The Effect of Diabetes and Limb Loss on Thermoregulation and Temperature Perception

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Abstract

The effect of diabetes and limb loss on thermoregulation and temperature perception

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Elevated residual limb skin temperatures inside the prosthetic socket are a source of thermal discomfort and likely responsible for profuse sweating, both reported as significant problems for lower limb amputees (Hagberg & Branemark, 2001; Meulenbelt, Geertzen, Jonkman, & Dijkstra, 2009). Simply donning the prosthesis causes a slight increase in skin temperature, which rises markedly during activity (Klute, Berge, Huff, & Ledoux, 2006). It takes doffing the prosthesis for the skin temperature to return to normal because poor thermal properties of the liner and socket severely impede heat transfer. Two local mechanisms that are important for maintaining thermal comfort are thermal perception (sensation of warm or cold) and control of peripheral blood flow (increasing or decreasing to dissipate or preserve heat). Diabetes is the reason for the majority of amputations each year (Dillingham, Pezzin, & Mackenzie, 2002) and has been strongly associated with altered thermoregulation (Wick et al., 2006). The goals of the proposed study are to design and build a research tool to measure thermal perception and blood flow and to use this device to determine if diabetic lower extremity amputees (LEAs) or traumatic LEAs: (1) are less sensitive to

warm or cold and/or (2) have an altered microcirculatory response to a thermal stimulus. We have tested the thermal perception and microcirculatory reactivity of the lateral aspect of the distal residual limb of 2 traumatic LEAs and 3 diabetic LEAs, and the homologous location of 4 healthy, intact controls. We measured local blood flow using a laser Doppler flowmeter (LDF) and warm and cold detection thresholds (WDT, CDT) with quantitative sensory testing (QST). We started at four initial temperatures (30°C, 32°C, 34°C, and 35°C) and used the Method of Limits algorithm with a ramp rate of 0.2 C/s (Divert, 2001; Shy et al., 2003), but modified it by holding the initial temperature and perceived temperature for three minutes to collect blood flow data. A linear mixed effects regression was used to compare the threshold and blood perfusion data by subject group, and a likelihood ratio test was used to distinguish trends in the shape of the variables relationship to initial temperature. The average difference in CDT temperatures between the traumatic LEAs and controls was -1.6°C (SD = 2.6°C) and between diabetic LEAs and controls was -1.8°C (SD = 2.6°C). For the cold test, compared to controls, average change in blood perfusion was 0.5 (SD = 2.2) profusion unit (PU) more and 2.3 (SD = 2.2) PU less for diabetics and traumatic amputees, respectively. The two amputee groups were combined for the WDT and change in blood perfusion because of insufficient power in both groups. For the warm test, average difference in WDT temperatures and change in blood perfusion for the amputees was 2.1°C (SD = 1.2°C) greater and 13.7 (SE 18.0) PU less than the controls. Differences in warm detection thresholds were close to significance (p=0.069). There was insignificant difference in cold detection, vasoconstrictor response to cold stimuli, and vasodilator response to warm stimuli for traumatic LEAs and diabetic LEAs as compared to healthy non-amputee controls. We conclude that amputees may have decreased sensitivity to warm temperature and there was no noticeable trend towards a change in cold perception, vasodilation or vasoconstriction. The implications of these findings may mean that amputees have altered perception of warmth.

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DEDICATION

To my loving and supportive husband, parents and siblings.

Chapter 1. Introduction

Warm and moist skin conditions inside the prosthetic socket may be responsible for thermal discomfort, formation of skin injury, or decreased performance due to a compromised fit of the prosthetic socket on the residual limb. (Hagberg & Branemark, 2001; Legro et al., 1999; Meulenbelt, et al., 2009; Naylor, 1955). Simply donning the prosthesis causes a slight increase in skin temperature, which rises even further with activity such as walking. Unfortunately, the current socket and liner systems are impermeable to moisture and work as thermal insulators by impeding conduction and radiation and completely eliminating convection and evaporation. Blocked heat transfer is compounded by loss of leg surface area consequent to amputation. The result is a buildup of sweat and heat inside the socket, conditions optimal for the development of uncomfortable and even harmful skin problems. The solution to both alleviating heat and allowing a skin injury to heal is often having the amputee take off their prosthesis. Disuse for an extended period of time can adversely affect the amputee's physical, mental, and emotional well-being.

Another avenue of heat transfer is through local thermoregulatory mechanisms. Control of peripheral blood flow helps mediate the rise and fall in body temperature by increasing or decreasing integument blood volume, where the heat can dissipate into the environment. Thermal perception is another powerful thermoregulatory mechanism. Perceiving warm or cold spurs behavioral changes such as putting clothes on or drinking cool water.

Unfortunately, it is unknown if and how the thermoregulatory system is altered by an amputation. With an amputation, vasculature, nerves and tissue are altered from normal states. Furthermore, the limb is exposed to unnatural pressures that may occlude microvasculature or constantly elevated temperatures that could cause subtle neural adaptations. Another compounding factor is the fact that the majority of amputees lose their limb as a result of diabetes, a disease strongly associated with metabolic, cardiovascular, and neurological dysfunction. We hope to better understand changes made to thermal perception or peripheral blood flow by an amputation, diabetes or both.

1.1 Purpose

For amputees, thermal discomfort is an issue inside the prosthetic socket, especially in hot humid summer months. Understanding how amputees perceive warm and cold will help in the design of an effective thermal intervention. Cutaneous blood flow volumes (expressed as perfusion units) are highly dependent on skin temperature. Therefore, measuring cutaneous vascular reactivity, defined as

the perfusion change from a baseline temperature to a perceived temperature, is a good indicator of underlying thermoregulatory health.

For this study we have two aims, each with two parts. The first aim is to understand how thermal sensitivity depends on the initial conditions of the skin for transtibial amputees versus controls. In the first aim we will compare thermal sensitivity of: (1) diabetic amputees to traumatic amputees and (2) diabetic or traumatic amputees to non diabetic intact controls. The second aim is to understand how vascular reactivity depends on the initial conditions of the skin for transtibial amputees versus controls. In the second aim we will compare vascular reactivity: (1) diabetic amputees to traumatic amputees and (2) diabetic or traumatic amputees to non diabetic intact controls.

The information obtained from this study will be used in the future design of a thermally comfortable socket. Without understanding the bounds of thermal perception and vascular reactivity, the design may not accomplish the intended means of noticeably cooling the socket. Additionally, insights of blood flow regulation will be useful in wound healing interventions for diabetic amputees.

Chapter 2. Background

2.1 Amputee Situation

Thermal discomfort and perspiration inside the socket are significant problems for lower extremity amputees (LEAs) (Dudek, Marks, Marshall, & Chardon, 2005; Hagberg & Branemark, 2001; Legro, et al., 1999; Meulenbelt, Dijkstra, Jonkman, & Geertzen, 2006). The most frequently reported problem in an open-ended survey of LEAs was that while silicone liners help suspension, coupling was compromised by sweat (Legro, et al., 1999). Another survey revealed that 72% of LEAs (n=90) reported heat and sweat as sources for a decreased quality of life (Hagberg & Branemark, 2001). It is clear that heat and perspiration are a major concern for amputees. What is unknown is what thermal discomfort means quantitatively for amputees and whether physiological changes make it harder to dissipate heat.

2.1.1 Residual Limb Skin Temperature

In 2006, our lab conducted two studies to assess the effect of activity on residual limb temperatures while in the prosthesis (Klute, et al., 2006). The protocol of the first study consisted of six transtibial, unilateral amputees donning a prosthesis, resting for 60 minutes, walking for 30 minutes on a treadmill at a self-selected walking speed and then resting for an additional 60 minutes. The results (Figure 2.1) showed that residual limb skin temperature elevated from 31.3±0.6°C to 31.7±0.9°C during the initial 60 minutes of rest, a 0.4˚C increase by simply donning the prosthesis. Once on the treadmill, the skin temperature rose rapidly to 33.9±0.9°C, which was a 2.2±˚C increase during the 30 minutes of activity. The temperature apex occurred after the subjects ceased walking and then fell slowly over the final 60 minutes, ending 33.1±0.7˚C, 1.4˚C higher than the pre-walking skin temperature. A 2.2°C increase in skin temperature could have a significant impact on the comfort of the leg and whole body, as previous investigators have found a 2°C change in skin temperature may represent a potentially large disturbance in the body's thermal balance (Savage & Brengelmann, 1996).

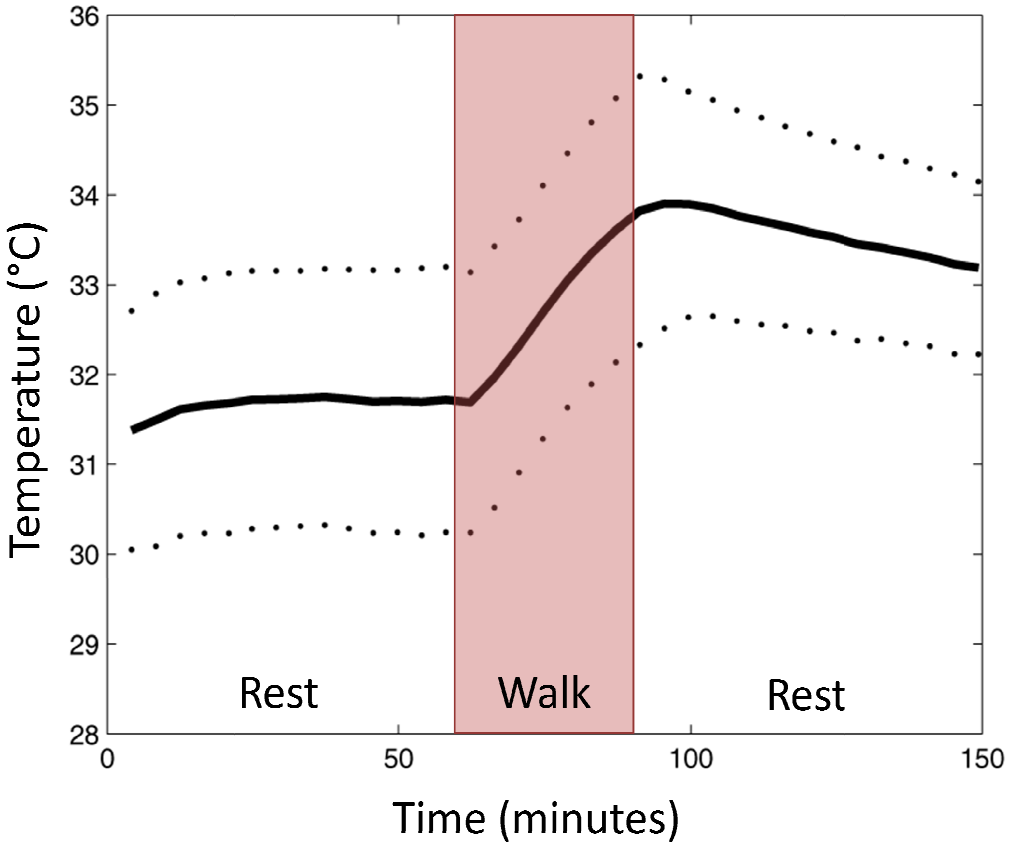

Figure 2.1. Residual limb temperature after donning the prosthesis, adapted from Huff et al., 2008. After donning the prosthesis, amputees (n = 5) rested for 60 minutes, walked for 30 minutes, and rested for an additional 60 minutes. The solid line is the mean skin temperature and the dotted line is the standard deviation.

In the second study, our lab monitored skin temperature inside the prosthesis, environmental humidity and temperature, step activity, and the subject's perceived thermal comfort over an entire day. The four subjects in the study were all left leg transtibial amputees of traumatic etiology. The results (Figure 2.2) showed that there was a strong association between step frequency and increased skin temperature. Skin temperature continued to rise throughout the day, with rest having an insignificant

cooling effect. The mean score for perceived thermal comfort (0 = cold, 5 = just right, and 10 = hot) coincidentally increased throughout the day, which may be associated with skin temperature, environmental conditions, or a combination of both.

The results from the two experiments indicate that residual limb skin temperature of transtibial amputees will naturally elevate when the prosthesis is donned, activity will exacerbate the problem, and skin temperatures increase throughout the day. Short periods of rest appear to be inconsequential to cooling. This explains the current practice for the amputee to sit, remove their prosthesis and wait for their limb to cool. The effect activity has on skin temperature while wearing clothing with poor heat transfer properties has also been demonstrated in a study of 8 young non-amputee men who walked on a treadmill and rested in intervals for 6 hours while wearing combat clothing and body armor (Gibbons, Illigens, Wang, & Freeman, 2010). In this study, the subjects walked for a period of time with a ventilated vest blowing ambient air on the torso and in the other they had no vest. The results showed that the areas that were not covered by the vest had little change in temperature and the chest and back maintained a significantly lower skin temperature under the body armor with the ventilating vest [T_{chest}: 35.33 (1.00)°C; T_{back}35.84 (0.88)°C] than when not wearing the cooling vest [T chest: 37.55 (0.51)°C.. Tback: 36.85 (0.83)°C].

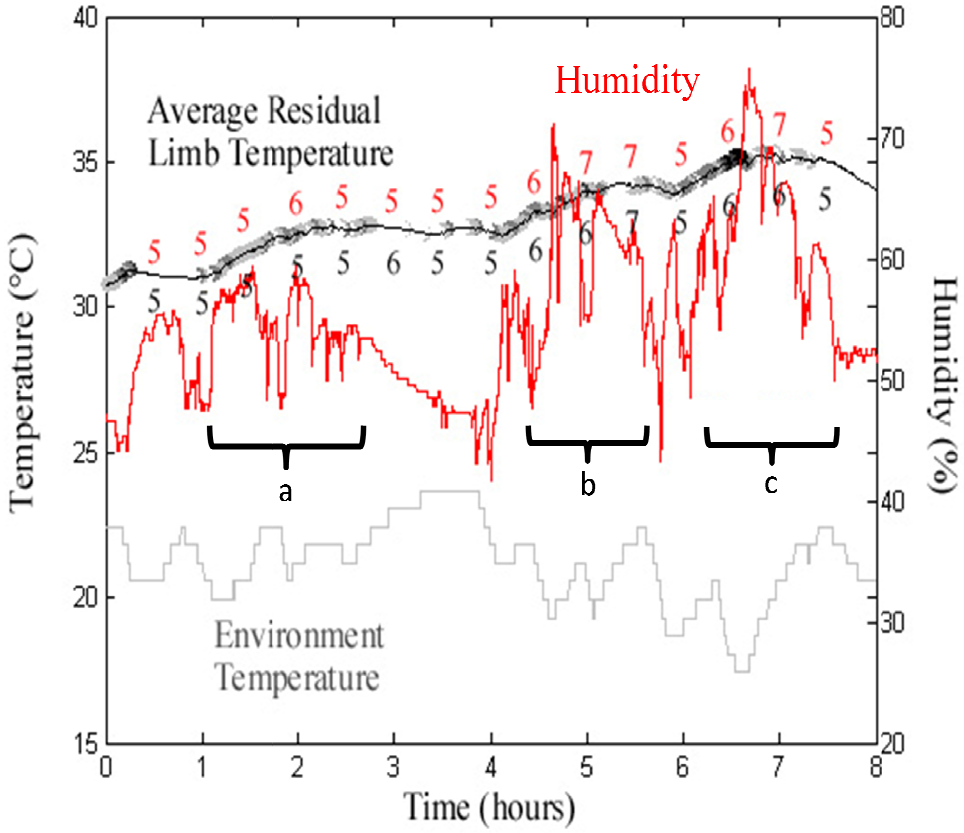

Figure 2.2. Average residual limb temperature of one subject over an 8 hour day, adapted from Klute et al. 2007. The residual limb skin temperature (black line) of one subject is shown to steadily increase throughout the day. Larger increases were observed when step count (black circles = 100 or more steps) was higher (a and b) but seem to plateau around 35°C (c). Whole body (red numbers) and residual limb (black numbers) thermal perception coincidentally increased as the environmental humidity (red line) spiked. The environmental temperature (gray line) had little effect as it stayed relatively consistent throughout the day.

2.1.2 Amputee Skin Injury Associated with Heat and Perspiration

Doffing the prosthesis is also a remedy for skin injury, another common problem among lower limb amputees. A study on amputee skin health (Meulenbelt, et al., 2009) found that 63% of amputees reported having at least one skin problem in the month prior to the questionnaire. In this study, the most frequently reported skin problem was profuse sweating (32%) followed by redness of skin persisting more than 1 minute after removing the prosthesis (29%) and sensitive skin (23%). Another study (Legro, et al., 1999) surveyed the issues of importance for lower limb amputees. One of the four most common issues was the avoidance of blisters and sores on the residual limb (96.7%). A common complaint recorded in the same study was that the adherence of the elastomer liner was compromised by sweat. While it is unclear whether skin redness is associated with thermoregulatory vasodilation, the warm socket environment is likely responsible for profuse perspiration and therefore has a significant impact on the quality of life for amputees.

Heat and moisture trapped inside the socket are anecdotally linked to the high incidence of friction blisters for amputees. Friction blisters result from shear forces that mechanically separate epidermal cells, forming a cleft that subsequently fills with fluid (Sulzberger, Cortese, Fishman, & Wiley, 1966). Investigators studying conditions most conducive to the formation of friction blisters found that the most influential factor is the skin's coefficient of friction (COF) (Naylor, 1955; Sulzberger, et al., 1966). Specifically, the COF tends to increase as the skin becomes more hydrated, being greatest and most prone to blistering in warm humid conditions. At a certain point, the risk of blistering drops because the skin wetness is too great and the skin transitions to a stick-slip behavior (Adams M 2007). As amputees begin to sweat, their risk of blistering increases. However, as the quantity of sweat increases, it no longer has the threat of causing a blister, but instead compromises adherence between the socket and the leg. At this point, the amputee is at risk of their prosthesis falling off.

This information supports hypotheses made by Meulenbelt and colleagues regarding protective and inciting determinants associated with skin injury for lower extremity amputees (LEAs) (Meulenbelt, et al., 2009). In this study, LE amputees (n=805) were surveyed on characteristics of their amputations and prostheses, levels of activity, stump and prosthesis hygiene, and skin problems one month prior to the questionnaire. The study found that older age, amputation caused by diabetes and/or peripheral arterial disease (PAD), and use of prosthesis for more than 8 hours a day indoors were not correlated with skin injury. Diabetes, PAD, and older age have all been linked to decreased sweat production and drier skin (Hachisuka, Matsushima, Ohmine, Shitama, & Shinkoda, 2001).

Some factors that provoke skin injury are washing the stump 4 times a week or more often, use of a liner, and smoking (Meulenbelt, et al., 2009). Warm conditions (the study was performed in the summer) may have evoked sweating and therefore influenced the washing frequency. Liners contribute to elevated skin temperatures and moist conditions by impeding heat and vapor transfer. Consequently, both hyperhidrosis and persistent heat rashes are two reasons to stop using a liner (Lake C 1997). Smoking can cause PAD and microcirculation dysfunction (Freiman A, 2004), which may affect the ability to thermoregulate.

In summary, residual limb skin injuries are probably due to exposure to several unnatural conditions, which makes skin problems a common issue for LE amputees. Profuse sweating and heat inside the socket have been reported as a significant issue of importance, anecdotally linked to the development of friction blisters, and inferentially associated with skin injury. Unfortunately for many, once a skin problem exists the treatment often requires that the amputee limit activity or doff their prosthesis for a period of time. Both remedies are inconvenient and have the potential to adversely impact the amputee's physical, mental and emotional well being (Levy, 1980, 1995; SW, 1983, 1999).

