Effects of Knee Extension Exercise on Microvascular Function: Implication for Diabetic Foot Ulcer Healing

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THESIS

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SUMMARY

This study showed that the skin blood flow response parameters to pressure and heat were useful in comparing microvascular nature in subjects with different characteristics in diabetes and healthy subjects. The results showed that peak and perfusion after pressure relief were reduced in the subjects with only diabetes as compared to the healthy subjects. This influence might be reduced owing to the early signs of impairment in the vasodilator component at the test site in the skin. In addition, increased skin blood flow responses were seen in the subject with history of DFU at all instances probably on account of impaired vasodilator component due to impaired axon-reflex in the presence of neuropathy. The results of this study demonstrated variable responses in the subjects with DFU and history with DFU, in the peak and perfusion of skin blood flow probably due to peripheral sympathetic nerve impairment in diabetes. The time to peak of the SBF which refers to the time taken to reach the maximum vasodilation after pressure relief, was one of the most important responses observed in this study that corresponded with the diabetic condition amongst the subjects. A higher time to peak was observed in the subject with DFU and history with DFU compared to other diabetic subjects and was the least in the healthy subject.

Reactive hyperemia post exercise intervention demonstrated an increase in the peak and perfusion in general, however these responses varied in the subjects with a more chronic condition of diabetes. The greater BMI may be a possible explanation for this variation. A biphasic response was observed in the skin blood flow responses to heat in a healthy subject in this study which has been reported as regular response to heat. Also, post exercise effects was not significantly correlated in changes in microvascular responses with diabetic characteristics. However, the consistency and impaired responses in this response requires further investigation.

1.0 INTRODUCTION

1.1 STATEMENT OF PROBLEM

Diabetes Mellitus (DM) is one of the most prevalent metabolic disorders which is characterized by elevated level of glucose in the blood, known as hyperglycemia resulting from deficiency in insulin secretion, its action or both (Nwankwo et al. 2014). The prevalence of DM is 9.4% in the population of United States, and amongst those, about 25.2% individuals aged 65 and older are affected with Diabetes (American Diabetic Association. 2016). The people affected with diabetes is increasing globally and remains the seventh leading cause of death due to its complications in the United States in 2015 (Yazdanpanah et al. 2015). Therefore, its care and early prevention has gained prior concerns over the recent years (American Diabetes Association, 2016). Diabetic foot ulcer (DFU) is one of the major complications that affects approximately 15% of DM population (Yazdanpanah et al. 2015). Although preventable, prevalence of this complication ranges between 4%-27% amongst all diabetic patients. Development of foot ulcers can indeed lead to infection, gangrene and hence results in greater risk of amputation, which is associated with the increased morbidity and mortality rate. It is estimated that lower limb amputation is 15 times higher in patients with diabetes compared to non- diabetic population and nearly 50-70% of lower limb amputations occur as a result of DFU (Yazdanpanah et al. 2015). Diabetic foot ulcers develop due to the concurrent mechanisms of several risk factors, causing skin and underlying tissue damage with or without the presence of bone exposure, in about one third to one half of people with diabetes (Nwanko et al. 2014, Yazdanpanah et al. 2015)

Diabetes induced autonomic neuropathy; a microvascular complication of diabetes forms the most common pathway in the development of foot ulcers that originates due to several impaired metabolic and vascular pathways(Cameron and Cotter. 1997, Cade. 2008). Dysfunction related to vasodilation due to the reduced synthesis of NO, leading to blood flow deficits and reduced nerve perfusion has been considered an important feature in the progression of diabetic neuropathy (Cameron and Cotter. 1997). Several microvascular literatures have addressed this concern, yet there are few dependent factors of microvasculature that still requires further investigation.

Owing to the chronic nature of DFU, recent practices including aggressive glucose control, debridement, offloading, topical hormones, non-epinephrine, hyperbaric oxygen therapy and growth factors, they often remain unhealed (Sheehan et al. 2003) . Exercises have known to accelerate skin blood flow due to muscle contractions to deliver oxygen to the working muscle (Flahr. 2010), reduce inflammatory properties (Peterson et al. 2005) and improve glycemic control (Elliott et al. 2009). Recent studies have employed different aerobic and resistance exercise protocols that have demonstrated positive outcomes in DFU healing, yet the mechanism of healing was not fully explained (Donna et al. 2010, Nwanko et al. 2014). A study has also shown exercises using a single large muscle group to increase blood flow in response to muscle contractions due to vasodilation (Osada et al. 2012). Therefore, an exercise paradigm involving muscle contractions can be used to evaluate microvascular skin blood flow in diabetes in preventing diabetic complications.

1.2 OBJECTIVE AND HYPOTHESIS

The objective of this pilot study is to evaluate the feasibility and effectiveness of an isolated thigh muscle exercise on the blood flow in individuals with diabetes. In order to reach the goal of the study, four aims were tested:

1) Examine and compare the SBF response to localized pressure in diabetic and healthy subjects before exercise. It is hypothesized that the reactive hyperemic response will be smaller in diabetic subjects as compared to healthy subjects before exercise training.
2) Examine and compare the SBF response pattern to localized heating in diabetic and healthy subjects before exercise. It is hypothesized that the heat hyperemia pattern is different between diabetic and healthy subjects.
3) Examine and compare the SBF responses to localized pressure in diabetic and healthy subjects after 12 weeks of exercise training. It is hypothesized that the reactive hyperemic response will increase in both the diabetic and healthy individuals.
4) Examine and compare the SBF response pattern to localized heating in diabetic and healthy subjects after 12 weeks of exercise training. It is hypothesized that the heat hyperemic response will increase in both the diabetic and healthy individuals.

1.3 SIGNIFICANCE

Although previous exercise studies demonstrated positive outcomes amongst diabetic subjects, the exercise effect on microvascular function and dosage of exercise remains unexplained. In this study, we examined the changes in the microvascular response before and after a standardized twelve-week knee-extension exercise training protocol, and the mechanism of exercise on diabetic blood flow would be better understood. Findings of this study may help to quantify the dosage of exercise in clinical practice and make it convenient to translate this form of exercise intervention in a clinical setting.

2.0 LITERATURE REVIEW

2.1 Diabetes and its complications

Diabetes is a group of metabolic disorder indicated by elevated levels of glucose in the blood, namely hyperglycemia which is a results due to impaired insulin secretion, action or both (Nwanko et al. 2014). It is strongly leads to several complications that are microvascular in nature such as diabetic retinopathy, diabetic neuropathy and diabetic nephropathy involving capillaries and macrovascular complications such as ischemic heart disease, peripheral vascular disease and cerebrovascular disease affecting large vessels; arteries and veins (Cade. 2008). Microvascular complications originate due to chronic hyperglycemia due to metabolic and structural derangements (Cade. 2008).

2.2 Diabetic Foot Ulcer Etiology

Diabetic foot ulcer is the most common and devastating complication of DM, that is multifactorial that may result in failure of healing and leads to lower limb loss and amputation (Yazdanpanah et al. 2015). The risk factors that could contribute in the etiology causing an DFU are uncontrolled hyperglycemia, duration of diabetes, peripheral neuropathy, structural foot deformity, older age, peripheral vascular diseases(Rogers et al. 2011).

