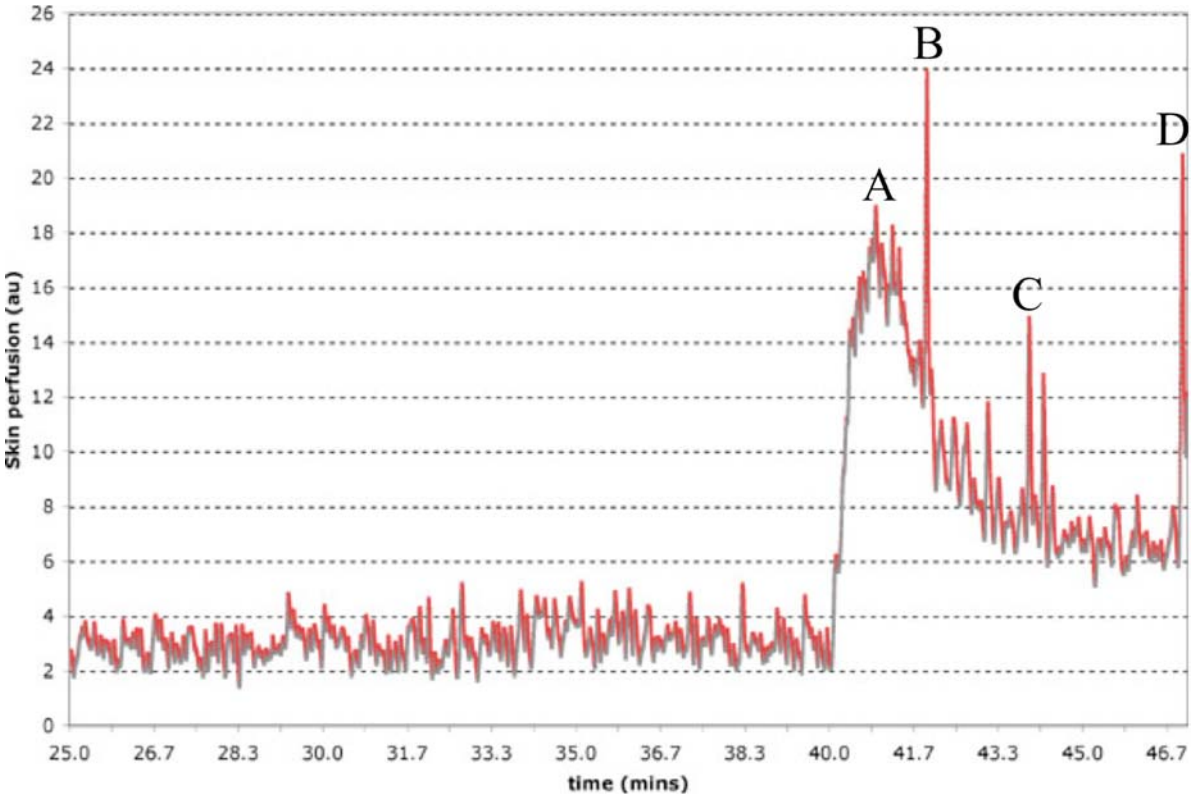

Figure 23. Skin perfusion of subject S15 during and after 60mmHg with noise

6.0 SUMMARY AND RECOMMENDATIONS

The results of this study showed that peak skin perfusion response after pressure relief in the cooling session was significantly attenuated. This indicated that local cooling influenced skin vasomotion under prolonged pressure. Such influence might be due to reduced metabolic vasodilator substances accumulating at the test site of the skin under local cooling. In addition, the attenuated perfusion response might also be beneficial to reduce skin ischemia reperfusion injury. The results denoted that local cooling had a protective effect on the skin under prolonged pressure. For six subjects, the results showed the potential effects of local cooling lasting over a 20-minute period; this was not seen for all subjects. Results of the study agreed with previous studies that skin temperatures naturally increase when in close contact with other materials for a prolonged period of time. In addition, the results also agreed with previous animal studies that local cooling provided a protective influence on the skin to prolonged pressure.

The integrated pressure and temperature control system developed in this study could precisely direct and maintain the pressure and temperature applied on the skin in the laboratory setting. The system also provided continuous non-invasive measurement of skin perfusion under pressure and temperature control for the investigation of the skin reactive hyperemic response.

In conclusion, the findings of this study recommended that local cooling on skin could provide protection of the tissue under prolonged pressure in young healthy subjects. Further investigations on populations at risk are required to understand the perfusion response toward

local cooling and prolonged pressure for these populations. The integrated system developed in this study was easy to use and could be modified for experimentation on at-risk populations.

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