2.1.3 Liner and Socket Thermal Properties

Currently, the standard fitted prosthetic system (**Figure 2.3**) includes a socket, liner and sock. The socket is a custom-fit hard outer shell typically made of thermoplastic or a laminate. The socket is used as a load-bearing component that connects the residual limb with the prosthesis. Amputees typically wear a liner between the socket and their residual limb. The liner is made of a silicone or similar viscoelastic material and acts as an interface to protect the skin from abrasion, add extra cushion, and improve suspension. Socks are a wool or cotton layer occasionally worn between the socket and liner to fill in space when the limb changes size and to provide more cushioning.

The custom fit prosthetic system greatly reduces, if not prevents, all methods of heat transfer. Most modern prosthetic socket layers are impermeable to moisture (Hachisuka, et al., 2001). Similarly, radiation and convection are completely eliminated by the multi-layered solid materials in the system. Therefore, the only method of heat transfer through the prosthetic system is via conduction. A recent bench test study tested the thermal conductivity of 23 liners and 2 socket materials (Klute GK, 2007). Overall, thermal conductivity coefficients were small ranging from 0.085-0.266 W/m-K for liners and 0.148-0.0150 W/m-K for socket materials, meaning both components are poor conductors and instead act as good thermal insulators.

Poor heat and moisture transfer properties of the prosthetic system are likely responsible for uncomfortable and unhealthy conditions inside the socket. Simply donning the prosthesis causes the

skin temperature to rise, possibly due to the fact that heat produced by basal metabolism is greater than heat lost into the environment through the prosthesis. Walking exacerbates the problem, where heat generated by muscle is substantially greater than the diminished heat transfer ability through the prosthesis. Furthermore, the inability of moisture to evaporate through the socket is likely the reason profuse sweating and loss of limb adherence have been reported as a problem amongst lower limb amputees (Legro, et al., 1999; Meulenbelt, et al., 2009). Heat transfer through the prosthetic system is not a viable option at this time; therefore the thermoregulatory mechanisms in the leg must acts as the dominant mechanism for relieving heat. We will begin to explore what role convective blood flow plays in heat transfer away from warm areas of the skin.

Figure 2.3. Prosthetic system typically includes a liner (gray), sock (white), and socket (black).

2.1.4 Perception

Thermal perception is the sensation warm or cold felt when the skin temperature increases or decreases, respectively (Hensel, 1973). These perceptions are mediated by separate neural pathways involving small afferent nerve fibers of the peripheral nervous system (Hensel, 1974). Cold receptors populate the skin only and are both C-fibers and thinly myelinated A delta fibers (faster conduction velocity; reaches brain within one second). The more ubiquitous warm receptors are unmyelinated C-fibers (low conduction velocity; reaches the brain within a few seconds) that populate both core and skin.

Afferent neural pathways can degenerate with disease, degrading sensation to stimuli like temperature, vibration and pain. Diabetic patients with or without symptoms of sensory neuropathy often have increased sensory thresholds (Dyck, Larson, O'Brien, & Velosa, 2000). Results from a study (n=48) performed by Loseth et al. (2008), showed that patients with diabetes mellitus and normal nerve conduction studies had significantly higher thresholds of cold perception on their distal calves than controls, whether they had symptoms of polyneuropathy or not. Another study performed by Shun et al. (2004), examined small-diameter sensory nerve degeneration in human diabetes by measuring sensory thermal thresholds and comparing them with results from nerve conduction studies. They found that 81.6% of diabetic patients had elevated warm threshold (45.9 ± 3.9°C versus 39.3 ± 2.3°C) temperatures on the foot dorsum relative to the control subjects. The abnormal rate of altered cold thresholds in the diabetic group was 57.6% (20.1 ± 1.0°C versus 28.5 ± 1.9°C). Individuals with Type 2 diabetes account 82% of all amputees in the U.S. (Ziegler-Graham, MacKenzie, Ephraim, Travison, & Brookmeyer, 2008). Therefore, a portion of the amputee patient population may not detect skin temperatures that would elicit a protective behavioral response, such as doffing the prosthesis when warm for a prolonged period of time. On the other hand, the neuropathic diabetic amputee population may not be good candidates for a cooling sockets, as they may not perceive and be bothered by thermal discomfort or the accompanied cooling.

Thermal thresholds have been studied as a means to understand phantom limb pain and nerve degeneration in the residual limb. In a large study (n=73 traumatic amputees), desensitization to temperature was noted in the residual limb of each amputee (Carlen, Wall, Nadvorna, & Steinbach, 1978). Thermal thresholds were assessed between the distal residual limb (DRL) and intact contralateral limb in two independent studies. The first, which tested upper extremity amputees, found some individual differences but no statistically significant differences for any of the thermal thresholds (Hunter, Katz, & Davis, 2005). The second found that lower limb amputees (n=42) had

significantly reduced thermal thresholds for cold perception relative to the intact contralateral limb (Harden, Gagnon, Khan, Wallach, & Zereshki, 2010). There were no noticeable differences in thresholds for warm perception, hot pain or cold pain.

2.2 Thermoregulation

Thermoregulation is the ability of an organism to stay within a thermally comfortable zone, regardless of environmental factors. During exercise or heat exposure, increased internal or skin temperatures elicit an amplified cutaneous blood flow (vasodilation) and sweating response vital to heat dissipation. Conversely, the response to cold exposure and falling body temperatures is vasoconstriction and shivering, essential heat preservation and generation, respectively. Changes in skin temperature also give rise to the thermal sensations warm and cold. These thermal perceptions are the impetus for behavioral change, a very powerful form of thermoregulation. Therefore, altered thermal perception and control of skin blood flow can substantially impair the ability to maintain normal body temperatures.

Type 2 diabetes mellitus (T2DM) is strongly associated with thermoregulatory impairment through metabolic, cardiovascular, and neurological dysfunction (Caballero et al., 1999; Johnstone et al., 1993; Veves et al., 1998). Epidemiological data from two heat waves revealed that individuals with T2DM are more susceptible to heat illness (heat stroke, heat exhaustion) when exposed to elevated ambient temperatures compared to their normal counterparts (Schuman, 1972; Semenza, McCullough, Flanders, McGeehin, & Lumpkin, 1999). These findings suggest a serious impairment in the ability of individuals with T2DM to regulate heat.

In recent years, the incidence of Type 2 diabetes mellitus (T2DM) has reached epidemic proportions (Zimmet, Alberti, & Shaw, 2001) and accounts for 82% of the amputee population in the United States (Dillingham, et al., 2002). With an amputation, vasculature, nerves and tissue are altered from normal states. Additionally, a substantial amount of surface area essential for heat dissipation has been lost or covered by a prosthetic system. It is known that non-vascular transfemoral amputees are sensitive to heat and sweat inside the socket (Hagberg & Branemark, 2001).

2.2.1 Skin Blood Flow Response

Peripheral blood flow is the main effector of the thermoregulatory system. The body takes advantage of circulatory convection to transfer heat from the core to the periphery and out to the environment. In thermoneutral environments, cutaneous blood flow level is approximately 5% of the cardiac output (~250 ml/min), equivalent to ~80 to 90 kcal/hr of heat produced during basal metabolism (Johnson

JM, 1996). During heat stress, elevated temperatures cause the blood flow level to increase substantially, using as much as 60% of the cardiac output over the whole body. Conversely, during cold stress, depressed temperatures lead to near zero levels of blood flow to the skin.

2.2.2 Cutaneous Vasodilation

Local warming of the skin elicits a biphasic response involving two independent mechanisms (Figure 2.5); a rapid initial peak in cutaneous blood flow that last for the first 3-5 minutes, followed by a plateau lasting 25-30 minutes. The initial phase relies primarily on local sensory nerves (Minson CT 2001; Arildsson M 2000) and is mediated by an axon (local) reflex that depends on two potent vasodilators: calcitonin gene-related peptide and substance P (Johnson JM 2010) Charkoudian N 2001; Minson CT 2001). The plateau that follows is mediated largely by nitric oxide (NO), a vasodilator produced in endothelial cells (Minson CT 2001; Kellogg DL Jr. 1999). The thermal hyperemia response is a sustained increase in skin cutaneous blood flow that achieves maximal vasodilation between 42°C and 44°C (Charkoudian N 2003). This maximal thermal vasodilation corresponds to a large diversion of a cardiac output to the skin, 80-90% of which is controlled by the active vasodilator system (Charkoudian N 2003). Therefore, impairments in control of the active vasodilator system could substantially inhibit the ability of the skin circulation to appropriately respond to whole body hyperthermia.

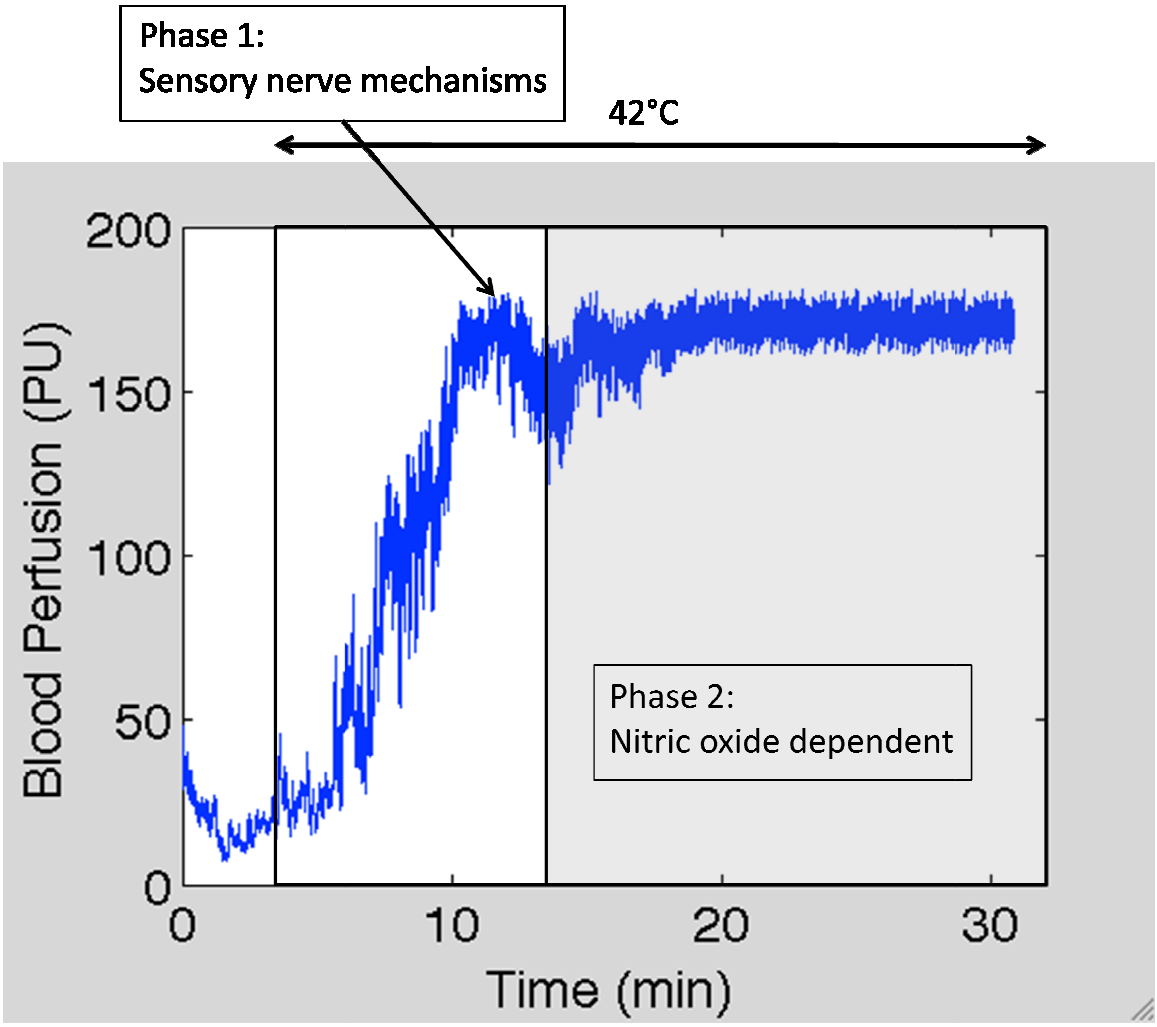

Figure 2.4. Cutaneous vasodilator response to 30 minutes of local warming. The first phase of vasodilation is mediated by local sensory nerves, while the second phase depends on nitric oxide.

The most common complication for diabetics is damage to the microcirculation and endothelial cells (Caballero, et al., 1999; Rendell & Bamisedun, 1992). Endothelial dysfunction can reduce the body's ability to produce and/or respond to NO, which results in an attenuated overall vasodilatory response at a given core temperature (Sokolnicki et al., 2009). For individuals with type 2 diabetes mellitus (T2DM), it has also been shown that the body exhibits a delayed onset of cutaneous vasodilation during whole body heating (Wick, et al., 2006). These alterations may have a profound effect on thermoregulation, which in itself is associated with morbidity and mortality in patients with T2DM.

It is unclear if and how the vasodilator response is changed for amputees. In 2008, Harden at al. compared the heat emitted from the surface of both the bare residual limb and contralateral intact limb of amputees with traumatic, dysvascular, and other amputation etiologies (n=36) using quantitative infrared telethermography (qIRT) (Harden, Gagnon, Gallizzi, Khan, & Newman, 2008). The investigators found the mean residual limb temperature (30.4°C [SD = 1.6]) significantly lower than the mean intact contralateral limb temperature (31.5°C [SD = 1.5]). No noticeable differences were found between the intact and amputated limb temperature of dysvascular amputees, although the sample size was too small to draw a conclusion. The results are an indirect indication of altered sympathetic and local vasoactive tone (Harway, 1986; Uematsu, Hendler, Hungerford, Long, & Ono, 1981). Unfortunately, the pathophysiological relevance of the temperature difference is unknown (Kristen, Lukeschitsch, Plattner, Sigmund, & Resch, 1984). Our research will help elucidate changes vasodilator responsiveness.

2.2.3 Cutaneous Vasoconstriction

The vasomotor responses to local cooling do not have the same distinctive phase demarcations as the response to local warming (Minson, Berry, & Joyner, 2001). The initial response is mediated by reflex vasoconstriction via norepinephrine and cotransmitters (Johnson JM, 1996; Stephens, Aoki, Kosiba, & Johnson, 2001; Thompson-Torgerson, Holowatz, & Kenney, 2008), causing the initial rapid drop in blood flow. When cold-sensitive afferent nerves sense low skin and/or internal temperatures, they affect the release of norepinephrine from sympathetic active vasoconstrictor nerves (Kellogg, 2006). Sustained local cooling causes a prolonged vasoconstriction through the inhibition of the NO system (Johnson & Kellogg, 2010).

Alterations in reflex cutaneous vasoconstriction function in diabetes remain unclear. Impaired vasoconstriction was noted in diabetics with autonomic neuropathy during prolonged whole body cooling, 16°C for 45 minutes or less, particularly in the foot, calf and forearm (Scott, MacDonald, Bennett, & Tattersall, 1988). No differences were observed between diabetics without neuropathy and nondiabetic controls. In the same study, cooling caused core temperatures to rise for the non-neuropathic diabetics and controls but either stabilized or fell for neuropathic diabetics. Relatively healthy individuals with T2DM also showed no difference in vasomotor response during baseline temperatures and rapid whole body cooling (10-12 °C for 3-4 minutes) as compared to the nondiabetic controls (Strom et al., 2011). This observation partially contrasts with results produced by Wick et al. (2006), who found higher cutaneous vascular conductance (blood perfusion over blood pressure) at rest compared with controls but similar conductance during 3 minutes of whole body

cooling. So far, it has been shown that relatively healthy diabetics show insignificant differences in vasoconstrictor response to cooling. It is unclear how tonic vasoconstriction is affected by diabetes and how vascular dysfunction progresses with more severe cases of the disease (Charkoudian, 2010).

As noted earlier, the residual limb of an amputee is significantly cooler than the contralateral intact limb when no prosthesis is worn. One theory that explains this alteration is an increased sensitivity of vasoconstrictor responsiveness with respect to skin temperature in the residual limb (Harden, et al., 2008)). Conversely, skin nerve sympathetic activity (SSA) during whole body cooling (14-16°C) showed abnormalities which suggest indirect inhibitory influences on cutaneous vasoconstrictor nerves (Fagius, Nordin, & Wall, 2002). Unfortunately, the SSA readings were only performed for one of the six subjects participating in the study. The reason is that the investigator could only find the peroneal nerve at the fibula head in the most recent amputee (2.5 year post-amputation). This supports the theory of a differential peripheral neuropathy of the distal residual limb (DRL) (Harden, et al., 2010). Our research will help explore the theory of dying back by testing vasoconstrictor response.

2.3 Summary

Elevated residual limb skin temperatures inside the socket are sources of thermal discomfort and likely responsible for profuse sweating, both reported as significant problems for lower limb amputees (Hagberg & Branemark, 2001; Meulenbelt, et al., 2009). Simply donning the prosthesis causes a slight increase in skin temperature, which rises markedly during activity (Klute, et al., 2006). (Klute, et al., 2006) These effects accumulate throughout the day and result in a substantially warmer residual limb at the end of the day. It takes doffing the prosthesis for the skin temperature to return to normal because poor thermal properties of the liner and socket severely impede heat transfer. In addition, heat inside the socket is likely responsible for profuse sweating, the most frequently reported skin problem among lower limb amputees (Meulenbelt, et al., 2009).

Two local mechanisms that are vital for maintaining thermal comfort are thermal perception (sensation of warm or cold) and control of peripheral blood flow (increasing or decreasing to dissipate or preserve heat). Diabetes is the reason for the majority of amputations each year (Dillingham, Pezzin, & Shore, 2005) and has been strongly associated with altered thermoregulation (Wick, et al., 2006). Additionally, vasculature, nerve and tissue are changed from their normal states because of the amputation itself. Therefore, little is known about how an amputation and diabetes effects thermal perception and peripheral blood flow control.

For this study we have two aims. The first aim is to understand how thermal sensitivity depends on the initial conditions of the skin for transtibial amputees versus controls. The second aim is to understand how vascular reactivity depends on the initial conditions of the skin for transtibial amputees versus controls. In the second aim we will compare vascular reactivity: (1) diabetic amputees to traumatic amputees and (2) diabetic or traumatic amputees to non diabetic intact controls.

Chapter 3. Thermode

3.1 Introduction

Thermal perception and vascular reactivity are two useful indictors of overall thermoregulatory health because they are relatively easy noninvasive tests (Holowatz, Thompson-Torgerson, & Kenney, 2008; Minson, 2010). Thermal perception is conventionally measured by finding the boundary (detection threshold) at which the individual goes from not feeling to feeling a thermal stimulus. Vascular reactivity is the magnitude and rate of the change in cutaneous blood perfusion in response to changing skin temperature.

A common tool to measure thermal perception is the thermode, a Peltier driven device designed to provide a noxious or non-noxious thermal stimulus over a range of 5-45°C. Numerous studies have used commercially available thermodes to assess and quantify sensory function in patients with neurological symptoms or those at risk of developing neurological diseases (Harden, et al., 2010; Løseth, Stålberg, Jorde, & Mellgren, 2008; Shun et al., 2004; Shy, et al., 2003). Typically, the thermode is placed on the targeted skin area and temperature is increased or decreased until the subject indicates feeling warm or cold, respectively. Abnormal nerve function is evaluated as significantly different warm and/or cold threshold values.