2.2.1 Diabetic Neuropathy:

It is typically defined as the presence of signs and/or symptoms of peripheral nerve damage in people with diabetes (Verrotti et al. 2014). Due to the diabetic condition, there is decreased ability

to vasodilate causing blood flow deficits that leads to reduced blood supply to the nerves and decreased nerve conduction (Cameron and Cotter. 1997). It may involve motor, sensory or autonomic nerves, and can also coexist periodically (Verrotti et al. 2014). It is often underestimated as it could potentially affect the autonomic nervous system leading to several organ dysfunctions and, most importantly it may be asymptomatic at the initial stages, compromising diagnosis and treatment in the later stages (Verrotti et al. 2014). This significantly contributes to micro-angiopathic comorbidities that lead to life threatening complications like cardiac dysfunction, ischemic heart disease and unexpected death due to alterations in the sympathetic tone. Diabetic neuropathy initially begins with the impairments in parasympathetic system causing autonomic dysfunction in gastrointestinal, cardiovascular, bladder, sexual and integumentary systems (Hosking et al, 1978). Diabetes induced neuropathy may also lead to damage of the sudomotor fibers and changes in vasomotor response to temperature changes, causing impaired sweating and temperature regulations (Hosking et al. 1978, Verotti et al. 2014). Owing to these changes, the foot loses its natural ability to moisturize, making the skin dry, along with the loss of sensation due to neuropathy, subsequently exacerbates the risk of ulceration (Hosking et al. 1978).

2.2.2 Pathogenesis of Neuropathy: Hyperglycemia, in diabetes interferes with the metabolic pathways that eventually affects the vascular component to the nerves and causes neuropathy. This includes two pathways; 1) glucose to sorbitol that leads to oxidative stress and abnormal NO synthesis, 2) the formation of Advanced end glycosylation (AGEs) due to polylol pathway. These metabolic pathways; AGEs, and polylol pathway interacts to induce oxidative stress. This process decreases the endothelial function due to increased activity of angiotensinII (AII) and angiotensinII type1, and decreased nitric oxide (NO) which in turn contribute to increased vascular smooth

muscle reactivity. Nitic oxide is responsible for vasodilation. Endothelial abnormality impairs the blood flow to the nerve and oxygen that produces schwan cell and neuronal dysfunction. Neuron dysfunction leads to nerve degeneration is responsible for neuropathy (Cameron and Cotter. 1997).

2.2.3 Diabetes and Inflammation

We have learnt that hyperglycemia forms the main source of complications in diabetes. Increased cytoplasmic and blood glucose overload interferes with several metabolic mechanism leading to chronic tissue damage. Along with glucose over load, there is excessive production of reactive oxygen nitrogen species (RONS), by the mitochondria that induces DNA damage and overstimulation of enzyme activity resulting in endothelial dysregulation and formation of AGEs. The production of AGEs causes alteration in protein content both in the extracellular and intracellular matrix. AGEs, then may react with specific receptors and produces a pro-inflammatory cascade consisting of (Tumor necrosis factor) TNF-α, TNF-β, (Interleukin) IL-1, IL-6, and (Vascular cell adhesion molecule) VCAM-1 and therefore increases oxidative stress. This inflammatory process continues repeatedly and causes secondary neuronal and vascular damage (Verrotti et al. 2014).

2.2.4 Foot deformity

Diabetic neuropathy results in diminished sensation, proprioception and deteriorated intrinsic muscles of the foot, which leads to the toes being curled up into a hammer toe or claw-foot deformity. This deformity may increase the pressure on the upper surface of the toes, under the metatarsal heads. Moreover, distal migration of the plantar fat pad covering the metatarsal heads could occur, with a flattened longitudinal arch (Reiber et al. 1999). As a result, bony prominences

develop in patients with foot deformities, especially under the metatarsal heads. Foot ulceration forms a greater risk in people lacking protective sensation due to repetitive shear stress and pressure. Charcot foot syndrome is the most commonly occurring foot disorder in people with diabetic neuropathy which is characterized by acute inflammation, varying degrees of bone destruction, subluxation and dislocation. Since diabetes is mediated through a process of uncontrolled inflammation, it leads to osteolysis in the foot and is responsible for the progressive fractures and dislocation by the simultaneous action of uneven pressures. The mechanism for the development in the bone and joint problems in diabetes has been documented due to a neutrally mediated vascular reflex leading to increased peripheral blood flow and active bone reabsorption contributing to progressive destruction. However, this relationship of blood flow and bone reabsorption requires further investigation (Reiber et al. 1999)

2.2.5 Trauma

Trauma was also a sufficient and important causative factor for foot ulceration. Due to advanced sensorimotor neuropathy, individuals with diabetes may lack the ability to identify any lower limb pressure, pain or trauma for instance, nail sticking out of shoe, if it is extremely tight. One study have showed that trauma contributed to 81% of causal pathways of DFU leading to diabetic limb amputation (Reiber et al. 1999)

2.3 CURRENT STRATEGIES IN THE MANAGEMENT OF DFU

2.3.1 Preventive Strategies

2.3.1.1 Education

Individuals with diabetes should be alerted about the risk factors and the significance of foot care, and to further prevent the complication of developing DFU and amputation. Self-monitoring, inspection of foot temperature, daily foot hygiene, use of appropriate footwear and glucose control should be greatly encouraged, which can be effective in reducing the morbidity and frequency of lower limb threatening complications due to hyperglycemia (Nathan et al. 2006).

2.3.1.2 Blood sugar control

Controlling glucose is the most influencing metabolic component in individuals with diabetes and it has been reported that most of the complications of uncontrolled blood sugar is the predominant cause of DFU (Alavi et al. 2014). HbA1c forms the best indicator of blood sugar control. Accelerated glycosylation of hemoglobin depends on a higher HbA1c level. Previous studies show that higher blood glucose levels decrease neutrophil function, including leucocyte chemotaxis, suppresses inflammatory responses and increases infection rate (McMurry.1984). Additionally, it was observed that 25% reduction in microvascular complications was associated with 1% mean reduction in HbA1c (Nathan et al. 2006). Also, It has been shown that for every 1% increase in HbA1c, there is s 25-28% increased risk in peripheral arterial disease (Yazdanpanah, Nasiri, and Adarvishi 2015). However, there is lack of literature that shows benefits of improved glucose control after the occurrence of a diabetic foot ulcer (Sigal et al. 2006).

2.3.2 Treatment Strategies

2.3.2.1 Debridement

It is known as the removal of dead and necrotic tissues, including infected and foreign materials from a wound, which is considered as a crucial step towards wound closure (Lebrun, Tomic- Canic, Kirsner. 2010). It also helps in decreasing bacterial growth and stimulates the production of local growth factors. Different types of debridement include autolytic, enzymatic, biological and mechanical (Jain. 2014). Surgical method of wound debridement involves cutting away the dead and infected tissue and encouraging bleeding to stimulate healing to convert chronic ulcer to an acute one (Attinger, Bulan, and Blume. 2000). Mechanical debridement technique involves the use of dressings, high irrigation, lavage and hydrotherapy (Schultz et al. 2003). Autolytic debridement is a method of accelerating healing of a wound that occurs naturally, if skin perfusion and venous drainage is adequately maintained (Robert G. Frykberg et al. 2006). Enzymatic debridement involves healing with enzymatic action with proteins, collagen and elastin. Despite these techniques of debridement, it is often preceded by the application of topical agents which may be expensive (Yazdanpanah, Nasiri, and Adarvishi 2015).