The work described in this thesis expanded on the standard thermode design by adding the ability to measure vascular reactivity. A laser Doppler flowmeter was embedded in the center of the temperature-changing surface to measure peripheral blood perfusion and calculate vascular reactivity. This allows us to accomplish our goals of the study, which is to measure the differences in thermal perception and microcirculatory response to thermal stimulus applied to the residual limb of traumatic and diabetic lower limb amputees.

3.2 Blood Flow Response and Thermode Design

Microvascular reactivity was measured using laser-Doppler flowmetry (LDF), which has been a widely used research and clinical tool to investigate skin microcirculation (Flynn, Edmonds, Tooke, & Watkins, 1988; Nilsson, Tenland, & Oberg, 1980; Rayman et al., 1986). The operating principle of this instrument is that monochromatic laser light undergoes a frequency shift (Doppler shift) when reflected off of blood cells moving at various speeds. Frequency and magnitude distributions of the returning wavelengths are directly related to the number and velocities of blood cells in the sample volume (Nilsson, et al., 1980). The reflected light is picked up by a returning fiber, analyzed and

expressed as a perfusion unit (PU). Blood flow data was collected with the PeriFlux System 5000 (Perimed, Järfälla, Sweden) at a sampling rate of 32 Hz. Before testing each subject, the instrument was calibrated using the Motility Standard provided by the manufacturer.

The exploded and assembled views of the thermode probe are provided in **Figure 3.1.** The thermode operates using the Peltier effect, converting voltage into a temperature difference. Power is fed into the Peltier element (CH-21-1.0-0.8; TE Technology, Inc, Traverse City, MI) by a remotely controlled power supply (1786B 0-32V, 0-3A; B&K Precision, Yorba Linda, CA) connected to a computer with a communication cable (USB Communication Cable IT-132; B&K Precision, Yorba Linda, CA). One side of the Peltier element is kept at a constant temperature by a water block connected to a liquid cooler with aluminum fins, thermoelectric plate and electric fan. The opposite side of the Peltier element (skin interface) becomes warmer or colder when power is applied, depending on the polarity of the voltage.

Polarity of the Peltier voltage is controlled by two single-pole double-throw (SPDT) electrical relays that are switched by a digital output voltage supplied by the data acquisition board. There is a positive voltage drop across the Peltier element for the each initial condition. The polarity flips for the cold ramp and threshold and returns back to positive when the test is over.

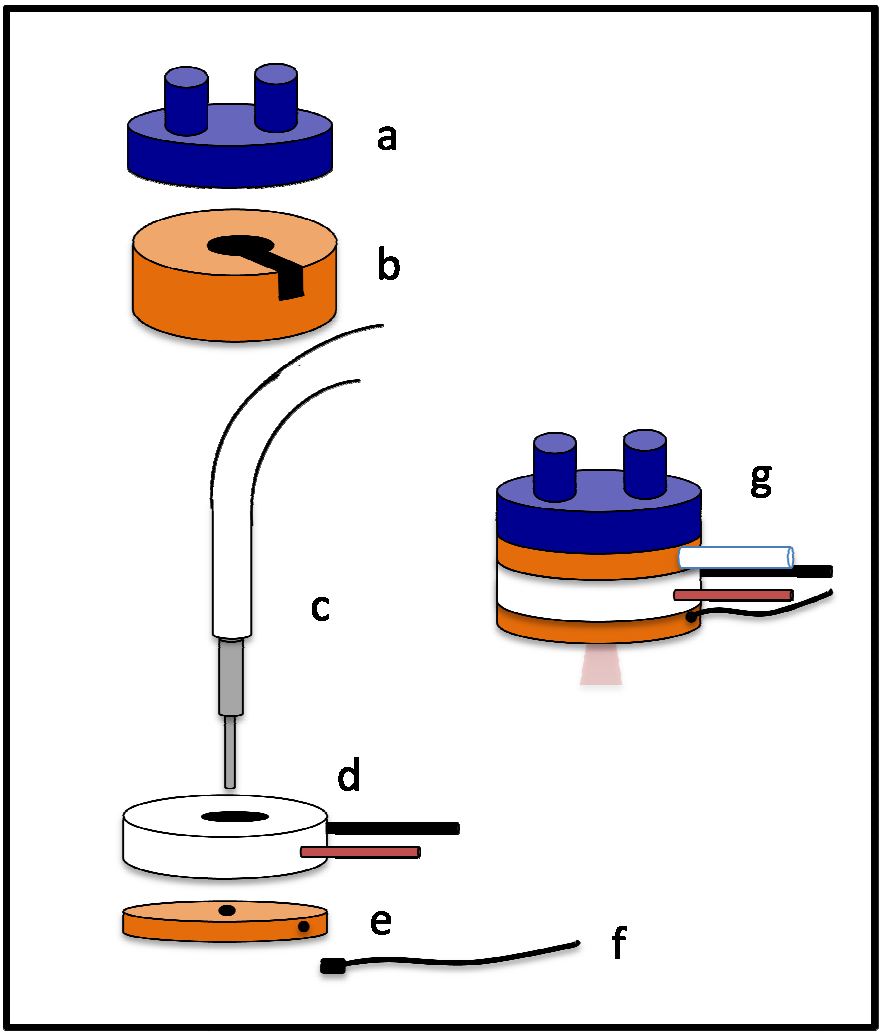

Figure 3.1. The exploded and assembled view of the thermode probe. The waterblock (a) and a piece of copper (b) that houses the laser Doppler flowmeter (LDF) (c) act as the heat sink for the Peltier chip (d). The other side of the Peltier chip heats up the copper interface (e) that rests on the skin during testing. Surface temperature of the probe is measured by a thermistor (f) embedded in the copper. The LDF goes in the middle of the Peltier chip and sits flush in a hole in the center of the copper. The assembled view (g) shows the laser beam below the probe.

As seen in **Figure 3.2** the power supply is controlled by a custom designed LabVIEW program. Voltage outputs are updated at a 5 Hz rate using hexadecimal commands. Temperature is relayed back to the program using a rice-sized thermistor (0.8 mm diameter, 9.5 mm length, 38-gauge wire, model MA100; Thermometrics, Edison, NJ) embedded in a thin (1.5 mm thick) copper interface between the Peltier element and skin. A 9.1 kΩ 5% precision resistor is in series with the thermistor to create a voltage divider as a way to determine the resistance of the thermistor, a variable resistor. The voltage drop across the thermistor and the entire circuit is sampled at 5 Hz with a data acquisition board (NI USB-6251 DAQPad; National Instruments, Austin, TX). The same thermistor configuration is used to measure skin temperature.

The thermode temperature (TT) sampled by the computer is compared with the desired set-point temperature (SP) produced by a computer algorithm. A proportional-integral-derivative (PID) controller calculates an error value (e):

$$e = SP - TT \tag{1}$$

based on the difference between the desired and actual temperature. The PID controller attempts to minimize the error by using the accumulation of the magnitude and duration of the past error, present error (e(t)), and the rate change (predicted future) of the error . The three terms are scaled and summed up, as shown in the equation below:

$$u(t) = K_p e(t) + K_I \int_0^t e(t)dt + K_D \frac{d\,e(t)}{dt} \tag{2}$$

where K_P is the proportional gain, K_I is the integral gain, and K_D is the derivative gain. The controller output (u) is augmented by a feed-forward temperature (T) dependent control variable (CV) to increase the speed of the response. The equation below represents the PID algorithm feedback plus feed-forward term:

$$u(t) = K_p e(t) + K_I \int_0^t e(t)dt + K_D \frac{d\,e(t)}{dt} + CV(T)\,. \tag{3}$$

Large swings in the output of the PID controller were clipped with a saturation equation

$$v(t) = \frac{1}{1 + e^{-0.05u(t)}} \tag{4}$$

where v(t) is the effective control signal.

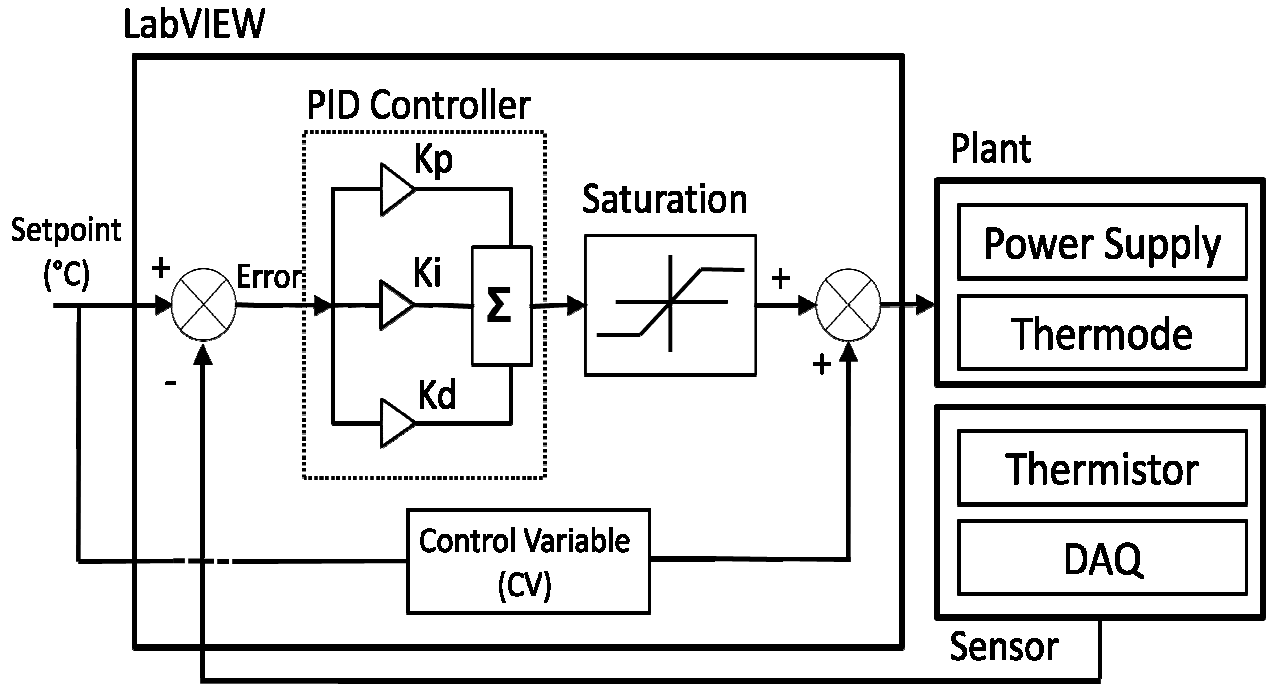

Figure 3.2. Thermode controller schematic diagram. The test operator sets an initial setpoint temperature into a computer program written in LabVIEW. The difference between the setpoint and the temperature at the surface of the thermode (error) is calculated and the output is filtered for noise with a saturation filter. A control variable is added to the controller output and the sum is communicated to the remotely controlled power supply as a new voltage.

3.3 Methods

The accuracy and responsiveness of the thermode temperature was directly dependent on the performance of the PID controller. Tuning the controller involved setting the K_p, K_I, and K_D gain values to get the best possible response to a step input for each initial condition. This was done by first setting the K_I and K_D values to zero and manipulating the K_P value. When the controller response was quick and still stable, the K_I value was adjusted to reduce the settling time. Finally, the K_D was adjusted to help decrease the overshoot. For the second set of tests, a trigger was added to turn the controller on within 0.5°C of the set point. The purpose was to reduce the error term as the temperature climbed from the water block temperature (20°C) to the initial temperature. We assumed that the residual limb has a similar thermal inertia as a large volume of water hence all tests were performed on a 6 liter MSR® Dromedary™ hydration bag with nylon shell.

The performance of the controller was assessed by analyzing the transient response, stability and steady state error for the eight conditions; four initial temperatures (30°C, 32°C, 34°C, and 35°C) for both warm and cold testing. Overshoot, rise time, settling time and transient error were parameters used to assess the transient response. The transient error is the accumulated difference between the thermode and setpoint temperatures during the final two minutes of the response. The final two minutes of the response were of interest to reach a steady-state temperature that best represents the blood flow response. Hence, the transient error (TE) was calculated over the last two minutes of the step input with the equation

$$TE = \sum_{t=60}^{180} abs(SP - TT) \tag{5}$$

where t is time (seconds) and SP and TT are the setpoint and thermode temperatures, respectively. Stability of the system was defined as the thermode temperature converging to the setpoint temperature. The steady-state error was found by taking the absolute difference between the setpoint and thermode temperatures at three minutes.

3.4 Results

The response had sustained oscillations when K_P was equal to 200 (unitless) and K_I and K_D were set to zero. We discovered that setting the K_P term at values equal to or below 100 produced stable, converging responses. As shown in **Figure 3.3a**, while the temperature response has substantial ringing when K_P is equal to 100 (K_I and K_D equal 0), the system is damped and converges to 32°C.

Rise time, settling time, overshoot, peak, steady-state error, and transient error were calculated for a variety of gain term combinations for each initial temperature. The results for the initial temperature of 32°C are shown in **Figure 3.3** and **Table 3-1**. The proportional term (K_p) had the greatest impact on performance, followed by the integral term (K_I) and then the derivative term (K_D). The settling time and steady-state error increased as the K_P term got larger. In general, the peak, percent overshoot and transient error all increased with increasing K_P values, although each parameter was greatest at a K_P equal to 50.

The results from tuning the K_P term led us to make two decisions: (1) to use 25 as the K_P value and (2) to find a way to reduce the error term from the initial step and in turn reduce the settling time. Therefore a trigger was added that switched the PID controller on when the thermode temperature reached within 0.5°C of the setpoint temperature. The function of the trigger is discussed in more detail later in this chapter but it should be pointed out that the K_I and K_D terms were determined with the new mechanism.

As expected, increasing the K_I term decreased the rise time and peak temperature. Settling time, steady state error and the transient error were lowest at a K_I value of 2 s, which became our new value. The final step was to adjust the K_D value. The largest K_D value (128 s) resulted in the best settling time, but produced large steady state and transient errors. The only two tests to produce unacceptable steady state errors were when K_P equaled 50 or 100. Therefore, the gains were set at 25 NU, 1 s, and 8s for K_P, K_I, and $K_{D,}$ respectively.

Table 3-1. Performance of temperature response while tuning the proportional-integral-derivative controller to a step input to 32°C. Proportional (K_P), integral (K_I), and derivative (K_D) gains were varied to determine the best combination for the optimal performance. The best performance was determined by finding the smallest rise time, settling time, percent overshoot, steady state error and transient error.

Gains			Rise Time (s)	Settling Time (s)	Overshoot (%)	Peak (°C)	Steady State Error (°C * 10^{-2})	Transient Error (°C)
K_P (NU*)	K_I (s)	K_D (s)						
25	0	0	22.2	111.6	4.35	33.39	5.8	185.3
50	0	0	13.2	120.6	11.12	35.56	26.6	412.7
100	0	0	8.4	NaN	7.98	34.55	45.1	378.1
25	0	0	10.6	60	1.95	32.63	5.3	119.1
25	2	0	6.6	54	2.27	32.73	3.6	77.0
25	4	0	6.6	73.8	2.11	32.68	7.2	107.4
25	2	8	6	52.8	2.27	32.73	0.1	98.4
25	2	32	6.6	73.8	2.11	32.68	7.2	107.4
25	2	128	8.4	49.2	2.29	32.73	4.0	98.8

*No Units

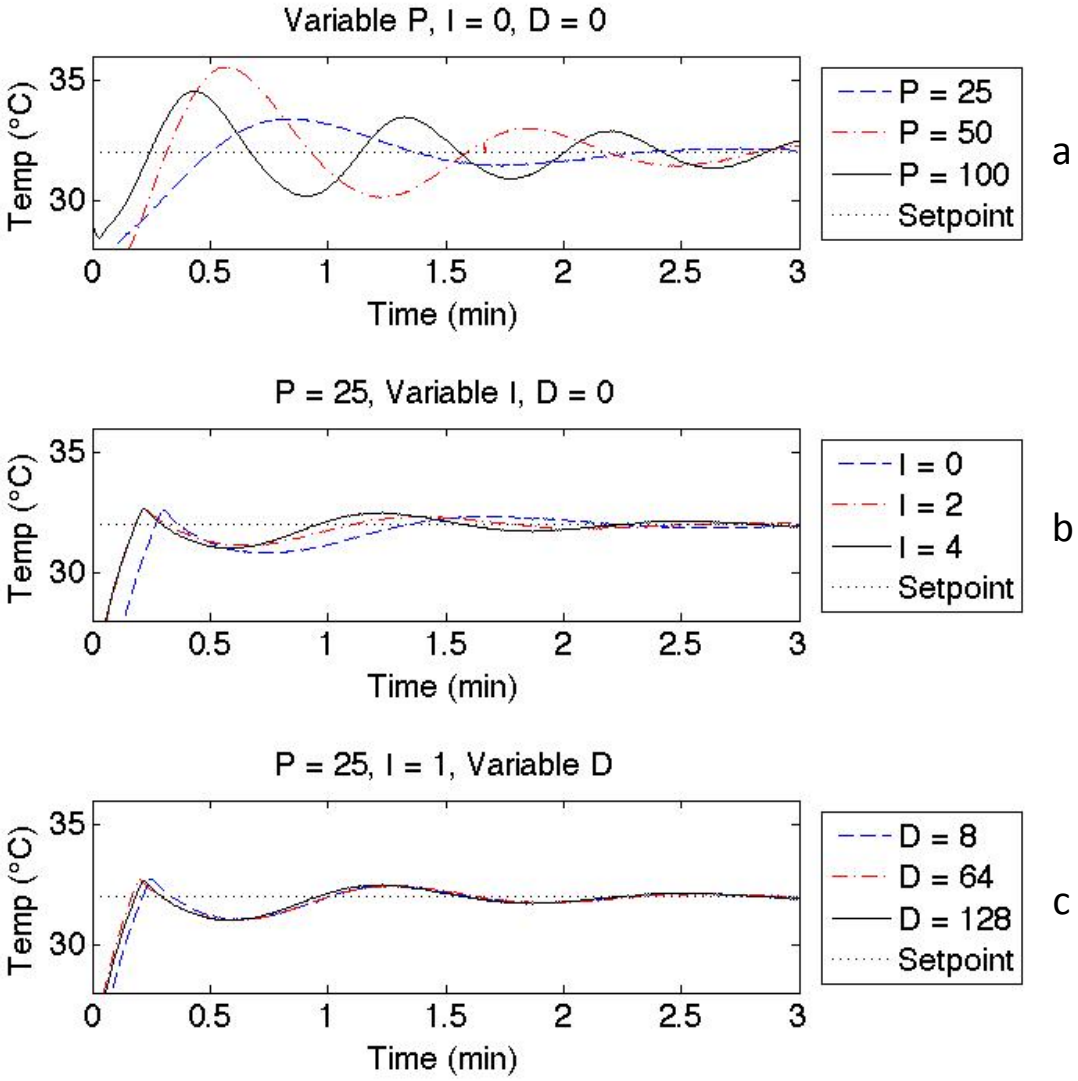

Figure 3.3. Thermode temperature response to a step input of 32°C while tuning the proportional-integral-derivative controller. The three gains (K_P, K_I, and K_D) were determined by analyzing the thermode temperature response to a step input of 32°C. Parameters that characterize the transient response were calculated for each response while varying the K_P term (a), the K_I term (b), and finally (c) the K_D term. The gains associated with the quickest and most accurate response were chosen for the remainder of the tests.