2.3.2.2 Offloading

Offloading techniques in diabetes is an important aspect in preventing DFU which commonly involves relieving the local pressures of the foot. This contributes to one of the most important characteristic feature in the management of DFU, mainly in the presence of neuropathy (Armstrong and Lavery. 2005, Armstrong et al. 2004). Owing to the insensate plantar surface caused by neuropathy, offloading is necessary as his or her proprioceptive feedback is not functional in judging whether the pressure has been relieved. Despite adequate perfusion, a DFU leads to chronic delay in healing, unless the foot is offloaded (Cavanagh and Bus et al. 2010). Techniques such as removable cast walker (RCW) and total contact cast (TCC) are suggested to relieve of plantar pressures (Mueller et al. 1989, Armstrong et al. 2004). Although both these casts have showed incredible healing, TCC makes it difficult to assess the wound regularly and also, requires a skilled technique in its application (Frykberg. 2002). Hence, this need of adopting a novel approach is essential in accelerating the healing of ulcer (Cavanagh and Bus et al. 2010).

2.3.2.3 Hyperbaric oxygen therapy (HBOT)

This approach involves the application of intermittent administration of 100% oxygen and has shown promising results in chronic non-healing DFUs (Oliveira et al. 2014, Strauss. 2005). In HBOT, the patient is required to breathe pure oxygen ranging from 1.4 to 3.0 absolute atmospheres, for 30 minutes, thrice in a 90-minute session with an interval of 5minutes in a hyperbaric chamber (Barnes. 2006). It has been demonstrated in a randomized controlled trial (RCT) by Londahl et al demonstrated improved outcomes as a group treated patients within 12 months. Another systematic review by Kranke et al found that treatment with HBOT resulted in a significantly greater rate of healed ulcers compared to the one treated without

HBOT. The mechanism of HBOT has been shown to relieve wound hypoxia, enhance perfusion, reduce edema, and decrease inflammatory cytokines, promote fibroblast proliferation and angiogenesis (Al-Waili and Butler. 2006, Thom. 2011). Despite the increased healing rates with HBOT, its use in the treatment of DFU remains questionable due to its scarcity in the availability and cost of care in the United States.

2.3.2.4 Negative Pressure Wound Therapy (NPWT)

This approach utilizes negative pressure that is applied locally to help accelerate healing in both chronic and acute wounds. It employs sterile polyurethane and latex free or polyvinyl alcohol foam dressing, which is fitted in the wound and sealed with adhesive drape on the surface of the skin. A negative pressure of 80-125mmHg is used in cycles or continuously. The fluid is suctioned from the wound and is collected into a container in the control unit. With this intervention, the wound appears to heal by removing exudate, edema, reducing bacterial colonization, enhancing blood circulation, cellular proliferation and improving oxygenation at the wound due to applied mechanical force. This method has been advocated in several RCTs as a safe and effective mode of treatment for DFU and enables faster healing time in wound closure. However, owing to higher expenditure in the material needs to treat wounds with NPWT compared to conventional approaches, the utilization of this technique becomes questionable (Vikatmaa et al. 2008, Armstrong and Lavery. 2005).

2.3.2.5 Growth Factors

Growth factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), insulin like growth factors (IGF1, IGF2), transforming growth factor b and

epidermal growth factor, have been used to promote fibroblasts, neutrophils and monocytes and other components of wound healing. In a randomized placebo controlled trial involving patients with full thickness DFU, Becaplermin was used which contains PDGF-BB, demonstrated completed closure vs placebo gel i.e., (50% vs 35%).Although, some studies have known to indicate that tumor infiltrating fibroblasts stimulates endogenous PDGF found in human melanoma cells. Hence, these factors should be used legitimately as there is a possibility in promoting factors.

Diabetic foot ulcer is considered as one of the most important form of chronic wound which is a critical concern to all the health care professionals. Owing to it's over complicated disease process, it has become very important in addressing this issue. Therefore, despite above treatment strategies, a difference approach. Most diabetic ulcers are challenging to treat due to its underlying pathophysiology (Frykberg et al. 2015).

2.4 EXERCISE TRAINING IN DIABETIC INDIVIDUALS

Exercise has been a general recommendation as an adjunct therapy to reduce hyperglycemia by increasing insulin sensitivity and decreasing plasma glucose, body fat and improve protection against developing cardiovascular complications (Sigal et al. 2006). Moreover, it is encouraged due to its low cost and non-pharmacological consideration increases its therapeutic concern. Along with Diabetes, there is also reduction in other associated metabolic

complications such as obesity through the exercise. Aerobic exercises using large muscle mass and resisted training (free weights, resistive loads) protocols are implemented to control diabetes (Sigal et al. 2006).

2.4.1 Mechanism involving glucose uptake in exercising muscle

Glucose acts as a fuel to meet the energy demands in a skeletal muscle from the regulating circulation. Resting muscle uses oxidation of fatty acids to meet the energy demands, but, in an exercising muscle, carbohydrates become the more important as the oxidative fuel. It was shown that glucose uptake is enhanced with increase in blood flow, oxygen consumption and CO2 in a contracting muscle, however, there are multiple factors that affect the uptake of glucose in a muscle during exercise. Absorption of glucose during exercise is encouraged through an increase in blood flow by the opening of capillaries, due to the wide opening of capillary surface area (Koivisto and Felig et al. 1978, Vranic and Berger. 1979)

2.4.2 Anti-inflammatory properties of exercise

Exercise has been prescribed as an important intervention in controlling diabetes and its associated complications due to its anti-inflammatory effects. Pro-inflammatory cytokines such as TNF-α, IL-6, IL-1ra and C- reactive protein are higher in aged and diabetic population. The presence of these factors is found to be associated with atherosclerosis, dementia and its increase is found to be observed more in smokers, obese individuals and type2 diabetes. It is identified that TNF-α is a stronger driver behind insulin resistance and dyslipidemia than IL-6 and presence of IL-6 is an indicator of a metabolic disorder. Regular exercise causes the suppression of TNF-α and increased circulatory levels of anti- inflammatory myokine; IL-6(Peterson and Pedersen. 2005). It was suggested in a study by Keylock et al that examined

the effects of aerobic exercise on cutaneous wound healing on aged mice. It was demonstrated that with exercise there was a significant trend to reduce size of the wound in aged mice by reducing IL-6, IL-1β and TNF-α. Hence, it was proved that exercises could induce anti - inflammatory response in the wound and help the heling process healing in old mice.

Exercise has extensively been advocated to heal wounds in healthy and diabetic individuals. In a study that was carried out on healthy older individuals with experimental wound puncture, investigated the effects of aerobic exercise (brisk walking, jogging, arm strengthening with warm up and cool down phase) on the healing of wound and measured cardiorespiratory endurance, salivary cortisol at rest and improvement post exercise. The participants experienced increased cortisol levels during stress along with wound healing. Exercise training protocols have also shown decreased wound size in Diabetic individuals. Dona Flahr et al demonstrated a home-based non -weight bearing exercise protocol (ankle exercises) healed foot wounds in older diabetic adults. Another RCT by Mueller et al, demonstrated improvement in the HbA1c values with non- weight bearing compared to eight bearing exercises in patients with diabetic peripheral neuropathy. Nwankwo et al, also documented accelerated healing of diabetic foot ulcer compared to standard medical care in individuals with diabetic foot ulcer and showed more control in HbA1c in the group that underwent exercise. All these studies have shown significant improvement in wound reduction and glucose control; however, these literatures still lack correlational evidence causing this improvement in wound reduction and legitimate exercise parameters effective to bring about this effect.

2.5 MEASUREMENT OF MICROVASCULAR RESPONSES

To investigate the microvascular function, a non- invasive measuring tool was used to examine these changes. Previous literatures have investigated vascular function in diabetic as well as healthy adults to investigate risk factors in cardiovascular health and predictive value of microvasculature complications in peripheral artery disease (Alex et al. 2016, Rousit and Cracowski. 2012). The outcome measures used by most of the studies to study microvasculature include reactive hyperemia to pressure (RH) (Tzen et al. 2010).and microcirculatory response to local heating, heat hyperemia(HH) (Lanting et al. 2017) In this study, RH and HH was evaluated with Laser Doppler Flowmetry.

2.5.1 Reactive Hyperemia and Heat Hyperemia

It is defined as the normal physiological response, which is characterized by a transient increase in blood flow after a brief period of tissue ischemia in different organs of the body (Levick et al. 2003, Wilkin. 1987) including skin, liver, brain, and heart. It is also a sudden increase in the skin blood flow that appears immediately after the release of vessel occlusion. The blood flow ascends form a biological zero (blood flow during vessel occlusion) to a peak then gradually descends to baseline value (blood flow before occlusion). It can be induced conveniently by blocking the SBF for as short as three minutes (Cracowski, Minson, Salvat. 2006, Tzen et al. 2010) and lasts about

one half of the time of the tissue ischemia. The skin reactive hyperemic response has been used in several studies investigating microvascular function in diabetic (Jan et al. 2013) in healthy adults to investigate drug effects over vascular function (Iredahl et al. 2015) in patients with peripheral arterial diseases post vascular surgery. Heat Hyperemia has also been advocated in many studies to assess neurovascular dysfunction in type 2 diabetes (Bandini et al. 2013),vasodilatory mechanism due to local heating in patients with desensitization (Ciplak et al. 2009), and to assess the association of diabetes and dermal microvascular dysfunction with LDF(Fuchs et al. 2017).

2.5.2 Vasodilation due to Reactive and Heat Hyperemia

Reactive hyperemia to pressure denotes the increase in the blood flow following a brief arterial occlusion. There are certain mediators that control this mechanism. Sensory axon -nerve reflex is partially involved in causing this response. The response is dependent on local mediators include Calcium -potassium channels, nitric oxide (NO) and the action of prostaglandins is conflicting (Roustit and Cracowski et al. 2012). However, (Wong et al. 2003) found using micro dialysis and Laser Doppler Flowmetry, that NO does not contribute to either the peak or the area under the curve, although maintains vasodilation after peak responses. Hence, it has been concluded that NO is not essential to observe a normal reactive hyperemic response (Engelke et al. 1996). In addition, prostaglandins appeared in playing a chief role in mediating peak vasodilation after arterial occlusion. Inhibition of reduced cyclooxygenase (COX), which is a prostaglandin did not cause an effect in the baseline blood flow, but reduced the peak and total reactive hyperemia. This denotes that the vasodilating prostaglandins formed in the ischemic period contributed to vasodilation after reestablishment of blood flow (Engelke et al. 1996). Despite its conflicting mechanism, reactive hyperemia would still be helpful in providing important information in microvascular function

(Wong et al. 2003). Heat Hyperemia (HH), has been stated as an independent tool with high sensitivity for detection of relative changes in blood flow. It is caused by vasodilation due to local skin heating. Due to vasodilation the rise in skin perfusion is directly proportional to the skin temperature and reaches maximum when temp reaches 41°C. The response shows two perfusion peaks which is mediated by independent mechanisms; 1) the initial peak predominantly depends on local sensory nerves and is facilitated by an axon reflex relying on calcitonin gene related peptide(CGRP) and substance P and plateau reached after the peak mediated by nitric oxide (Fuchs et al. 2017).

2.6 MEASURING TECHNIQUE OF MICROVASCULATURE

Laser Doppler Flowmetry (LDF)

LDF can monitor skin microcirculatory and provide perfusion response by employing a Doppler shift of laser light as it hits moving red blood cells along with white light reflectance spectroscopy that is based on the theory of optical fiber (Rousit and Cracowski. 2012). It is a non-invasive and reliable tool to assess the risk of microvascular dysfunction (Schubert and Fagrell. 1991a, Tzen et al. 2010). The measurement is carried out by projecting a laser beam to skin, then scattered light is collected by optical fibres (Togawa& Tamura. 1997b).

3.0 METHODS

3.1 RESEARCH DESIGN

This feasibility study utilized a single-subject pre-test post-test intervention design. The participants went through a pre- exercise test, then, a 12- week exercise training followed by a post exercise test.

3.2 PARTICIPANTS

Subject recruitment was followed after the study protocol was approved by the Institutional Review Board of the University of Illinois at Chicago (IRB # 2016-0874). Six participants (three male and three female) ranging in age from 40 to 60 were recruited, with two of them being healthy (non-diabetic), two with T2DM without current or history of DFU, one with T2DM and DFU history, and one with T2DM and current DFU (Wagner Scale 1). Subjects were recruited from the greater Chicago area via research flyers. All interested participants were initially screened through a telephonic interview about their medical history. Inclusion criteria are: age 40-70 years, BMI: 18-40kg/m^2. Participants were excluded if they had any sort of cardiovascular conditions or abnormalities, lower limb musculoskeletal issues that would prevent them from performing knee extension exercise, respiratory disorders, and arteriovenous insufficiencies like intermittent

claudication, renal disorders, and other infectious diseases. The participants involved in the study were engaged in light to moderate level physical activity like walking (15-30mis /day), jogging, cycling and stair climbing.

3.3 RESEARCH PROCEDURE

This study was divided into four visits: Screening, baseline peak power test, 12-week exercise training and post peak power test and totally included 40 visits (Figure 1**)**. Five participants were able to successfully complete all the visits and the subject with DFU only completed the screening and the baseline measurement visit. A complete description of each visit is described as follows:

Visit 1# screening: Consent for the study was obtained only after which all the other protocols were performed. Venipuncture was carried out in which, 12-hour fasting blood was drawn in a 10ml SST tube, centrifuged and sent to the laboratory to measure fasting plasma glucose, cholesterol levels, and 5ml in an EDTA tube to measure HbA1c. Resting EKG with 12 leads was performed as a diagnostic protocol to rule out any existing cardiac issues in all participants. Skin blood flow responses to pressure (reactive hyperemia) and heat (heat hyperemia) was recorded at the great toe (lateral aspect of the first metatarsal) and heel (at the junction of the Achilles tendon and the calcaneus) with the help of Laser Doppler Flowmetry (LDF). Reactive hyperemia and heat hyperemia were recorded with a pressure indenter mounted on the LDF probe and heating probe respectively. This protocol was performed every 2 weeks till the 40^{th} visit. Physical Activity Readiness Questionnaire (PARQ) was obtained for the purpose of safe exercise prescription along

with medical history, drug history, amount of physical activity, resting heart rate and blood pressure.

Visit 2 # Baseline measures: It consisted of protocols like, foot sensory examination, DEXA scan, and the Ankle-Brachial index (ABI).