After tuning the proportional term (K_P), it was evident that the response time was too slow and the transient and steady state errors were too great. Therefore, a trigger mechanism was added to improve the program and controller efficiency. The input into the Peltier chip was set to maximum voltage (1.5 V) when the test began, which caused the temperature to increase rapidly. Once the thermode temperature reached within 0.5°C of the setpoint temperature, the program added the PID controller to bring the thermode temperature to the setpoint temperature. The results (**Table 3-2**) show that by initiating the PID controller when the thermode temperature reached within 0.5°C of the setpoint temperature, each aim was accomplished. It is evident that the transient error of the temperature responses (**Figure 3.2**) is much lower when the controller begins within 0.5°C of the setpoint temperature. The transient error is reduced by 35.5% when K_P = 25 and 74.2% when K_P = 50. The biggest improvements in settling time, overshoot, and peak temperature were also observed when K_P equaled 50. Peak temperature was reduced by 3.6°C, settling time improved by a minute and 40 seconds, and percent overshoot dropped from 13.9% to 2.5%. The same trends were observed when K_P = 25. The only exception to the general optimized performance was that the steady state error was larger when the trigger was initiated and K_P equaled 50. However, the temperature was 0.11°C higher than the setpoint temperature, which does not represent a larger error.

Table 3-2. Performance of thermode temperature response when the proportional-integral-derivative controller is engaged from the beginning of the test or initiated within 0.5°C of the setpoint temperature.

Trigger	Gains			Rise Time (s)	Settling Time (s)	Overshoot (%)	Peak (°C)	Steady State Error (°C * 10^{-2})	Transient Error (°C)
	K_P (NU*)	K_I (s)	K_D (s)						
Off	25	0	0	22.2	111.6	4.35	33.39	5.8	185.3
	50	0	0	11.7	167.7	13.9	36.4	0.9	624.6
On	25	0	0	10.6	60	1.95	32.63	5.3	119.1
	50	0	0	9.5	68.4	2.5	32.8	11.1	160.9

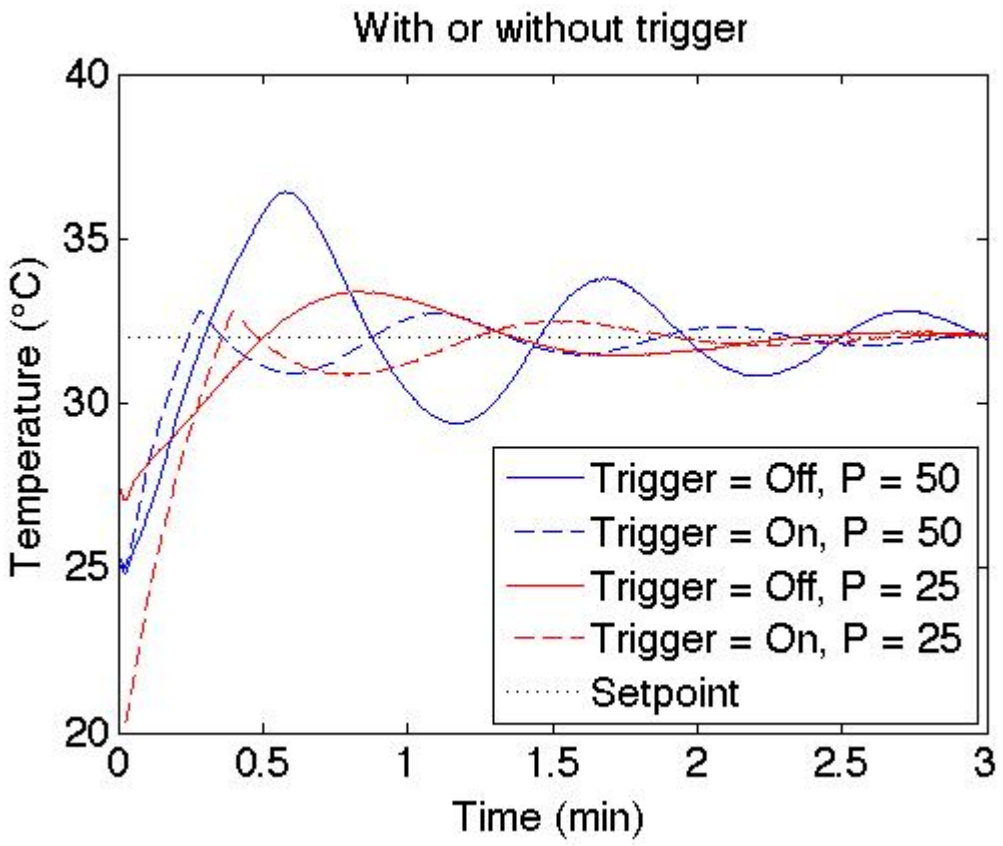

Figure 3.4. Thermode temperature response to a step input of 32°C while testing the mechanisms that switches the proportional-integral-derivative (PID) control on or off. The trigger is a switch inside the LabVIEW™ code that turns the PID controller on when the thermode temperature reaches within 0.5°C of the setpoint temperature. The thermode temperature response was measured for both conditions, with and without the trigger initiated. The starting temperature was not controlled, which may affect results.

Finally, the performance of the PID controller was assessed for each initial condition with K_P, K_I, and K_D gain terms of 25, 2 seconds, and 8 seconds, respectively. The results (**Table 3-3**) show increasing rise time and settling time as the initial temperature gets larger. The most noticeable increase in settling time occurs between 32°C and 34°C, likely related to the longer rise time. Overall, each response settled in a sufficient amount of time with little transient error, as seen in **Figure 3.5**.

Table 3-3. Thermode performance with varying setpoint temperatures.

Temperature (°C)	Rise Time (sec)	Settling Time (sec)	Overshoot (%)	Peak (°C)
30	8.5	17.0	2.2	30.7
32	9.6	22.8	3.3	33.1
34	19.4	54.5	2.6	34.9
35	7.6	56.5	0.9	35.3

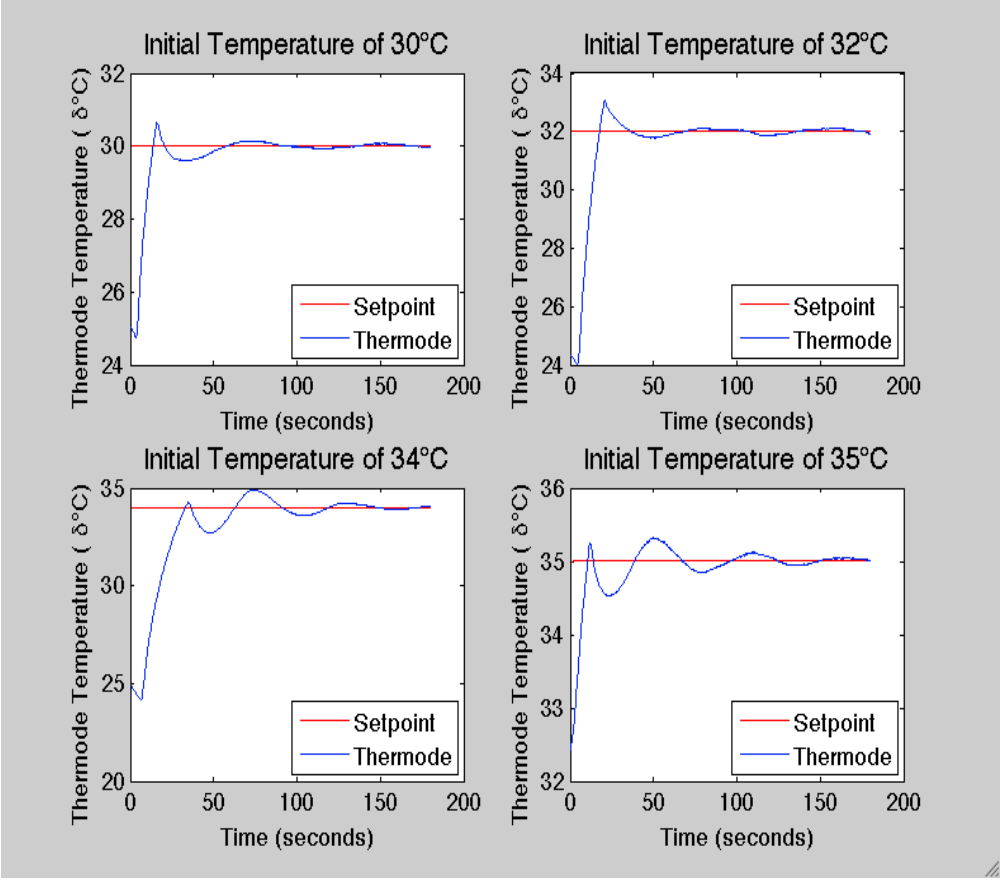

Figure 3.5. Thermode temperature performance for each of the initial temperatures and trigger mechanism engaged.

3.5 Discussion

The tuning results for the PID controller revealed that the thermode reaches the set point temperature the quickest when the K_P, K_I, and K_D terms are 25, 2 seconds, and 8 seconds, respectively. Of the gains, the proportional term (K_P) had the greatest impact on the performance, followed by the integral term (K_I) and finally the derivative term (K_D). The trigger function, which turned the PID controller on when the thermode temperature reached within 0.5°C of the setpoint temperature, had the greatest impact on the controller performance by reducing the settling time up to 74% and improving all transient response parameters. Finally, the performance of the thermode improved as the setpoint temperature decreased, being greatest for the two lower initial temperatures, 30°C and 32°C, as there were slightly smaller rise and settling times.

There were several limitations to this study. First, manual tuning was time consuming, which made waiting for the thermode to find a steady-state temperature between trials difficult. Therefore, the results described above do not reflect true comparisons because the step input varied slightly for each trial. Once the trigger mechanism was added, each response was consistently assessed for a step input that increased by 0.5°C, which eliminated problems associated with variable inputs. Secondly, the water bath did not account for the convective heat transfer provided by cutaneous blood flow at the testing site. When tested on a human subject leg, the thermode was less accurate and slower at higher temperatures. The reason is that the vascular system works as its own temperature controller, increasing blood flow where a local area is warmed to carry heat away via convection. Therefore, we adjusted the proportional gain to equal 100 to improve accuracy and decrease settling time. A few samples of the temperature response with the new proportional gain are shown in **Figure 3.6** and **Appendix A** (**Figure A.1** and **Figure A.2**). Finally, it was difficult to measure for all of the setpoint temperatures reached in the human subject tests. The final setpoint the thermode had to reach was dependent on how sensitive the subject was to warm or cold. As a result, the thermode reached temperatures as high as 42.2°C for warm detection and as low as 22.1°C for cold detection tests. Therefore, the use of the trigger mechanism and feed-forward term were critical to the performance of the thermode over a wide range of temperatures.

The optimal behavior of the PID controller was to track the test algorithm temperature (3 minutes initial, ramp, and 3 minutes final (**Figure 3.6**)) with little transient error and overshoot and a quick settling time. The goal was to achieve a steady state temperature within the first minute of the initial and final setpoint because the last two minutes of each stage were used to calculate blood flow. Large oscillations in the skin temperature would likely cause a dynamic blood flow response that could be

misinterpreted as a significant transient error in the perfusion data. Quick settling times were easy to achieve at lower setpoint temperatures, but the large step change to higher temperature (34°C and 35°C) never settled. Therefore, a trigger mechanism was added to reduce the error term from the initial step.

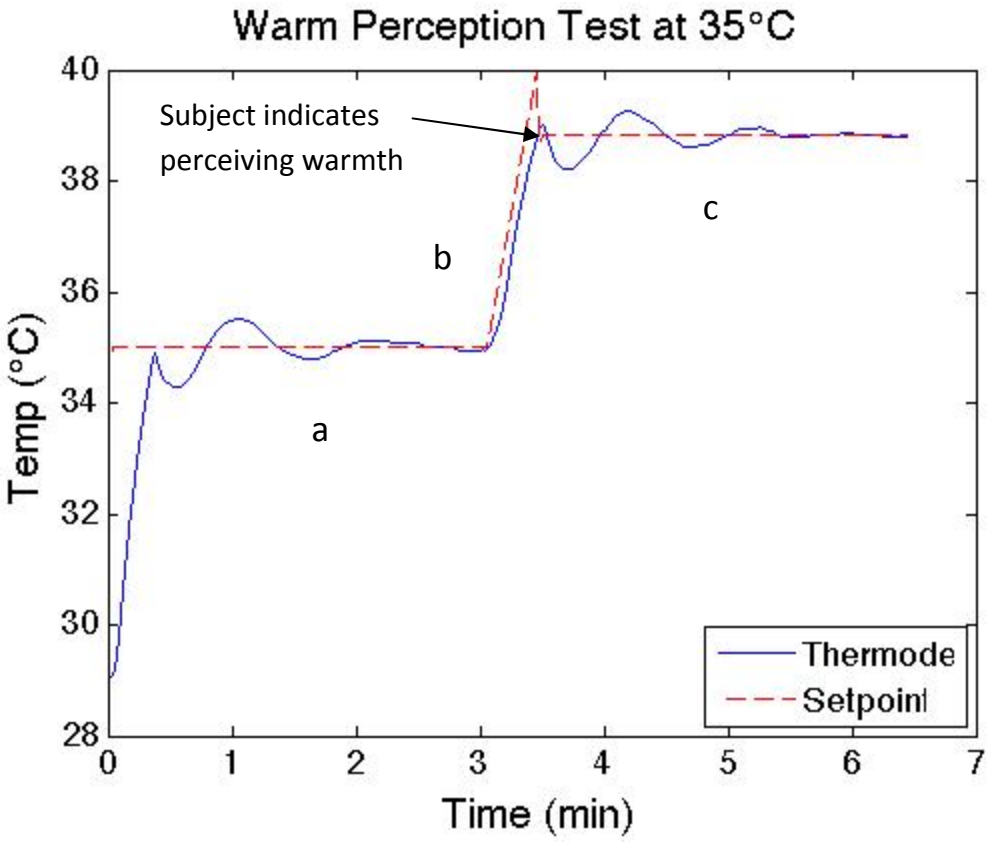

Figure 3.6. A sample performance of the thermode performing a full test. The test algorithm consists of a setpoint temperature (red dashed line) being set to (a) an initial temperature for three minutes, (b) the temperature ramping up or down, and (c) the subject pressing a button which holds the new perceived temperature for an additional three minutes. The optimal thermode response (blue solid line) is to track the setpoint with as little error as possible.

As stated above, the trigger mechanism was added to reduce the initial error (difference between the setpoint and thermode temperatures) from the large step input. Initiating the PID control within 0.5°C of the setpoint was particularly important for the higher initial temperatures (34°C and 35°C) that had larger step changes, and in consequence larger errors. By delaying the PID controller, we were able to

reduce settling time and improve overshoot and reduce the transient and steady state errors. It should be noted that there is a drop in the temperature when the PID controller is engaged, as seen in **Figure 3.6**. A feed-forward term redirects the temperature projection in the opposite direction to minimize the overshoot. This modification is particularly important after the ramp, as some subjects perceived temperatures relatively close to pain thresholds. Therefore, the feed-forward term prevented the temperature from reaching warm or cold temperatures that would cause pain to our subjects. The other consideration for the design of the control system was the fact that the ramp of the thermode may lag the setpoint temperature. Therefore, the setpoint temperature adjusts to the thermode temperature at the moment the subject presses the button. This new setpoint temperature is held for an additional three minutes at which point the test ends.

3.6 Conclusions

The optimal behavior of the thermode is to track the temperature set by the computer algorithm, used in human subject testing. We were able to accomplish this by manually tuning the PID controller for each gain term. The optimal gain combination obtained while measuring on the water bath was 25, 2 seconds, and 8 seconds for terms K_P, K_I, and K_D, respectively. However, the proportional term was adjusted to 100 after testing on a human subject leg revealed the response was too slow. Finally, the addition of the trigger mechanism had the greatest impact on optimizing the performance of the thermode controller. By turning the PID controller a close range of the setpoint temperature, we were able to minimize error, increase settling time, and reduce the overshoot. The trigger mechanism also proved to be vital in maintaining a consistent performance, regardless of the setpoint temperature.

Chapter 4. Human Subjects Testing

4.1 Introduction

Simple sensory tests, called quantitative sensory testing (QST), are a well-accepted method for detecting local thresholds to evaluate small nerve fiber health. These thresholds are compared with a control to determine whether sensation is felt normally or not (Kandel ER, 2000).

Many technical details may influence temperature threshold levels. The initial and the adapted skin temperature under the probe (Pertovaara & Kojo, 1985), the rate of temperature change (Divert, 2001), the testing algorithm, patient's reaction time and focus (Shy, et al., 2003), and the density of the receptive field at the testing site all have an impact on the threshold results. The size of the probe affects the number of thermoreceptors innervated, where sensation is heightened with the summation of a larger area of receptors (Hilz, Stemper, Axelrod, Kolodny, & Neundörfer, 1999). Warm sensation thresholds are mediated by afferent fibers with conduction velocities of the same range (C-fibers) whereas cool thresholds are signaled via faster conduction afferent fibers (Pertovaara & Kojo, 1985). Pressure of the attached thermode does not significantly affect the reproducibility of the thermal sensory threshold measurements (Pavlaković et al., 2008).

The two goals of our study are to determine how the etiology of an amputation and possible adaptation to the socket and liner system effect thermal perception and vasomotor response. The area of interest for testing was the anterior-lateral aspect of the distal residual limb and the homologous area for controls. Due to the fact that we cannot test perception inside the socket, we arbitrarily chose four initial temperatures that fall within or close to the thermoneutral zone that have been observed inside the socket (G. K. Klute, Huff, K., & Ledoux, 2007). The testing algorithm we chose was the method of limits because it is simple, quick (Fruhstorfer, Lindblom, & Schmidt, 1976; Hilz, Glorius, & Berić, 1995), and allowed us the ability to measure perception and vascular reactivity simultaneously. We chose a rate of 0.2°C/s; the rate is associated with maximal skin thermal sensitivity and stimulates deep cutaneous sensory nerves (Divert, 2001).

The second goal of our study was to measure the local vasomotor response to a changing temperature. A local vascular response coincides with thermal thresholds. Therefore, the imbedded laser Doppler flowmetry probe measured the rate of change and difference in magnitude of cutaneous blood perfusion from the first 3 minutes to the last.

4.2 Materials and Methods

Patient Recruitment

Nine subjects were recruited from the Veterans Affairs Puget Sound Health Care System (VAPSHCS). All subjects met the following inclusion criteria: 1) between 18 to 80 years of age, 2) ambulate without upper extremity aids, 3) able to walk consistently for at least 30 minutes on a treadmill, and 4) be cognitively intact so as to understand the research protocols in which they are participating. Control subjects were healthy non-amputees who met the above criteria. Subjects were excluded if: 1) there is an active tumor or treatment of tumor, 2) their residual limb was ulcerated, 3) they were unable to feel the monofilament against their skin in the areas where the thermistors will be placed, 4) they do not meet the inclusion criteria. Each subject provided informed consent of the protocol approved by Human Subject Review Committee of the Veterans Administration Institutional Review Board.

Methods

All testing was done in one session at the Veterans Affair Puget Sound Health Care System (VAPSHCS) Motion Analysis Lab (building 1, room 1D-105) in Seattle. Subjects were asked to sit and doff their prosthesis, if applicable.

Once seated for 10 minutes, the subject read (or was read) the temperature sensitivity test instructions (**Appendix B**). The investigator used Velcro® straps to attach the thermode to the lateral aspect of the residual limb or either leg for the intact subjects, as seen in **Figure 4.1**. A grain-sized temperature sensor was taped 2 cm away from the thermode for a local skin temperature reading. The total time needed to tape the temperature sensors and read the instructions was adequate for the skin temperature and blood flow to reach a basal state.