Visit 3 # Pre -Peak Power Test: All subjects who qualified the first 2 visits, participated in the exercise testing protocol. Initial heart rate and blood pressure were recorded in the supine position followed by the same in the exercise position (high sitting). Blood sugar with the finger stick is recorded prior to the testing. If the blood sugar pre- exercise was less than 100mg/dl, a carbohydrate snack or a juice box was offered to raise the sugar levels. The participants were instructed to perform knee extension using the ergometer at 60RPM. The peak power is achieved, when the subject reaches the threshold by the addition of weights equivalent to 5 watts at an interval of 2 minutes at 60 RPM. A warm up and a cool down period involved performing knee extension without the weight before and after the testing respectively. Heart rate, Blood pressure, and rate of perceived excursion (RPE) scale was recorded after every increase in the weight. All the participants reached their peak within 15 minutes and the 50% peak power was calculated based on 50% of the achieved power. Post-test vitals (HR and BP) was recorded in all participants and post blood sugar was recorded only for only diabetic participants.

Visit 4 # Exercise Training: The exercise visits began from the 4th visit, 3 times /week for 12 weeks. All participants visited the study location 3 times/ week for 30 minutes with a warm up period and a cool down period for 2 minutes. Heart rate, blood pressure was monitored, pre, during, post exercise for both healthy and diabetic groups. Blood sugar was also monitored pre, during (15 min), and post exercise for patients with diabetes. The exercise protocol was followed according to the appropriate exercise considerations by the ACSM guidelines. If the blood sugar pre- exercise

was less than 100mg/dl, a carbohydrate snack or a juice box was offered to raise the sugar levels. The participant was exercised at 50% of the submaximal exercise test. The rate of the perceived excursion was recorded at 10 and 20 minutes. Participants had to maintain 60RPM by looking at the monitor to ensure consistent speed at all intervals.

Post Peak Power Testing: A post peak test was performed at the 41st visit, after 12 weeks of training to measure a change in the peak power. It is performed similar to the peak power testing.

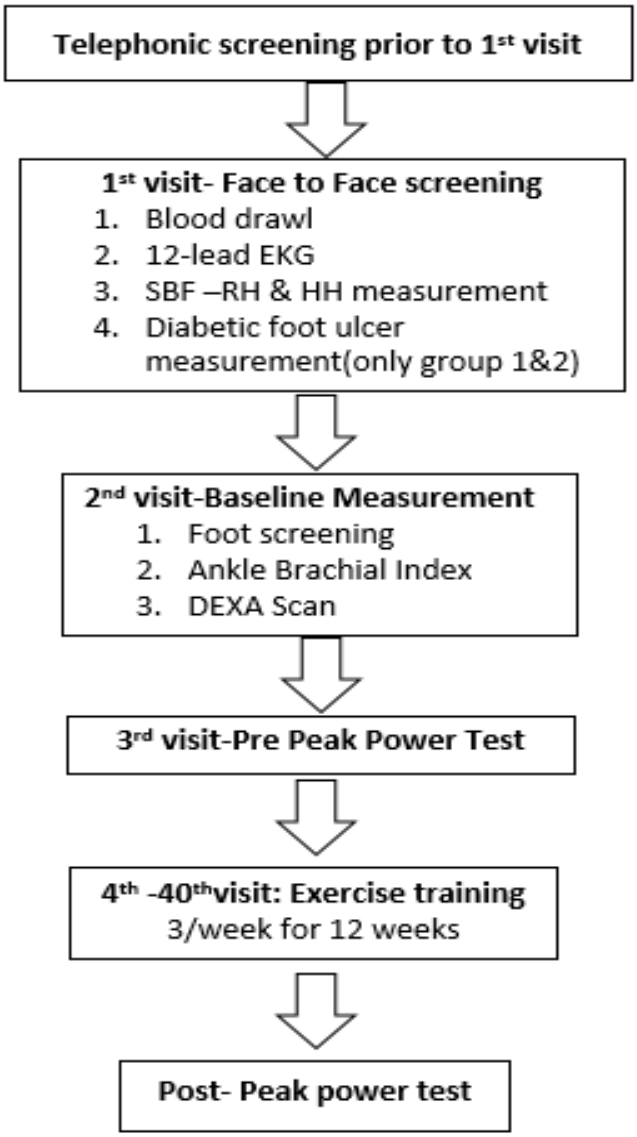


Figure 1.Schematic representation of study procedure

3.4 INSTRUMENTATION

3.4.1 Pressure Indenter

Localized pressure (60 mmHg) was applied for 3 minutes using a pressure indenter at the great toe and heel (high risk areas of DFU) to induce a reactive hyperemic response. This model was constructed using a 3-D printer. This is attached to the LDF probe with a spring to record skin blood flow signals to induce controlled pressure (Zanca, 2006).

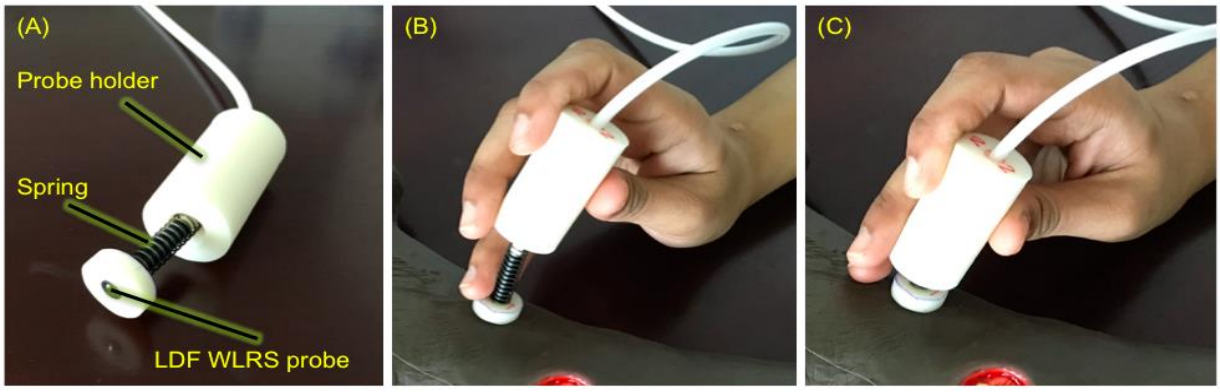


Figure 2. (A) hand- held LDF probe with pressure indenter, (B) no pressure, (C) with pressure application.

3.4.2 Laser Doppler Flowmetry (LDF)

The non-invasive LDF (CP2T-1000 probe, Moor Instrument, Wilmington, DE) was used in the study. It is an FDA approved device that provides noninvasive measurements of skin blood flow at a depth of about 1mm via fiber and laser optics technology. The LDF probe is positioned to record the skin blood flow responses with temperature at the same time. Skin blood flow and StO_2 is monitored with the help of LDF. Heat hyperemic response is assessed with the help of LDF with heater ring. The skin perfusion data was presented in real time through data acquisition software

known as Labchart (AD Instruments) on a computer. Reliability of this instrument has been proved by previous studies that have worked with this model (Tzen et al, 2010).