Four sensitivity-testing conditions (initial temperatures of 30, 32, 34, and 35°C) were tested for both the warm and cold perception tests. As shown in Figure 3.1, each trial consisted of three stages: (1) the initial temperature was held for three minutes, (2) the temperature ramped up or down at a rate of 0.2 °C/s until the subject indicated perception, and (3) the final temperature was held for an additional three minutes after subject indicated feeling warm or cold. Maximum blood perfusion was measured by ramping the temperature up from 32°C to 42°C over ten minutes and holding for an additional ten minutes. Data from the maximum blood flow tests was used to compare maximum vasodilation for each group. During the maximum blood perfusion, the subjects were given an optional questionnaire (**Appendix C**).

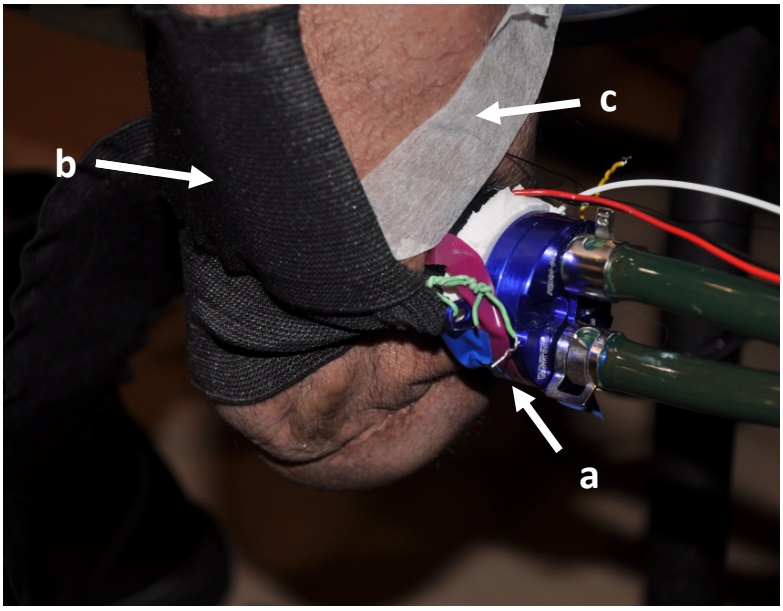

Figure 4.1. The thermode (a) was attached to the residual limb of a transtibial amputee subject with a Velcro strap (b). A grain-sized thermistor was taped (c) to the skin next to the thermode to measure residual limb skin temperature.

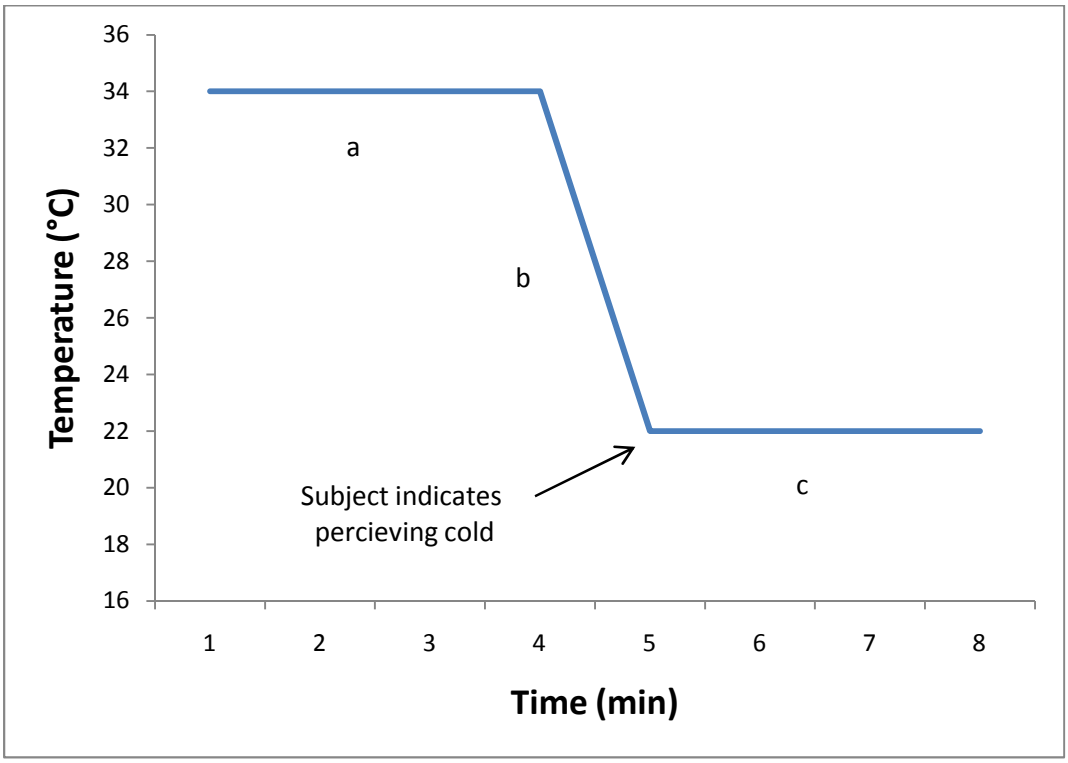

Figure 4.2. Cold perception test when the initial temperature is 34°C using a modified version of the Method of Limits testing algorithm. The temperature of the thermode will be held for a 3-minute acclimatization period (a), ramped up (warm perception test) or down (cold perception test) (b) until the subject indicates feeling "warm" or "cold", respectively. The duration of the ramp depended on how long it was before the subject indicated perception. Once the subject has reached their threshold, the temperature will be held for 3 additional minutes (c).

Analysis

Thermal thresholds were calculated by finding the difference between the initial and final thermode temperatures. Mean thresholds and threshold standard deviations were calculated for each initial temperature and warm and cold test for the control, diabetic amputee and traumatic amputee groups.

Before vascular reactivity was determined, the laser Doppler flowmeter data was filtered using a low-pass Butterworth filter with a cutoff frequency of 0.5 Hz. The cutoff frequency was determined by performing a fast Fourier transform (FFT) (**Figure 4.2)** of a few samples of noisy data. All blood flow data had a sampling rate of 5 Hz, sampling period of 0.2 seconds, and a length of time that depended on how long the temperature ramped before the subject indicated perception.

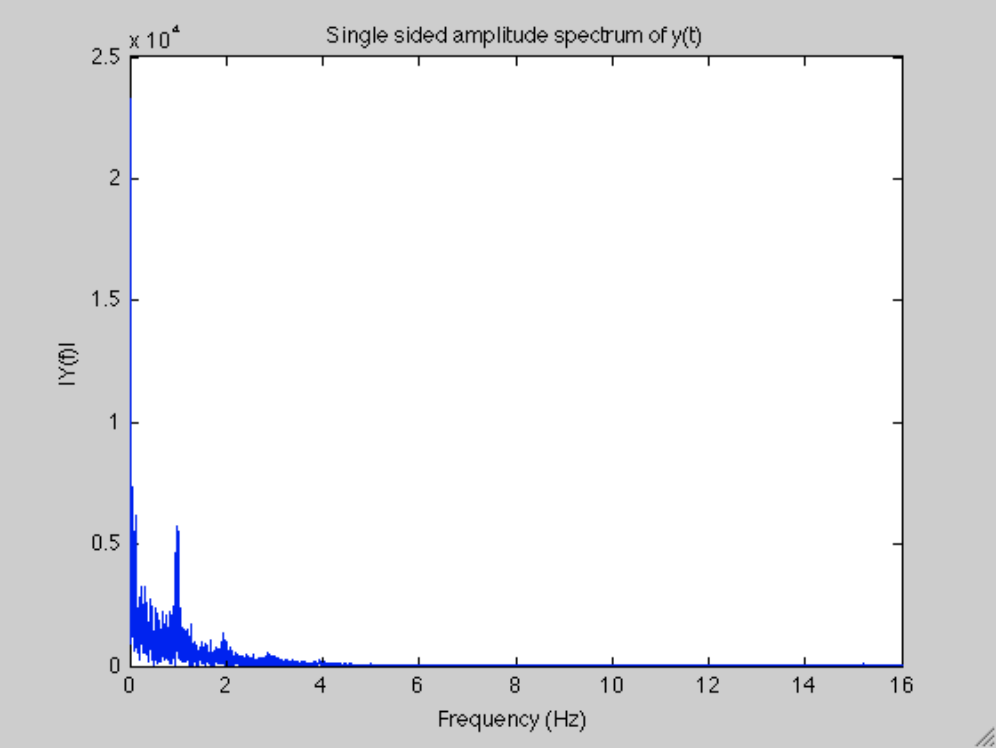

Figure 4.3. The absolute value of the magnitude of frequencies (Hz) in a fast Fourier transform of a sample of blood perfusion data.

After filtering, vascular reactivity was found as the mean difference between the last two minutes of the initial two minutes, as shown in **Figure 4.3**.

Linear mixed effects regression was used to determine if there were differences in the temperature threshold by group (diabetic amputee vs. traumatic amputee vs. control). The model was as follows:

where

- Y=temperature threshold,
- T_1, T_2, and T_3 equals 1, if initial temperature (IT) equals 32°C, 34°C, and 35°C respectively and equals 0 if temperature IT equals 30°C,
- G_1 and G_2 equal 1 if group equal diabetic and traumatic LEAs, respectively, and 0 for the control group,
- β_0 equals the mean temperature threshold for the control group at IT 30°C.
- β_1, β_2, and β_3 equal the average difference in temperature threshold for IT 32°C vs. 30°C, 34°C vs. 30°C and 35°C vs. 30°C, respectively.

- β_4 and β_5 equal the average difference in temperature threshold for diabetic vs. control and traumatic vs. controls groups, respectively.
- b_i equals the difference in mean temperature threshold at IT 30°C for subject i.
- ε = residual error.

Two diabetic LEAs and one traumatic LEAs were unable to participate in the warm perception tests because of lack of sensitivity to warmth. Therefore, the diabetic LEAs and traumatic LEAs were combined for warm thermal threshold and warm vascular reactivity tests, as only one subject from each group participated in these trials. Differences in group by vascular reactivity (final minus initial blood perfusion) were modeled similarly, with blood perfusion change as the dependent variable (Y) and a model covariate added to account for the influence initial blood perfusion has on the outcome. A group composed by increasing initial temperature interaction term was tested for significance using a likelihood ratio test. The results determined if the threshold temperature or blood perfusion trajectory (defined by increasing initial temperatures) differed by group. Analyses were carried out using R 2.11.1 (R Development Core Team, 2010), and specifically, the LME4 package (Bates & Maechler, 2010) to estimate the linear mixed effects models.

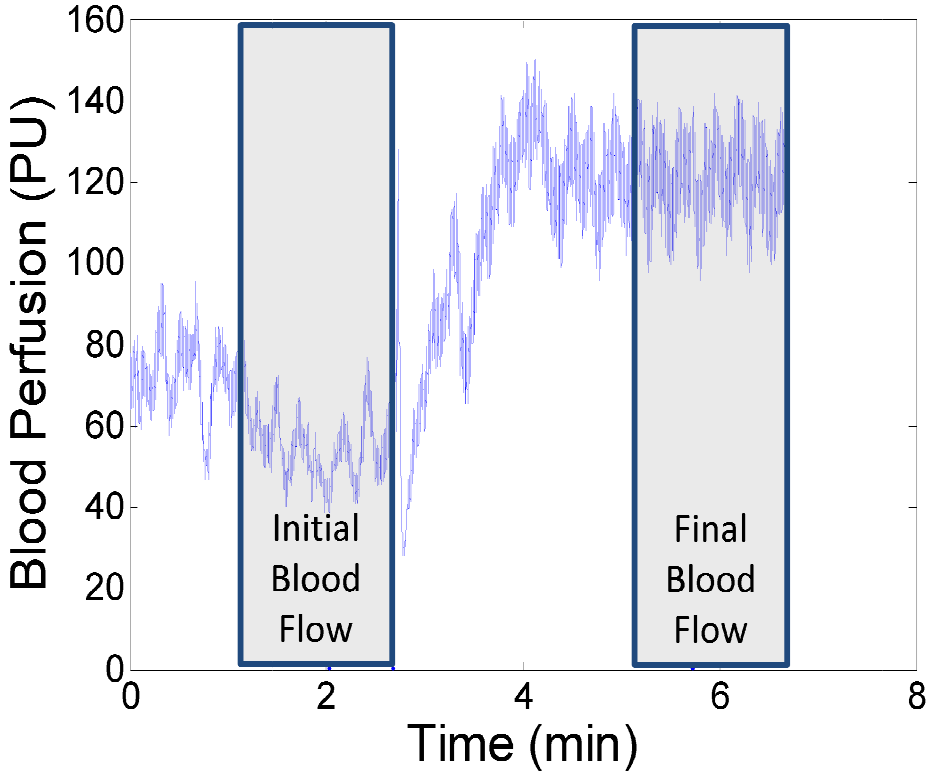

Figure 4.4. Vascular reactivity is the change in blood perfusion (PU) as the skin temperature goes from the initial test temperature to the threshold temperature. This graph shows an ideal sample of blood perfusion data for a warm detection test. The shaded areas represent the initial and the final blood flow testing periods used to calculate the mean PU magnitude change.

4.3 Results

4.3.1 Demographic Data

Nine subjects were recruited from the Veterans Affairs Puget Sound Health Care System (VAPSHCS). Subjects were divided up into three groups (**Table 4-1**): diabetic and traumatic lower extremity amputees (LEA), and healthy intact controls. The mean age of the traumatic LEA and control group were similar, but both were lower than the mean for the diabetic LEA group. The reason for this result is a consequence of the broad inclusion criterion. Mean weight and height were comparable for all groups.

Regarding the two amputee subpopulations, the mean duration of amputation for the diabetic LEAs was almost double the length for traumatic LEAs. The reason for this is likely related to the fact that the mean age of the diabetic LEAs was older than the traumatic LEAs. All subjects were transtibial

amputees, with one diabetic subject having both legs amputated. The etiology of amputation for one diabetic LEA subject was due to arthritis and not diabetes. This subject was the only one who did not report having neuropathy. Amputees were asked to rate (0=not bothered, 10= unbearable) how troubled they were by heat and sweat in the summer and winter months. On average, the traumatic LEAs reported being moderately troubled (6) by heat and sweat in the summer and negligible problems (0.5) in the winter. Only one diabetic LEA reported being troubled by heat or sweat. Sweat was more commonly reported as the bigger issue.

Subjects were excluded from all cold or warm perception and vascular reactivity tests if they could not sense 18°C or 43°C, respectively. As a result, one traumatic LEA and one diabetic LEA did not participate in the warm perception tests and one diabetic LEA did not participate in the cold perception tests.

Table 4-1. Subject demographics.

	Traumatic LEA	Diabetic LEA	Control
	(n=2)	(n=3)	(n=4)
All			
Age (year)	37.5 ± 7.8	66.7 ± 1.5	31.3 ± 4.4
Weight (kg)	75.1 ± 6.1	71.7 ± 29.3	70.3 ± 13.1
Height (cm)	179.1 ± 1.8	175.3 ± 7.1	171.2 ± 9.9
Amputees			
Duration of amputation (year)	12 ± 5.7	23.3 ± 13.3	N/A
Troubled by sweat or heat in the: (0 = not bothered and 10 = unbearable)			
Summer	6 ± 0.0	1.7 ± 2.9	N/A
Winter	0.5 ± 0.7	1.3 ± 2.3	N/A
Bigger issue:			
Heat (n)/Sweat (n)	0/2	1/1	N/A
Cold (n)	0	1	N/A
Diabetics			
Type of diabetes (T1DM/T2DM)	N/A	(n=1/n=2)	N/A
Duration of diabetes		19.3 ± 9.5	
Existence of neuropathy (%)		66.7	
Duration of neuropathy (year)		15.0 ± 5	

4.3.2 Thermal Perception Threshold Data

All subjects performed the thermal quantitative sensory tests to the best of their ability. Cold detection and/or warm detection tests were stopped if the individual was deemed insensitive; one was unable to detect the thermal stimulus by 43°C for warm and 18°C for cold and another diabetic and one traumatic amputee were excluded from just the warm detection tests. One diabetic amputee was excluded from both warm and cold detection tests.

Table 1 shows the average temperature thresholds by group, with initial temperatures adjusted to reflect linear mixed effects model estimates. For the warm test, amputees had an average threshold temperature an average of 2.1°C (SD = 1.2°C) higher than the controls with an overall association between group and temperature threshold just below significance (p = 0.069). For the cold test, temperature thresholds for the diabetic and traumatic groups were 1.8°C (SD = 2.6°C) and 1.6°C (SD = 2.6°C) degrees lower than those for the controls. These thresholds differences were not statistically significant (p=0.6).

In general, all three groups had decreased sensitivity to cold and increased sensitivity to warm as initial skin temperature increased. As shown in **Table 4.4,** the range of insensitivity to temperature is narrowest for controls, followed by traumatic amputees and diabetic amputees. The shape of the cold thresholds by initial temperature trajectories did not differ significantly (p = 0.6) between groups. Cold thresholds stayed relatively constant between initial temperature 30°C and 32°C and then sloped downward for initial temperatures 34°C and 35°C. In contrast, there was a significant difference (p = 0.003) in the shapes of the warm threshold temperature by initial temperature trajectories for each group. For the controls, when the initial temperature increased from 30°C to 32°C, the temperature threshold decreased an average of 2°C, with similar temperature thresholds for initial temperatures 34°C and 35°C. Diabetic LEA and traumatic LEA groups had similar warm perception thresholds for 30°C and 32°C, but had a 4°C threshold at the initial temperature 34°C. For all tests, cold perception thresholds were lower and warm perception thresholds higher for the diabetic and traumatic LEA groups than the controls. The most noticeable differences were at the initial conditions 34°C for traumatic LEA and 35°C for diabetic LEA. The trajectories of cold thresholds did not differ between the groups.

Table 4-2. Average temperature thresholds [standard errors] by group from linear mixed effects models for thresholds on group adjusted for initial temperature.

	Control (n=4)	Diabetic (n=2)	Traumatic (n=2)	p
Warm test	4.7 [0.7]	6.8 [1.0]*		.069
Cold test	-4.5 [1.5]	-6.3 [2.1]	-6.1 [2.1]	.607

*Two subjects had missing data, one in the diabetic group and one in the traumatic group. Consequently, the 2 groups were combined.