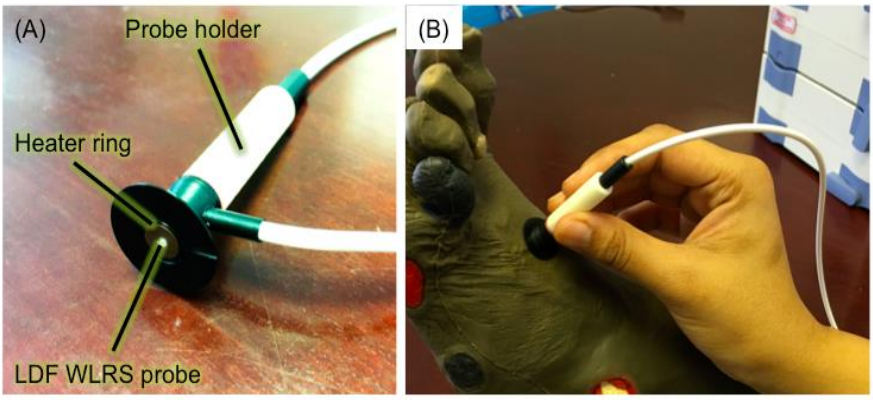


Figure 3 (A) hand held LDF-WLRS probe with heater ring, (B) HH measure.

3.4.3 Doppler Ultrasound

The Edan SonoTrax Doppler was used to measure and rule out peripheral vascular diseases on the subjects that were enrolled into the study. It was used to measure the Ankle –Brachial Index. It is calculated by dividing the higher brachial systolic blood pressure by the higher ankle pressure (tibialis posterior or dorsalis pedis)

3.4.4 Monofilament Sensory Testing

Foot sensation was examined to evaluate loss of protective sensation in Diabetic /Neuropathic or in patients with Diabetic foot ulcer. It is evaluated by Semmes-Weinstein 5.07(10 gram) monofilament. It is randomly tested at 10 sites with eyes closed around the foot area (front and back). It is interpreted by the subject's feedback on feeling the monofilament, if not it is indicated by loss of sensation at that sites. Affection of more than 8 sites indicates high risk of neuropathy, skin changes or foot deformity.

3.4.5 Heart- rate monitor

Digital Polar Heart rate monitor (Polar Equine Health Check FT1 Heart Rate Monitor 93045117) was used to accurately measure resting, during exercise exertion levels, and also its recovery post exercise

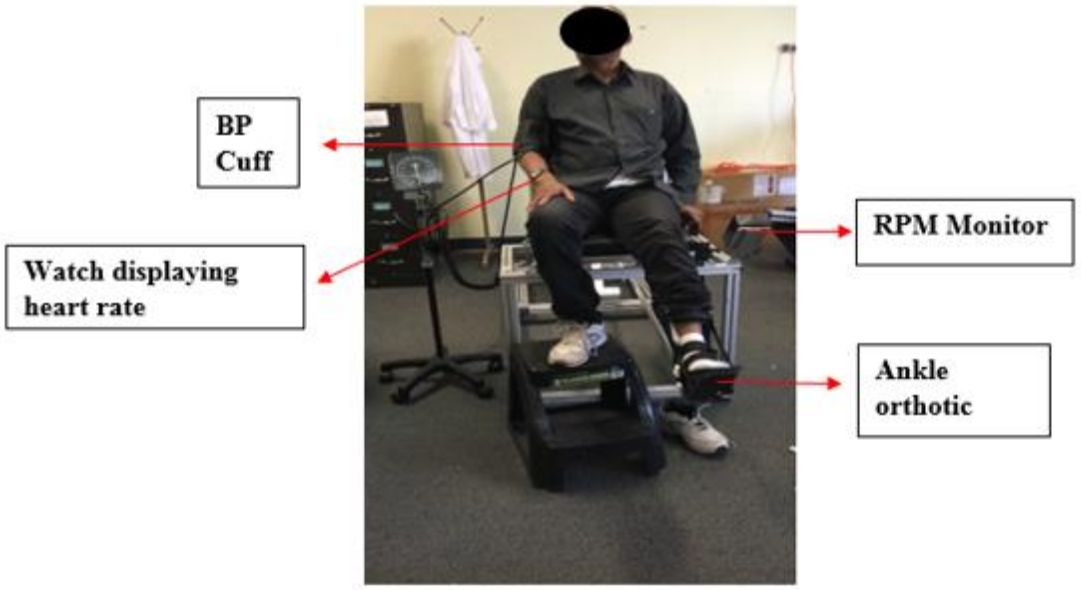
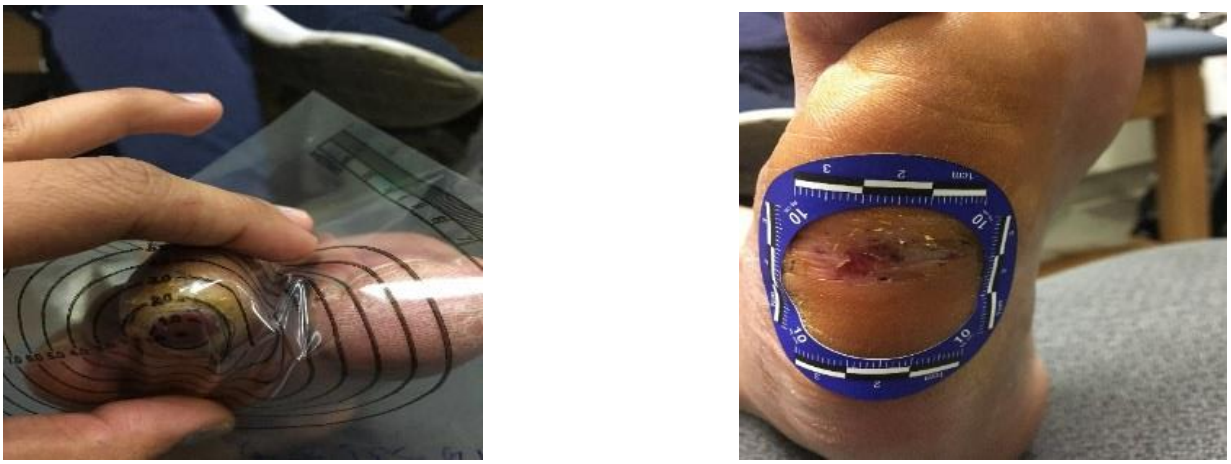


Figure 4. The exercise set up

3.4.6 Wound measuring scale

The wound was measured by the bull eye transparent film by drawing the wound over the film and calculates the diameter of the wound. On the other hand, the wound scale (By KISS Healthcare) was attached around the circumference of the wound and the wound is measured with the help four miniature linear scales. This was used to provide to standardize wound photography even with the help of a regular camera, to reduce subjectivity in wound photographs, and to maintain proper wound documentation for research and health care records.



A **B**

Figure 5. A represents the planimetry method and B represents the wound measuring scale (KISS Healthcare) method.

3.5 DATA COLLECTION

Demographic data (height/weight), and medical history was obtained before the experiment for eligibility of subjects. Body Mass Index (BMI) was calculated based on height and weight using the equation

$$\text{BMI= weight in pounds)/ (height in inches)}^{2} * 703$$

Experimental Data included diabetic history, wound measurements with digital images, ABI, monofilament testing, pre and post peak test, skin blood flow responses: reactive and heat hyperemia, exercise training parameters: HR (Heart rate), Blood pressure, blood sugar. The skin blood flow responses were collected at 20Hz sampling rate. The reactive hyperemia was induced by inducing 60mmHg pressure with the help of a pressure inducer and consists of four parameters, which was investigated (Carolan-Rees, Tweddled, Naka, & Griffit. 2002) and reported by previous researchers (Tzen et al, 2008). Figure 6. Is the skin reactive hyperemic response of subject G1_04. The reactive response is characterized by the following: “A” is the baseline of skin blood flow, “B” is the peak perfusion after pressure relief, “C” is the duration from pressure relief to peak, and “D” is the total perfusion area after pressure relief.

The following are the parameters that were analyzed in the perfusion response after 60mmHg pressure relief

bSBF_Great Toe: the mean value of skin blood flow data collected in the first 1 min before pressure application at great toe

bSBF_Heel: the mean value of skin blood flow data collected in the first 1 min before pressure application at heel

pSBF_Great Toe: (Peak SBF after 60mmHg pressure): the spike in SBF right after pressure relief at the great toe

psbf_Heel: (Peak SBF after 60mmHg pressure): the spike in SBF right after pressure relief at the heel

Npeak_Great Toe: [normalized peak SBF after 60mmHg pressure, (pSBF-bSBF/bSBF)*100: The spike in SBF after pressure relief normalized to bSBF.

Npeak_Heel: [normalized peak SBF after 60mmHg pressure, (pSBF-bSBF/bSBF)*100: The spike in SBF after pressure relief normalized by bSBF.

aSBF_Great Toe (perfusion area after pressure): The area of perfusion after the release of pressure at great toe

aSBF_Heel (perfusion area after pressure): The area of perfusion after the release of pressure at heel

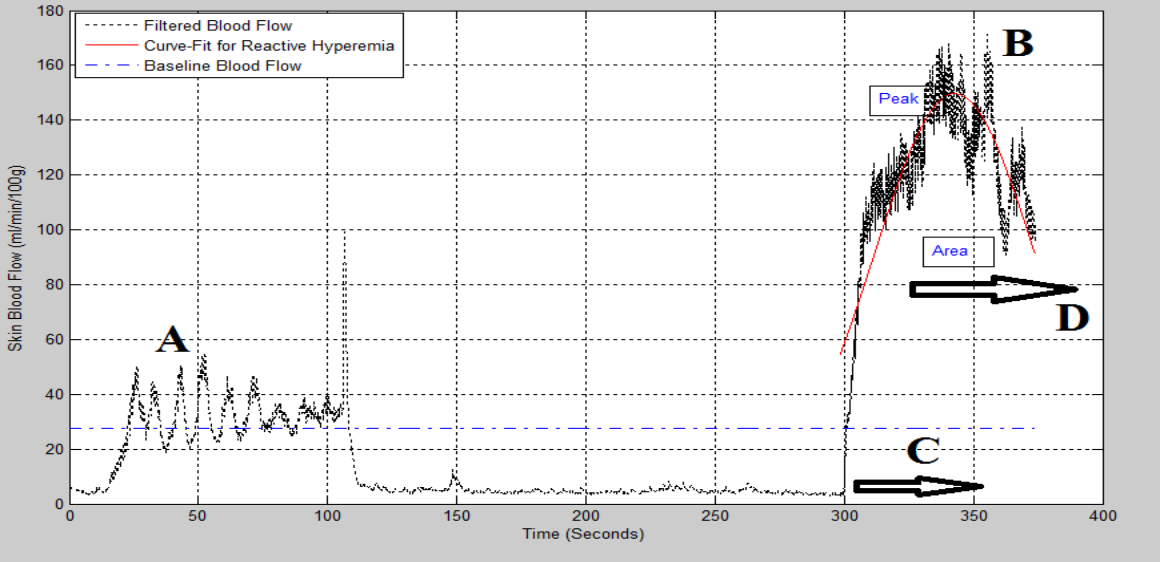


Figure 6. Skin reactive hyperemic response

Figure 7. represents the heat hyperemic response, although very scarcely used in previous literatures to study skin blood flow responses (Minson et al. 2010), a bi-modal pattern and its parameters has been determined (Minson et al.2010, Sorensen et al. 2016). Heat hyperemia was induced by imparting local heat at 41°C, after which, following are the parameters of a heat hyperemic response: "A" is the baseline skin blood flow, "B" is the initial peak determined as the highest 30 sec period of flux occurring within the first 5 min of localised heating, "C" is the nadir which is determined as the lowest 30 sec duration of within 5 min of local heating and "D" Plateau is the last 5 mins of stable flux prior to maximal flux.