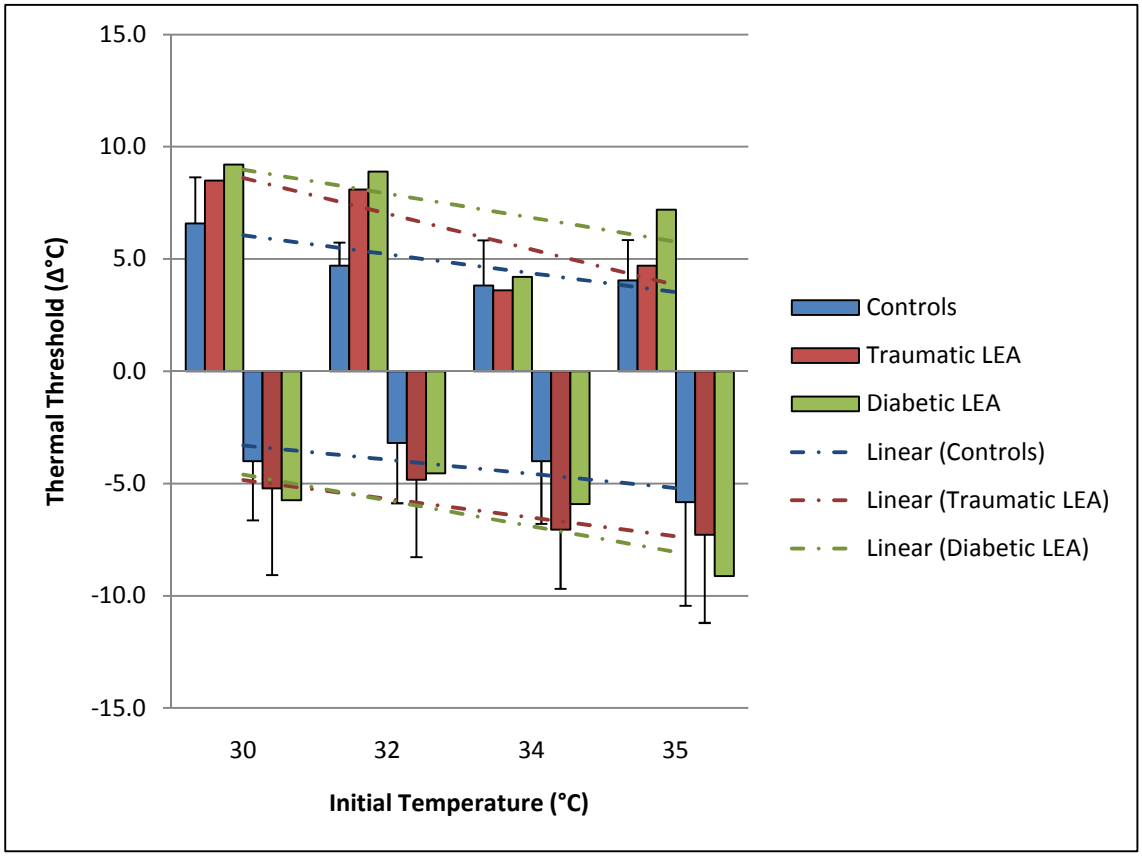

Figure 4.5. Temperature perception thresholds for varying initial temperatures. Linear trend lines (dashed) provide a view of the sensitivity associated with the warm (positive values) and cold (negative values) perception thresholds.

4.3.3 Microcirculatory Response Data

The vascular response was only measured for subjects who could participate in the thermal perception tests. The results from the test are shown in **Table 4-3**, and correspond to the mean magnitude change of the blood flow after reaching steady state at the initial temperature and the threshold temperatures.

For the warm test, average change in blood perfusion for the amputees was 13.7 (SE 18.0) PU less than the controls (**Figure 4.5**). For the cold test, compared to controls, average change in blood perfusion was 0.5 (SD = 2.2) PU more and 2.3 (SD = 2.2) PU less for diabetics and traumatic amputees, respectively (**Figure 4.6**). For both warm and cold tests, there were no significant differences in the vascular reactivity by group ($p > 0.3$). There was also no evidence that the change in blood perfusion trajectory across increasing initial temperatures differed by group (group by initial temperature interaction $p>0.7$). No discernible pattern for the warm response for traumatic LEA group or either response for the diabetic LEA group.

Table 4-3: Average change in blood perfusion [standard errors] by group from linear mixed effects models of blood perfusion change on group adjusted for initial temperature and initial blood perfusion.

	Control (n=4)	Diabetic (n=2)	Traumatic (n=2)	p
Warm test	14.2 [10.4]	0.5 [14.7]**		.359
Cold test	-1.3 [1.3]	-0.9 [1.8]	-3.7 [1.7]	.399

*Two subjects had missing data, one in the diabetic group and one in the traumatic group. Consequently, the 2 groups were combined.

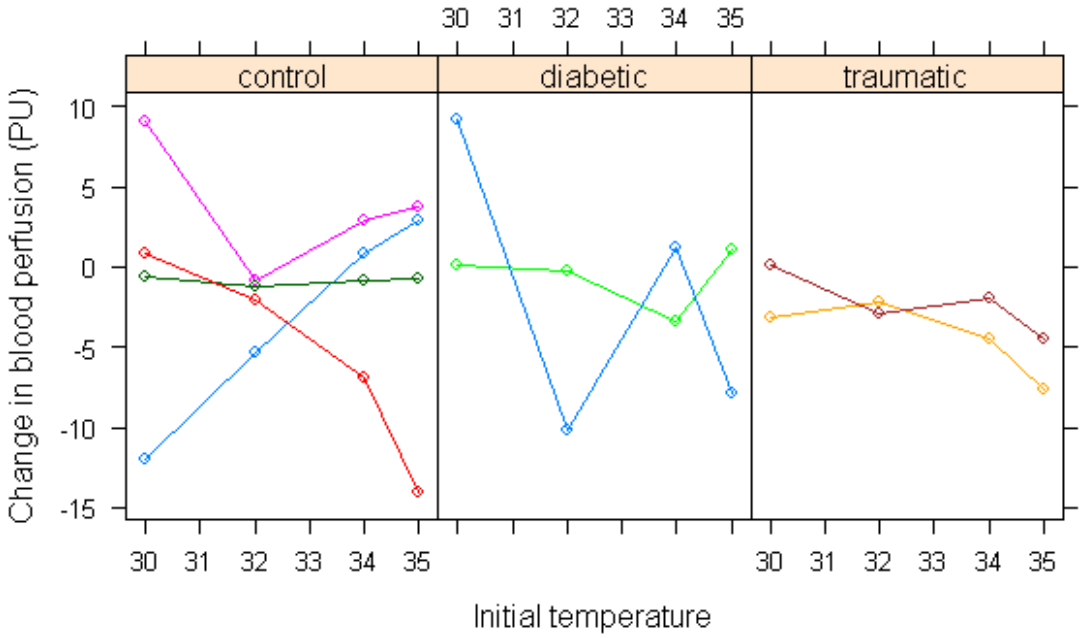

Figure 4.6 Warm test change in blood perfusion by initial temperature by group. Each line represents a single subject.

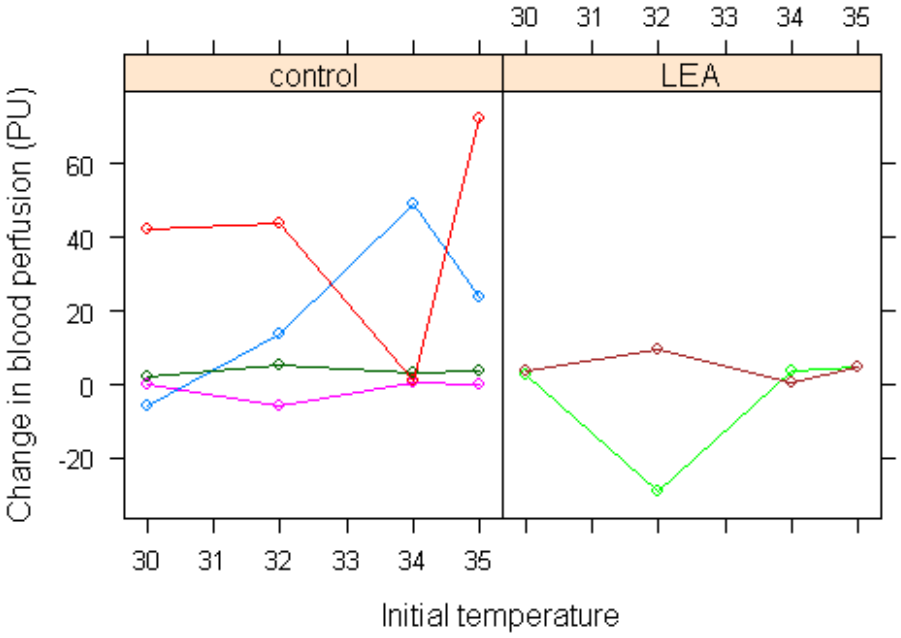

Figure 4.7 Cold test change in blood perfusion by initial temperature by group. Each line represents a single subject.

The local reflex maximum initial peak of the maximum blood flow response was also measured to further understand the microcirculatory health of the amputees. The results are shown in **Figure 4.7**. The figure shows that the traumatic LEA and controls group have similar responses, but the diabetic response is reduced. However, we found no significance between the three groups ($p = 0.4$).

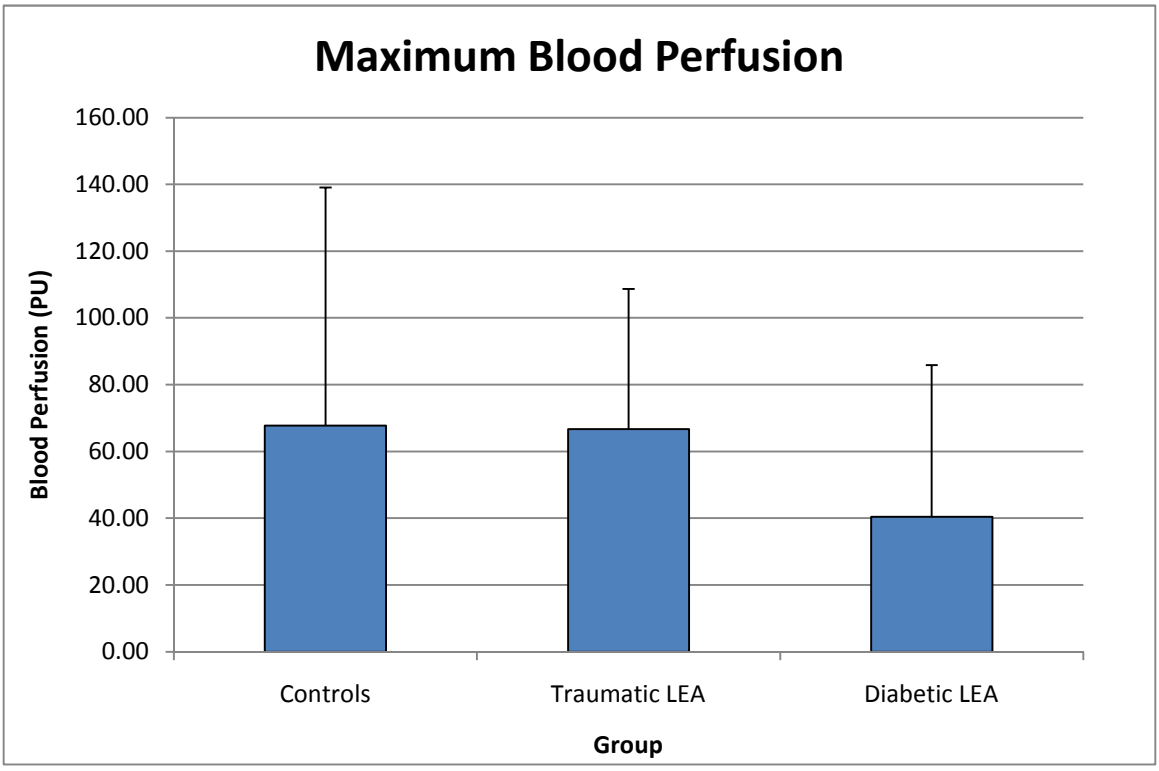

Figure 4.8. Average maximum blood perfusion of the controls, traumatic LEAs and diabetic LEAs.

4.4 Discussion

In this study, we compared the thermal thresholds and vascular reactivity of four controls, two traumatic LEAs, and three diabetic LEAs. Our goal was to determine if there was a significant difference in warm and cold sensitivity and the local thermoregulatory response between the diabetic LEAs, traumatic LEAs, and controls. The major findings for thermal perception were 1) a trend towards decreased thermal sensitivity is to warm, and 2) no insignificant difference in cold detection for traumatic LEAs and diabetic LEAs as compared to healthy non-amputee controls. There were no differences found in the vasoconstrictor response to cold stimuli and vasodilator response to warm stimuli for traumatic LEAs and diabetic LEAs as compared to healthy non-amputee controls.

There were several limitations to this study. The sample size for all groups was small and may not be reflective of the target population. This was especially true for the diabetic LEAs, who were insensitive to temperature more frequently than traumatic LEAs or controls. Also, without the inclusion of diabetic non-amputees, it is hard to distinguish whether the amputation, the disease, or both factors affect changes in peripheral nerve function. Therefore, the addition of a diabetic non-amputee sample population would have enabled an interesting comparison on the effects of amputation on diabetic perception and thermoregulation. Beyond expanding the sample population, the other large limitation was the inability to make perception or blood flow measurements while amputees were wearing their socket. We tried to overcome this obstacle by measuring perception and vascular reactivity at a range of initial temperatures previously observed inside the socket (G. K. Klute, Huff, K., & Ledoux, 2007). Unfortunately, our thermal thresholds may be higher than those perceived inside the socket, as sensitivity is heightened when a large area of thermoreceptors is innervated (Hilz, et al., 1999). Large areas of heated or cooled tissue may also accentuate the local vasomotor response, yielding greater changes in blood flow than observed in this study. On the other hand, a tightly fitting socket may diminish peripheral blood flow. Finally, the final notable limitation is the brevity of each blood flow test. The fact that we combined the perception and vascular reactivity tests together limited our testing time. Perception tests are psychophysical in nature, which means they require the concentration of the subject. Long testing times would likely result in higher thresholds. However, we were not able to achieve full blood flow steady state for either the initial or final temperature.

4.4.1 Thermal Perception

Our quantitative sensory tests attempted to reveal decreased sensitivity to both warm and cold for traumatic LEAs and diabetic LEAs when compared to non-amputee controls. The association between groups and thresholds was just below significance for the warm tests ($p=0.069$) and had no significance ($p= 0.6$) for cold tests. This may be influenced in part by the relatively small sample population ($n=4$ controls, $n=5$ amputees) for this study, as well as the high variability inherent to perception tests of the lower limb (Bartlett, Stewart, Tamblyn, & Abrahamowicz, 1998). A power analysis revealed that 31 to 39 subjects would be necessary for finding significance for 5% precision level, 90% of the time for cold threshold testing. The same analysis revealed that 7 subjects would be necessary to reveal significance for warm threshold testing at the precision level mentioned above. Regardless of testing for statistical significance, the difference between average thresholds for the controls and amputees may reveal clinical relevance for both warm and cold, as previous investigators

have found a 2°C change in skin temperature may represent a potentially large disturbance in the body's thermal balance (Savage & Brengelmann, 1996).

The effect initial skin temperature has on thermal sensitivity was also observed by Divert et al (2001), who used the same ramp rate as this study. The investigators measured warm and cold perception on the forearm of 33 young (aged 20-23 years old) volunteers. During the test, temperatures were increased incrementally by 1°C between a 29 to 38°C temperature range. The results (**Appendix D, Table D-1** and **Figure D.1**) showed similar trends as our control population, including a decrease in warm detection thresholds and increase in cold detection thresholds with increasing initial temperatures. Sensitivity to temperature decreases distally (Hagander, Midani, Kuskowski, & Parry, 2000), which may explain the higher threshold (4-8°C) temperatures we observed on the lower leg of the control group as opposed to the forearm (1-3°C). A change in sensitivity was also observed in both amputee populations, although there was a significant difference ($p=0.003$) in the shape of the warm threshold trajectory (**Appendix D, Figure D.2)**. Both diabetic and traumatic LEAs had little change in sensitivity at the lower initial temperatures (30 and 32°C) and then a substantial increase at 34°C. Interestingly, sensitivity decreased between 34°C and 35°C. The shape of the cold threshold trajectory did not differ between either amputee group or controls (**Appendix D, Figure D.3)**.

Cold perception thresholds observed in our present study contrasted with reports of decreased sensitivity to cold among amputees (Harden, et al., 2010). A previous study found statistically significant ($p=0.012$) differences in cold perception of the residual (affected) limb as compared to the non-amputated contralateral (unaffected) limb of 44 upper and lower extremity amputees. The investigators tested thresholds at an initial temperature of 32°C and found the average cold thresholds were felt approximately 1.5°C lower on the affected leg as than the unaffected leg. Despite not finding significance, our study found similar mean differences between the controls and diabetic LEAs (1.6°C) and traumatic LEAs and diabetic LEAs (1.3°C) when the initial testing temperature was 32°C. There were negligible differences between the traumatic LEAs and controls (0.3°C). Possible reasons for discrepancies include a differing methodology, number of subjects, and control population. Specifically, ramp rate (0.2°C/s vs. 1°C/s), initial temperature (a range of temperatures vs. 32°C) and number of subjects (9 vs. 44) varied. Methodologically, the previous study made comparisons between the residual limb (affected limb) and non-amputated contralateral limb (unaffected limb). We expanded the sensitivity measures to include three additional initial temperatures to understand how perception varies with differing skin temperatures. The average mean differences between controls and traumatic LEAs and diabetic LEAs were 1.6°C and 1.8°C over the

entire testing range, respectively. Our control population consisted of non-amputee healthy subjects because across-subject comparisons are a common methodology in quantitative sensory testing (Dyck, et al., 2000; Gruener & Dyck, 1994; Løseth, et al., 2008) and we were concerned the neuropathy in the intact limb of our diabetic LEA subject population would act as a confounding factor. Finally, the ramp rate chosen for this study is slow enough to stimulate the superficial and deep cutaneous thermoreceptors, causing increased sensitivity. The differences in methodology and the subject population may increase the inter-subject variability and reduce our ability to find significance in the different means. In a study of upper extremity amputees with recent (≤6 months) limb loss, individual differences were found in the cold thresholds of the affected and unaffected limb, but no significant trend was observed (Hunter, et al., 2005). Our findings of decreased warm perception for both amputee populations supported results found in previous studies (Braune & Schady, 1993; Hunter, et al., 2005).

The finding of elevated warm thresholds may be attributed to the warm sensing nerves adapting to the temperature during the slow ramp selected for this study. Harding et al. (2005) observed that rates up to 0.3°C/s resulted in higher warm thresholds but had no effect on cold. Furthermore, the residual limb is exposed to unnaturally warm temperatures throughout the day when wearing the prosthesis, which may cause the warm thermoreceptors to shift their peak firing rate. The combination of a shift in peak firing rate and because thermoreceptors respond more favorably to rapid temperature changes may be responsible for the high warm thresholds for the lower two initial test temperatures.

Degradation of small nerve fibers has frequently been seen in individuals with diabetes mellitus. Hypoesthesia, especially for temperature, pain and vibration modalities, is a well-known consequence of diabetes mellitus (Singleton, Smith, & Bromberg, 2001). Loseth et al. (2008) studied the thermal thresholds and intraepidermal nerve fiber (IENF) density of the distal leg of intact diabetics with normal nerve conduction studies and either the presence or absence of symptoms indicating neuropathy. They found the IENF density was significantly reduced and cold perception thresholds were elevated in both symptomatic and asymptomatic patients compared to controls. Warm perception thresholds were elevated in only the individuals with existing symptoms of neuropathy. In our study, one diabetic amputee was insensitive to cold only and one was insensitive to both. One traumatic LEA was insensitive only to warm temperatures. Both diabetic amputees who could not sense at least one of the modalities reported having neuropathy. It is possible that neuropathy was a contributing factor to the loss of sensitivity for all three subjects.

Understanding how skin temperature affects perception is important for the design of an effective cooling socket system. From our study, we understand that a large difference (~5°C) between the residual limb skin temperature and the cooling temperature is necessary to notice an effect. Furthermore, the skin temperature rises throughout the day, which means the temperature difference has to be even greater to be perceived. The study also revealed that amputees likely perceive temperature after sweat onset, as evidenced by the finding that local heating of the forearm by 5°C caused a marked localized increase in sweat rate (Ogawa & Asayama, 1986).

A thermally comfortable socket may not be necessary for all amputees. Surprisingly, amputees are more often insensitive to warm than cold, which could imply that certain populations of amputees do not experience thermal discomfort. In the questionnaire given during this study, both amputee groups were asked to rate (0 = not bothered, 10 = unbearable) how troubled they are by heat and sweat in the summer and in the winter. Each traumatic amputee indicated being moderately bothered (6) by heat and perspiration in the summer and negligibly bothered in the winter. On average, the diabetic LEA group indicated not being bothered by heat or sweat in the summer or winter. One diabetic LEA responded with the fact that their limb felt cold the majority of time. These amputees were the same subjects who were not able to participate in the warm perception tests because of lack of sensitivity to higher temperatures. Therefore, a cooling socket would not be appropriate for all amputees or at all times of the year. It will be important to determine who is eligible for a cooling socket by inquiring about discomfort issues and testing perception. The next question to answer is whether a temperature controlled socket is necessary for limb health.

4.4.2 Microcirculatory Response to Thermal Stimulus

Our vascular reactivity tests attempted to show an attenuated blood flow response to warm or cold thermal stimuli for diabetic LEA and traumatic LEA as compared to controls. Due to high variability as a result of our small sample size, the association between subject group and magnitude change in blood perfusion was weak to nonexistent for both the average warm ($p = 0.359$) and cold ($p=0.399$) tests. Also, there was no trend found in the shape of the trajectory of initial temperatures related to blood perfusion. A power analysis revealed that between 13 to 27 subjects would be necessary for finding significance for cold and warm testing, respectively, for 5% precision level, 90% of the time.

The vasodilation results reported here are the first for residual limb blood flow reaction to thermal stimuli below the typical hyperemia stimulus (42-44°C). However, our findings do not support attenuated blood flow responses found in either non-amputated diabetic individuals or post-surgery lower extremity amputees. Reduced thermal hyperemia has been observed in both individuals with

type 1 diabetes mellitus (T1DM) (Wilson, Jennings, & Belch, 1992), type 2 diabetes mellitus (T2DM) (Wick DE, 2006, Caballero AE, 1999; Veves A, 1998; Williams SB. 1996), and dysvascular individuals with above knee and below knee amputations (Fairs, Ham, Conway, & Roberts, 1987) when the skin was heated to 42°C. Wick et al (2006) found that forearm blood flow for the subjects with T2DM was 79% of the control value. Our findings for the diabetic LEAs group were substantially lower (19.2% of the control), but this may be attributed to either the amputation itself or the severity of the disease as the diabetics in the study were excluded if they had cardiovascular disease or peripheral distal neuropathy. Site difference may also contribute to the difference, as we tested the lower limb and not the forearm. Our traumatic LEA data was more consistent with results found by Fairs et al. (1987), who found maximum thermal hyperemia on the medial and lateral lower limb amputees to be 39.3% and 48.2% of the homologous location on the control limb. Our findings on the lateral aspect of the residual limb were most similar for traumatic LEAs. However, the demographic of their study was recent amputees of dysvascular etiology as opposed to our group of traumatic and diabetic amputees who lost their limb an average of 12 and 22 years previously, respectively. Duration since amputation may affect the vasodilatory response, as would the higher thermal stimulus (mean traumatic LEA was 37.2°C and mean diabetic LEA was 38.0°C) and shorter testing periods.

The vasoconstriction results here have been both reinforced and contradicted by existing literature. Quantitative infrared telethermography (qIRT) measurements revealed that the residual limb skin temperature is 1.3 to 3.0°C lower than the contralateral limb when the amputee is not wearing a prosthesis (Harden, et al., 2008). One theory for a cooler residual limb is that sympathetic hyperactivity causes pronounced vasoconstriction (Harden, et al., 2008), therefore effecting the basal vasoconstrictor tone. This theory supports our findings, in which the vasoconstrictor response caused a significant decrease in peripheral blood flow for both traumatic LEAs and diabetic LEAs. The contradictory results are from measurements of vasoconstrictor reflexes showed that the complicated T1DM group had a poorer response to cold at the big toe than the uncomplicated group or the controls (Wilson, et al., 1992).

This is the first study examining the impact an amputation in combination with diabetes or trauma has on thermoregulation. Impaired thermoregulation in intact individuals with T2DM has been observed both in epidemiology reports (Schuman, 1972; Semenza, et al., 1999) and through microvascular reactivity tests (Wick, et al., 2006). For traumatic amputees, it has been reported through a survey as an inability to stay thermally comfortable (Hagberg & Branemark, 2001). Thermal discomfort may not be directly linked to impaired thermoregulation, but the inability to maintain a balance between

heat production by the muscles and metabolism with heat dissipated through the socket system. However, diminished peripheral blood flow response could lead to a decrease in convective heat transfer, theoretically the engaged thermoregulatory mechanism when the socket is on. Ineffective heat transfer may also be responsible for reports of profuse sweating, as perspiration assists heat transfer through evaporation.

The implications of impaired thermoregulation may be more of a nuisance for younger, more active amputees who use their prosthesis more frequently and intensely. Skin temperatures inside the socket have been shown to rise significantly with activity (G. K. Klute, Huff, K., & Ledoux, 2007). Elevated skin temperature, moisture, and greater forces from walking and running may make the macerated residual limb skin susceptible to ulceration or blister formation. A blister or ulcer may result in pain and discomfort and could limit use of the prosthesis until it heals.

Impaired vasodilation may have more severe implication for dermal wound healing for diabetic amputees. A recent survey revealed that 63% of a random sample of amputees were troubled by residual limb problems at any one time (Meulenbelt, et al., 2009). These problems include delayed wound healing and recurrent skin ulcerations. Chronic stump ulcers have been reported up to 20% of the amputees surveyed in another study (Byung-Jin, 2003). Unfortunately, diminished blood flow and tissue oxygenation in the distal LEA make healing difficult (Vigier et al., 1999). Our study found that although diminished, problems associated with wound formation may be ameliorated by provide local warming to the limb, and healing may be remedied by keeping the limb warm and providing greater PU for diabetic LEAs. With increased localized blood flow, the limb would likely be more protected from ulcers and, in the event of skin injury, would speed up recovery time.

4.5 Conclusion

In conclusion, we report that diabetic LEAs and traumatic LEAs have altered thermal perception to warm temperature. Using thermal QST, we found that both amputee groups were less sensitive to warm than controls over a wide range of skin temperatures. For all groups, sensitivity to warm increased and cold decreased, as the skin temperature got warmer. However, there was a noticeable difference in the effect initial temperature had on sensitivity to warmth for the combined amputee group as opposed to the controls. There was no noticeable difference for the blood perfusion change from baseline to threshold temperatures and cold thresholds for diabetic LEAs and traumatic LEAs as compared with controls.

From this study, we have an understanding of how amputees perceive temperature on the residual limb. Although not statistically significant, there is a strong trend indicating amputees are less sensitive to warm than controls. This means residual limb skin temperatures may increase to relatively high temperatures before the amputee notices and their temperature regulation system acts to alleviate the heat. Possible side effects of insensitivity to heat are profuse sweating and skin injury, both issues reported as causing discomfort to lower limb amputees. Too much sweat may result in loss of adherence of the prosthesis and would result in pistoning or the limb falling off. Therefore, a cooling socket is necessary to keep the limb comfortable and safe. Fortunately, amputees do not exhibit desensitization to cold. Therefore, a cooling socket may not only prevent the skin temperature of the residual limb from increasing to the level of sweat onset, but may also be noticeably cold and comfortable. A new temperature-controlled limb technology may also benefit amputees who develop dermal wounds. Ulcers and other skin injuries are relatively common amongst amputees. Increasing local temperature may increase blood flow to an injured area and could promote wound healing.

Chapter 5. Future Work

This is the first work aimed at understanding thermal perception and microcirculatory health for the purposes of building a more comfortable and potentially healthier socket. Therefore, understanding the bounds of perception and the influence thermal stimuli have on blood flow are only a small part of the foundation necessary for designing an ideal socket. In future work, we will expand on perception, explore more thermoregulatory mechanisms in the residual limb, and build and test new socket and liner technologies. Additionally, we will try to better understand the diabetic leg and develop possible interventions to prevent the formation or promote the healing of skin injuries. In this section, I will touch on each theme addressed above.

There are many forms of perception that influence the way an amputee experiences their prosthetic limb. Temperature perception is an important impetus for behavioral change, such as doffing the prosthesis before skin temperatures are warm enough to elicit a sweat response. If taking the limb off is unrealistic, then perceiving either the loss of limb adherence or frictional forces from pistoning may allow the amputee to stop walking before the formation of a blister or the prosthesis falls off. It would also be beneficial to understand the temperature perception preference in the design of a smart thermally comfortable residual limb to ameliorate thermal discomfort. Specifically, it would help to understand if the amputee would prefer to perceive coldness on their limb or be content with the socket removing enough heat to avoid perception of warmth.

Thermoregulation inside the socket is largely a mystery. We have begun to explore how residual limb blood flow changes in response to a thermal stimulus. This is one of many indicators of overall microcirculatory health. In the future, it would be beneficial to measure vascular reactivity while the limb is inside the socket. The residual limb is exposed to a considerable amount of compressive forces when loaded with body weight (S. Zachariah, 2004; S. G. Zachariah, Saxena, Fergason, & Sanders, 2004) and large areas of skin are elevated in temperatures while inside the socket (Peery, Ledoux, & Klute, 2005). The influence of these factors will be accounted for by embedding the laser Doppler flowmeter (LDF) in the liner to measure blood flow. Perspiration is also a means of thermoregulation. Understanding the quantity and skin temperature associated with onset of sweat will help in the design of liner and socket technologies that reduce skin moisture and inadvertently help maintain adherence. Accomplishing these design goals will hopefully help reduce the amount of skin injury and maintain a well-fitted socket.

Finally, perception, sweat, skin temperature, and blood flow information will be used in the design of a comfortable, adaptable socket system. Socket designs will focus on keeping the limb cool and dry.

One aspect of the development will be to test different cooling paradigms to see which reduces sweat production and is most noticeably cool to the amputee. Another more challenging aspect will be to develop a technology that adds minimal weight to the overall system. The technologies will be tested using a bench-top device and then on human subjects performing multiple tasks. The goal is to develop a socket system that an amputee can wear comfortably throughout the day.

It is estimated that one in four diabetic individuals may require amputation because of the severe peripheral vascular disease (PVD) (Newton et al., 1999). In the United States, the rate of such amputations has increased by 27% over the last decade (Dillingham, et al., 2002). Therefore, further research in vascular reactivity is imperative to understand this growing population. Continued work should focus on figuring out how to provoke the largest blood flow response with either occlusion or temperature. Increased blood flow to an area of injury may reduce recovery time. Even more important is the prevention of skin injury. Developing new technology to sense the formation of blisters or ulcers inside the socket will reduce concomitant issues and prolong the use of the prosthesis.

In summary, more research must focus on ways to maintain limb comfort and health inside the socket for lower limb amputees. Understanding perception, sweat response, skin temperature, and blood flow will aid in the design of a more comfortable and safer socket system. These parameters may also contribute to finding ways to reduce the rate and promote healing of skin injuries for the growing diabetic population.

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Appendix A: PID Controller Results

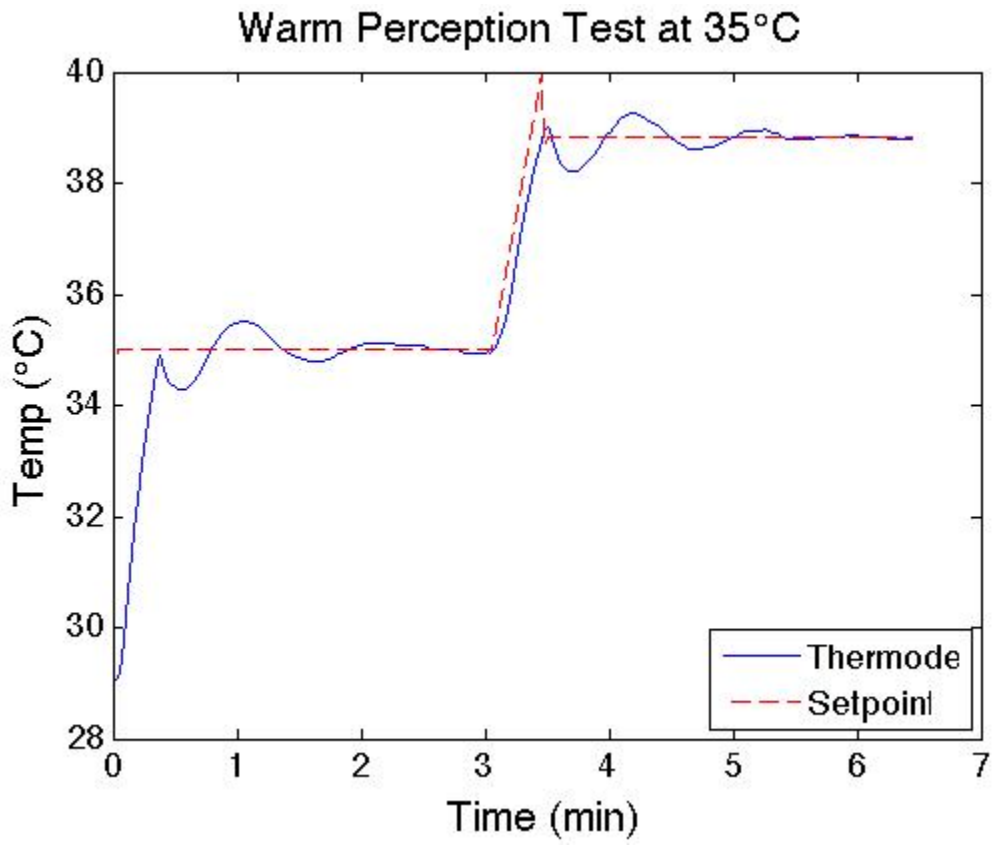

Figure A.1. Warm perception test when the initial temperature was set to 35°C.

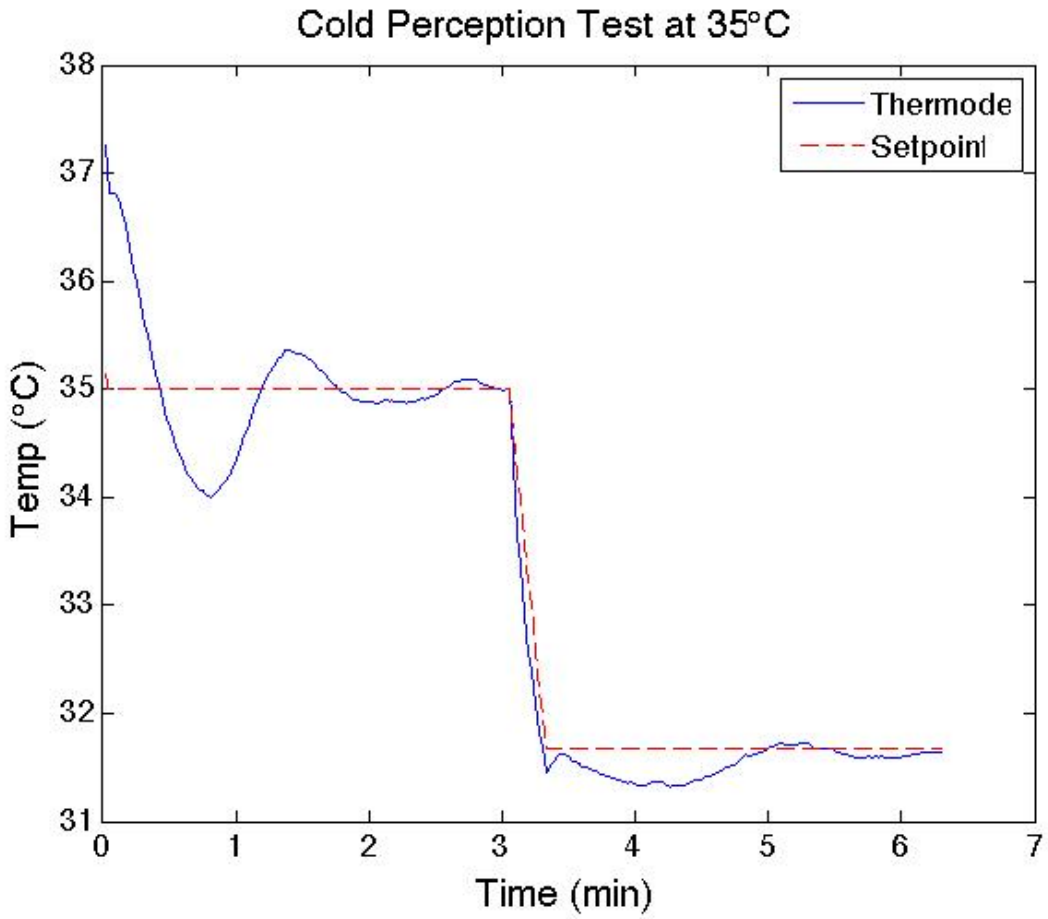

Figure A.2. Cold perception test when the initial temperature was set to 35°C.

Table A-1. Thermode performance when the PID is engaged form the start of the test.

Temperature (°C)	Rise Time (sec)	Settling Time (sec)	Overshoot (%)	Peak (°C)	Settling Time Threshold (N/A)
30	7.4	88.3	2.8	30.8	0.1
32	9.3	122.1	7.0	34.2	
34	12.1	110.0	5.6	35.9	
35	11.2	111.9	6.1	37.1	

Table A-2. Thermode performance when the PID is engaged after temperature trigger.

Temperature (°C)	Rise Time (sec)	Settling Time (sec)	Overshoot (%)	Peak (°C)	Settling Time Threshold (N/A)
30	8.5	17.0	2.2	30.7	0.1
32	9.6	22.8	3.3	33.1	
34	19.4	54.5	2.6	34.9	
35	7.6	56.5	0.9	35.3	

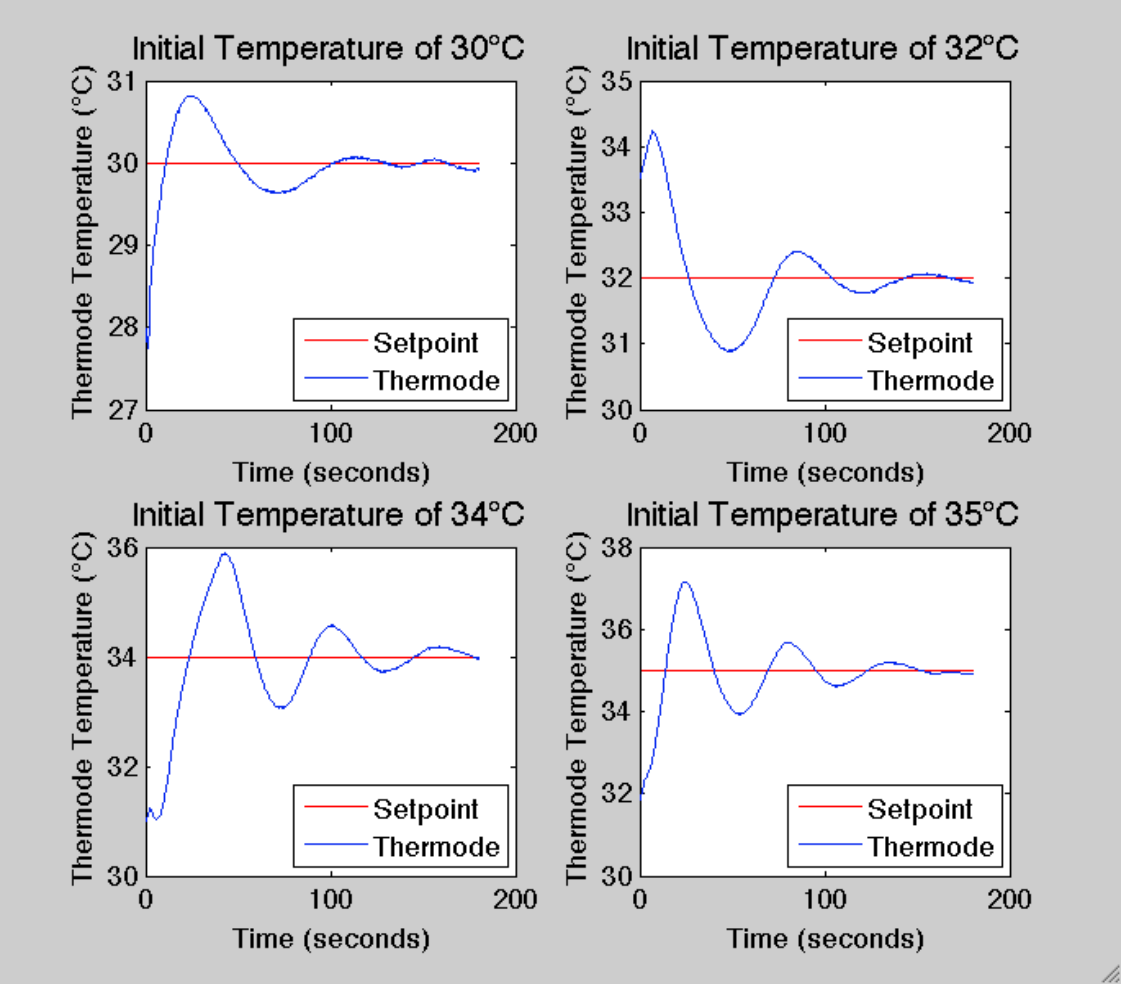

Figure A.3. Thermode temperature performance for each of the initial temperatures with full participation from the PID controller.

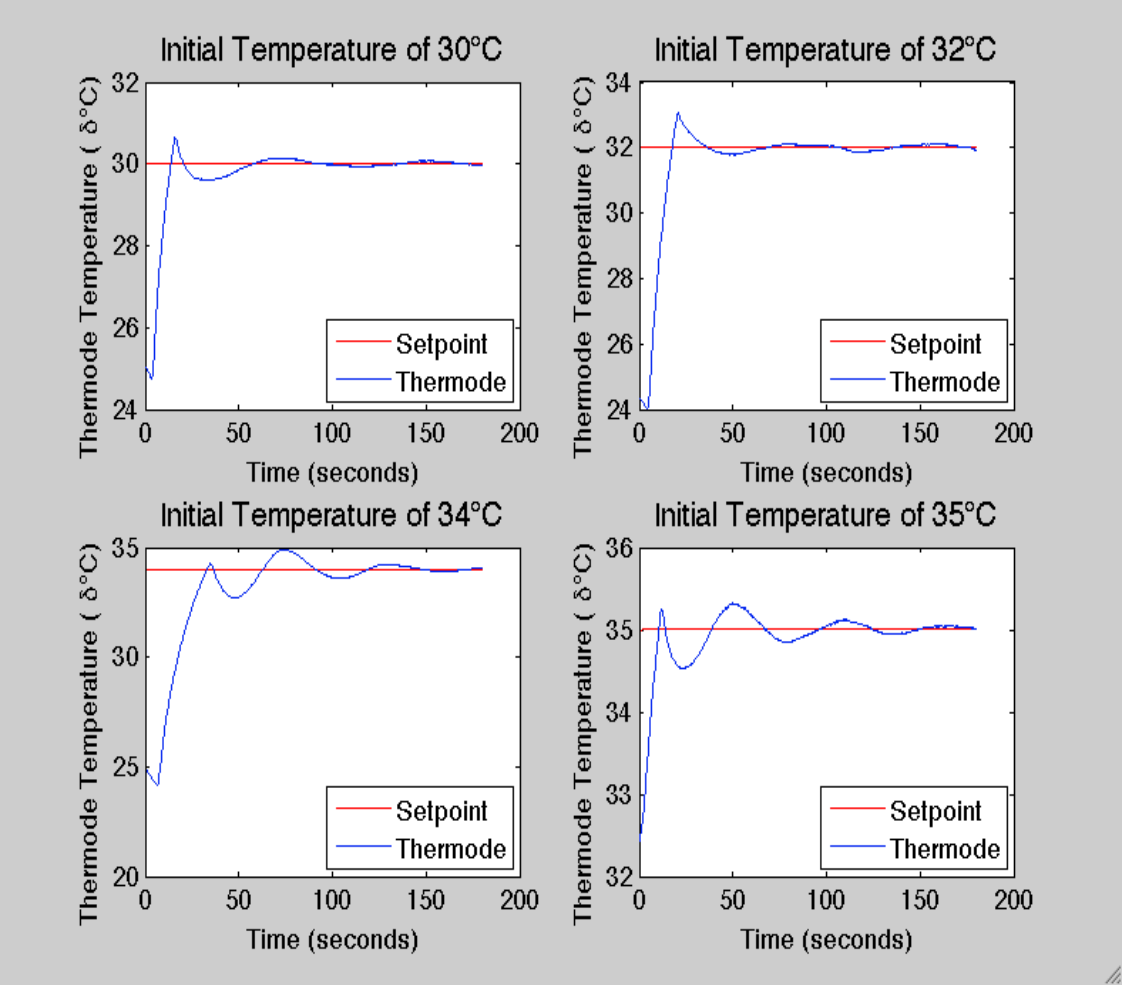

Figure A.4. Thermode temperature performance for each of the initial temperatures with delayed participation from the PID controller.

Appendix B: Temperature Sensitivity Test Instructions

You are about to participate in a study, which will see how sensitive your skin is to temperature. Imagine you set your hand on a cold countertop. Your skin will sense the low temperature and tell you that the counter is cold.

The way we tell how sensitive you are is by finding the point where you go from not feeling to feeling a temperature. This point is found by putting this small sensor on your skin and changing its temperature at a steady rate until you feel a new sensation (warm or cold).

When the sensor changes from not being felt to feeling warm or cold, you will let us know by pressing this RED button. If you do not feel anything, you will not press any button. Try hard to press the red button only when you feel warm or cold. This will require you to pay attention throughout the test.

If the sensor feels like an uncomfortable temperature, you will either press this BLACK button or remove the whole thing by unvelcroing the strap. Under no circumstance will the sensor hurt you. There are extra safety precautions to ensure this. If you feel like you do not want to continue the test for any reason, either press the black button or indicate to the investigator that you are finished.

We will test your sensitivity eight times. The initial temperature of the sensor will be varied randomly as will the direction of temperature change. In the end, we will have tested your sense of warmth and your sense of cold four times each. There will be a 2 minute break between each test to let you rest and regain concentration.

Any questions?

Appendix C: Questionnaire for Temperature Sensory Testing

1. Age:

2. Height:

3. Weight:

4. Please list the state(s) or country(ies) if outside the U.S. where you spent your first 6 years of life.

For the amputees:

5. Number of years after amputation:

6. Level of amputation:

7. Reason for amputation:

8. Are you troubled by heat and sweat in the summer? (Please use a number on the scale of 0 to 10 where 0 = not bothered and 10 = unbearable)

9. Are you troubled by heat and sweat in the winter? (Please use a number on the scale of 0 to 10 where 0 = not bothered and 10 = unbearable)

10. Would you say heat or sweat or both are the bigger issue?

For the diabetics:

11. Type of diabetes mellitus: (Type 1 or Type 2)

12. Duration of diabetes (Years):

13. Existence of peripheral neuropathy (Yes or No):

14. Duration of peripheral neuropathy (Years of N/A):

15. Are you taking any medication for neuropathy (Yes, No, or N/A):

Appendix D: Results from the thermal perception tests

Table D-1. Warm detection thresholds (WDT) and cold detection thresholds (CDT) for traumatic and diabetic lower extremity amputees (LEA) and healthy intact controls. Thresholds are the difference between the final and initial temperature and are represented as the mean [std] for each group.

Test	Initial Temperature (°C)	Traumatic LEA (°C)	Diabetic LEA (°C)	Healthy Intact Controls (°C)
WDT		(n=1)	(n=1)	(n=4)
	30	8.5	9.2	6.6 [2.0]
	32	8.1	8.9	4.4 [1.0]
	34	3.6	4.2	3.8 [2.0]
	35	4.7	7.2	4.1 [1.8]
CDT		(n=2)	(n=2)	(n=4)
	30	-5.2 [3.9]	-5.8 [2.2]	-3.8 [2.6]
	32	-4.8 [3.4]	-4.5 [2.3]	-3.2 [2.7]
	34	-7.1 [2.6]	-5.9 [0.7]	-5.1 [2.5]
	35	-7.3 [3.9]	-9.1 [4.7]	-5.8 [4.6]

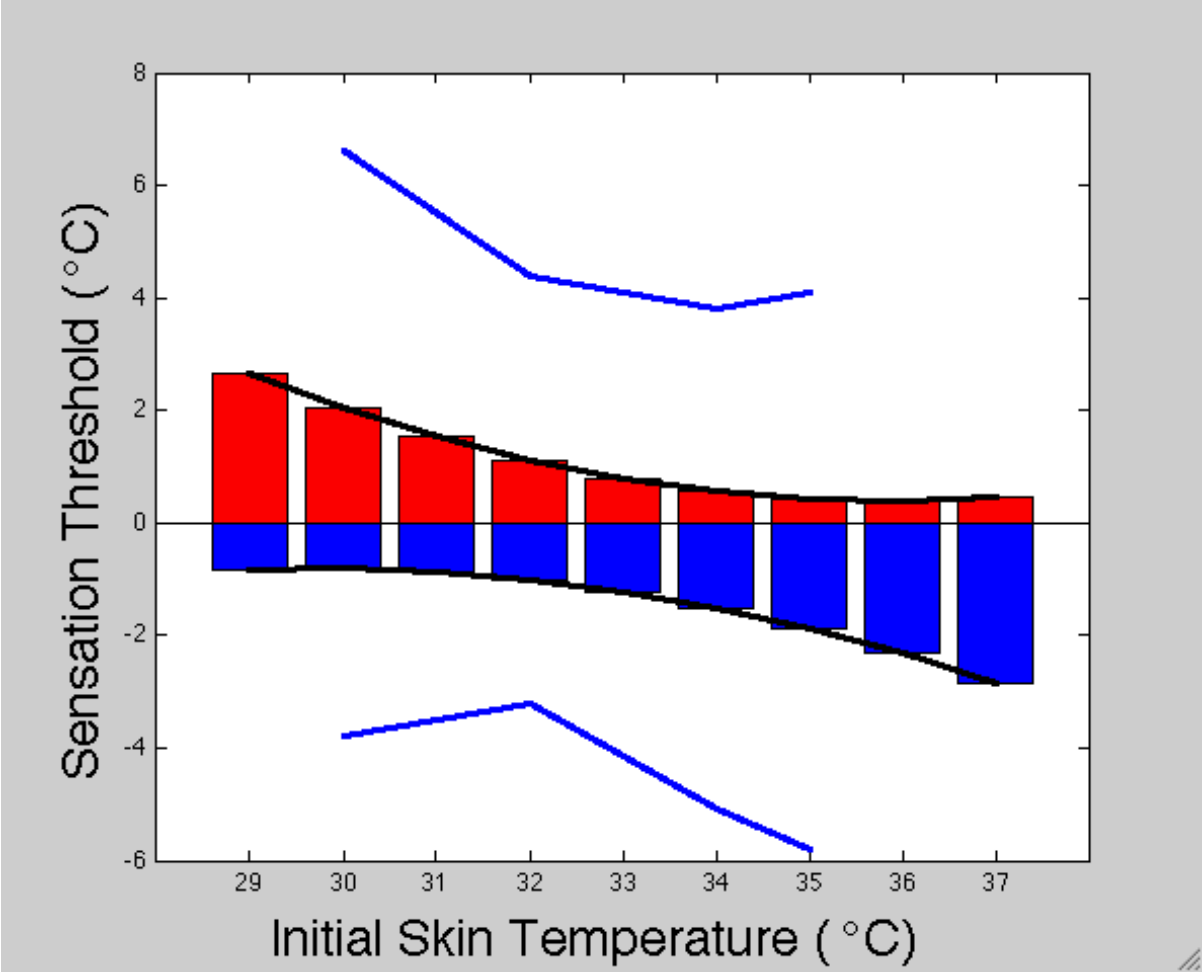

Figure D.1. The effect initial temperature has on warm (red) and cold (blue) thermal sensitivity. The thresholds (perceived temperature – initial temperature) were measured on the forearm of 33 young (20-23 years old) male and female volunteers. The range of temperatures that the subjects do not perceive temperature fall between the trend lines for the previous study (black line) and current study (blue line).

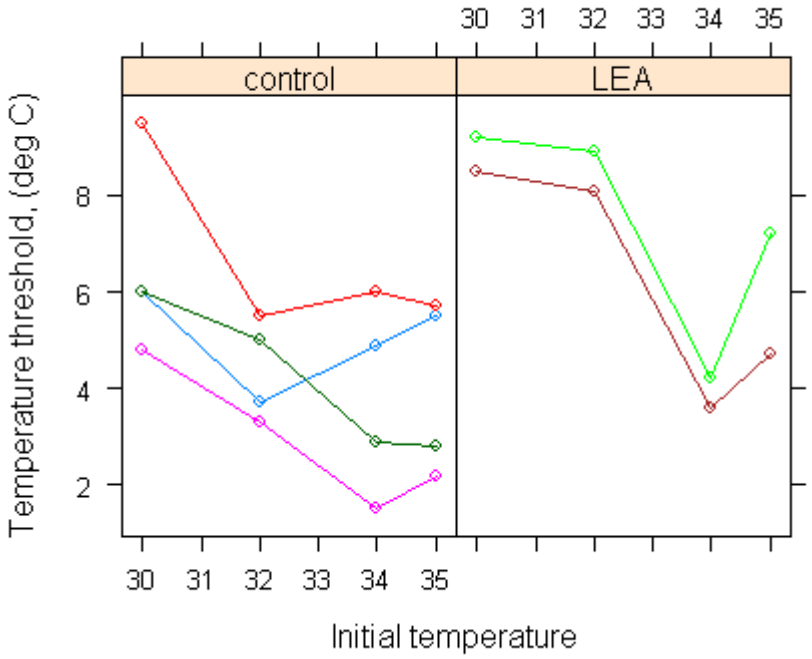

Figure D.2: Warm test temperature threshold by initial temperature by group. Each line represents a single subject.

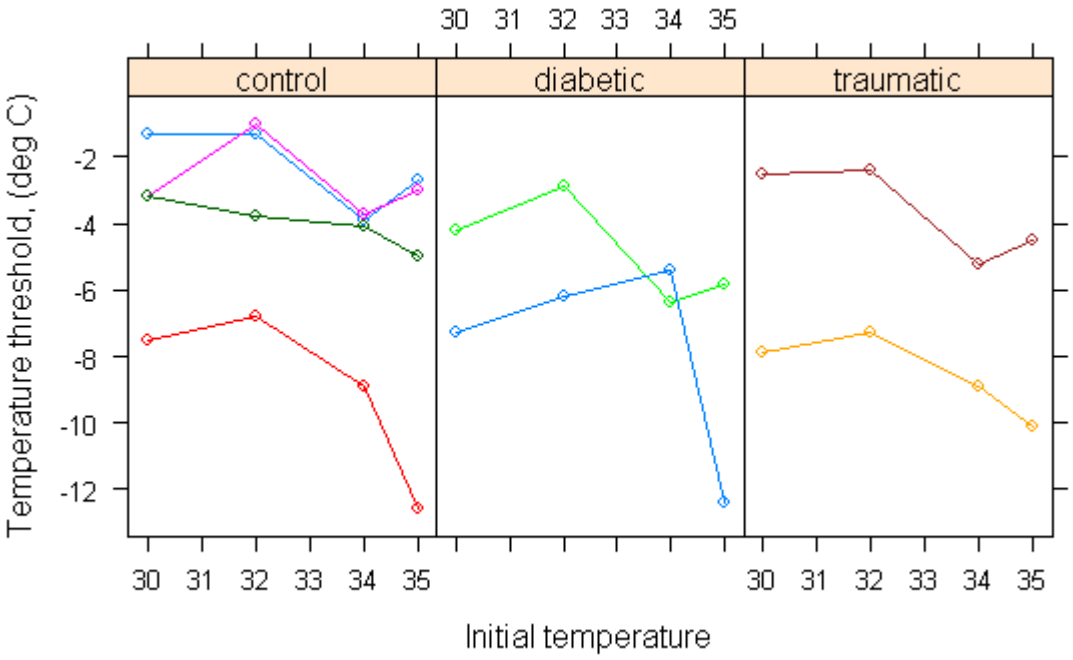

Figure D.3: Cold test temperature threshold by initial temperature by group. Each line represents a single subject.

Appendix E: Results from the vascular reactivity

Table E-1 The mean [standard deviation] blood perfusion (PU) after settling on the initial temperature.

Initial Temperature (°C)	Controls (PU)	Traumatic LEAs (PU)	Diabetic LEAs (PU)
Cold	(n=4)	(n=2)	(n=1)
30	16.2 [14.3]	8.0 [0.6]	9.0 [1.4]
32	8.0 [4.4]	9.0 [0.4]	12.0 [7.0]
34	9.1 [8.0]	8.6 [3.5]	21.2 [11.2]
35	16.3 [14.9]	13.2 [1.5]	11.6 [2.5]
Warm	(n=4)	(n=1)	(n=1)
35	16.3 [14.9]	13.2 [1.5]	11.6 [2.5]
30	9.9 [8.0]	4.4	14.4
32	11.1 [8.7]	7.6	36.0
34	11.5 [5.9]	7.2	9.4
35	16.3 [14.9]	7.3	8.7

Table E-2 The mean [standard deviation] blood perfusion (PU) after settling on the final (threshold) temperature.

Initial Temperature (°C)	Controls (PU)	Traumatic LEAs (PU)	Diabetic LEAs (PU)
Cold	(n=4)	(n=2)	(n=1)
30	15.5 [9.4]	6.5 [1.8]	13.6 [5.1]
32	5.6 [3.0]	6.4 [1.0]	6.8
34	8.1 [8.0]	5.4 [1.8]	20.0 [14.4]
35	14.3 [17.3]	7.1 [0.6]	8.2 [3.9]
Warm	(n=4)	(n=1)	(n=1)
35	19.5 [22.5]	8	17.2
30	25.3 [23.8]	17	6.8
32	25.0 [24.5]	7.7	12.9
34	33.9 [367.2]	12	13.5
35	19.5 [22.5]	8	17.2

Table E-3 The mean [standard deviation] blood perfusion (PU) change from initial to final temperatures.

Initial Temperature (°C)	Controls (PU)	Traumatic LEAs (PU)	Diabetic LEAs (PU)
Cold	(n=4)	(n=2)	(n=1)
30	-0.7 [8.7]	-1.5 [2.3]	4.6 [6.5]
32	-2.4 [2.0]	-2.6 [0.6]	-5.2 [7.0]
34	-1 [4.2]	-3.2 [1.8]	-1.1 [3.2]
35	-2 [8.3]	-6.1 [2.1]	-3.4 [6.3]
Warm	(n=4)	(n=1)	(n=1)
35	9.7 [22.0]	3.7	2.8
30	14.2 [21.2]	9.4	-29.2
32	13.5 [23.7]	0.5	3.5
34	25.0 [33.3]	4.7	4.8
35	9.7 [22.0]	3.7	2.8