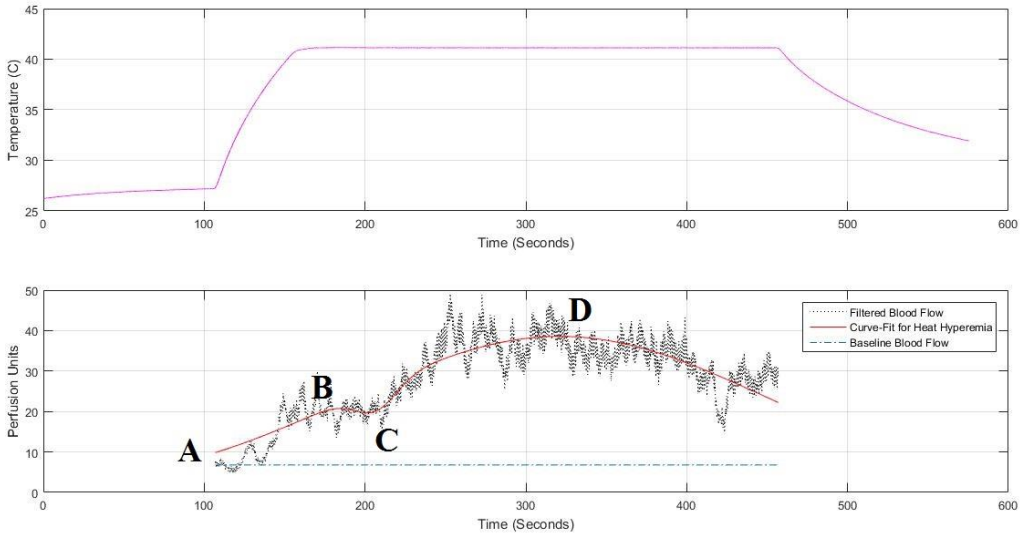


Figure 7. Heat Hyperemic Response

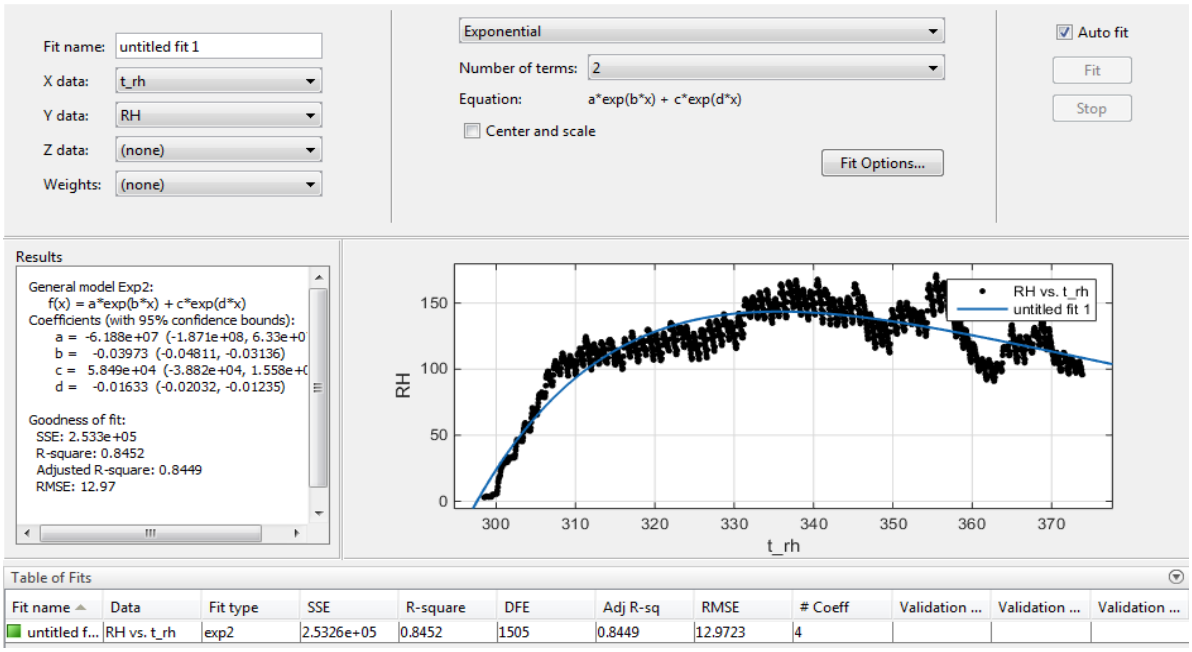
3.6 DATA ANALYSIS

To obtain the parameters of reactive and heat hyperemic response, the skin blood flow signals were processed through the bi-exponential curve fitting tool in MATLAB (MathWorks.Inc). The reactive hyperemic parameters were calculated based on the formula and codes listed in Figure 8 & Figure 9. The heat hyperemic response was calculated based on the formula:

% perfusion during HH= avgHPU -avgBPU/ avgBPU*100

```
7555      %% Areas, Time to Peak, Normalized peak, Peak value,
7556
7557      %Using trapz command and Trapezium integral method
7558 -    results = [];
7559
7560 -    for i = 1:1:length(RH)
7561 -        results(i) = fitresult((i/20)+t_rh(1));
7562 -    end
7563
7564      %for finding the peak
7565 -    [max_value, index_number] = max(results);
7566
7567      %Time to reach the peak
7568 -    time_peak = ((index_number-1)/20);
7569
7570      %Normalized Peak
7571 -    normalized_peak = ((max_value - baseline)/baseline)*100;
7572
7573      %curvefit = results * ones(size(t_rh))
7574      %Area under the Curve Fitting curve
7575 -    Y = trapz(t_rh, baseline_rh);
7576 -    Z = trapz(t_rh, results);
7577 -    area_with_curve = Z-Y;
7578
7579      %Area under the curve with original data
7580 -    Y1 = trapz(t_rh, baseline_rh);
7581 -    Z1 = trapz(t_rh, RH);
```

Figure 8. Data analysis with Matlab coding



Fit name	Data	Fit type	SSE	R-square	DFE	Adj R-sq	RMSE	# Coeff	Validation ...	Validation ...	Validation ...
untitled f...	RH vs. t_rh	exp2	2.5326e+05	0.8452	1505	0.8449	12.9723	4			

Figure 9. Curve fitting tool for plotting area under curve

Since this is a feasibility study with small sample size, data was represented in 3 formats: (1) graphical representation of reactive and heat hyperemic response parameters among all subjects, and before and after exercise training, and (2) visualization of localized heating on SBF. (3) To determine the relationship between the diabetic characteristics and microvascular function, Spearman rho correlation coefficient tests were computed using the SPSS 24 software.

4.0 RESULTS

4.1 PARTICIPANTS

A total of six subjects were recruited including two healthy subjects (G5_01, G5_10), two subjects with T2DM (G4_01, G4_03), one subject with T2DM and history of DFU (G3_03), and one subject with T2DM and current DFU (G1_04). The demographic data is listed in Table.1

Table.1 Demographic Information of all subjects

ID	Age (*years*)	Gender	Race	BMI (kg/m^2)	BP (*mmHg*)
G5_01	53	F	AF-AM	36	120/75
G5_10	41	F	HISP	30.1	120/80
G4_01	58	M	AF-AM	29.1	120/80
G4_03	56	F	AF-AM	30.6	130/80
G3_01	61	M	CAUC	34.3	140/98
G1_04	52	M	ARAB	33.3	135/80

1

Table. 2 shows the Fasting plasma glucose level, HbA1c, body fat percentage, and the lipid profile for all subjects. The HbA1c levels and fasting plasma glucose was the highest in the subject with current DFU followed by the one with history of DFU compared to the diabetic and healthy group. Total fat % and BMI ranged between 35%-51% and 29-36 respectively.

1 AF-AM: African-American, HISP- Hispanic, CAUC- Caucasian, ARAB- Arabian

Table. 3 represents the diabetic history, ulcer history, foot sensory examination and comorbidities of the diabetic subjects.

Table. 2 Metabolic profile for all subjects

ID	Fasting Plasma Glucose(mg/dl)	HbA1c (%)	Total Fat (%)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
G5_01	97	5.5	50.4	90	212	66	128
G5_10	92	5.6	45.6	130	143	43	74
G4_01	76	6.6	41	83	192	79	96
G4_03	108	8.2	39.1	102	139	41	78
G3_01	206	8.8	37.8	116	199	66	110
G1_04	235	10.8	41.4	180	129	35	58

Table. 3 Diabetic &Foot History for all diabetic subjects

ID	G4_01	G4_03	G3_01	G1_04
Diabetic Duration	1 year	6 years	6 years	14 years
Diabetic Control	Metformin	Metformin &Insulin	Metformin &Insulin	Metformin &Insulin
Ulcer history	none	none	Past DFU at right great toe	Current Ulcer at right ball of 2nd toe and lateral aspect of foot
Foot History	none	Bunion surgery at left toe	Amputation of left toe due to water blister	Charcot foot
Foot sensory exam	none	normal	6/10(B/L forefoot)	10/10(B/L)
Comorbities	none	Hypertension& Cholesterol	Cholesterol	Hypertension
ABI	1.07	1.01	1.3	1

4.2 WOUND MEASUREMENT

The subject with DFU had wounds at two sites, one at the right ball of second toe and lateral aspect of foot. The wound was measured with two kinds of wound measuring scales; transparent bull eye film and wound measuring scale (KISS Healthcare Inc) (Figure 10& Figure 11). Table 4 represents the wound characteristics of the subject with DFU

Table 4.Wound characteristics in the DFU subject

Wound Location	Lateral Aspect of foot	Great Toe
Measurements	Length: 3.1cm	Length:1.6cm
	Width: 1.3cm	Width: 1.3cm
	Depth: 0cm	Depth: 0.2cm
Wagner Scale	1	1
Dressings used	Betadine and Gauze	Betadine and Gauze

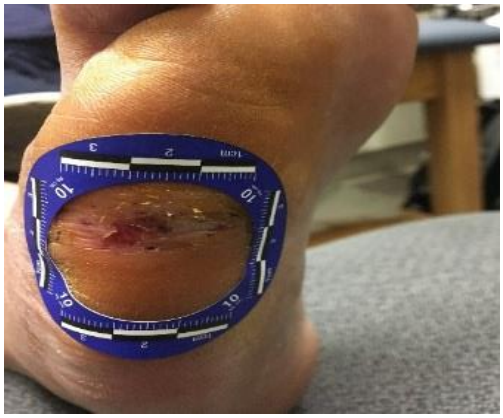


Figure 10. Wound measured by wound scale (By KISS Healthcare)

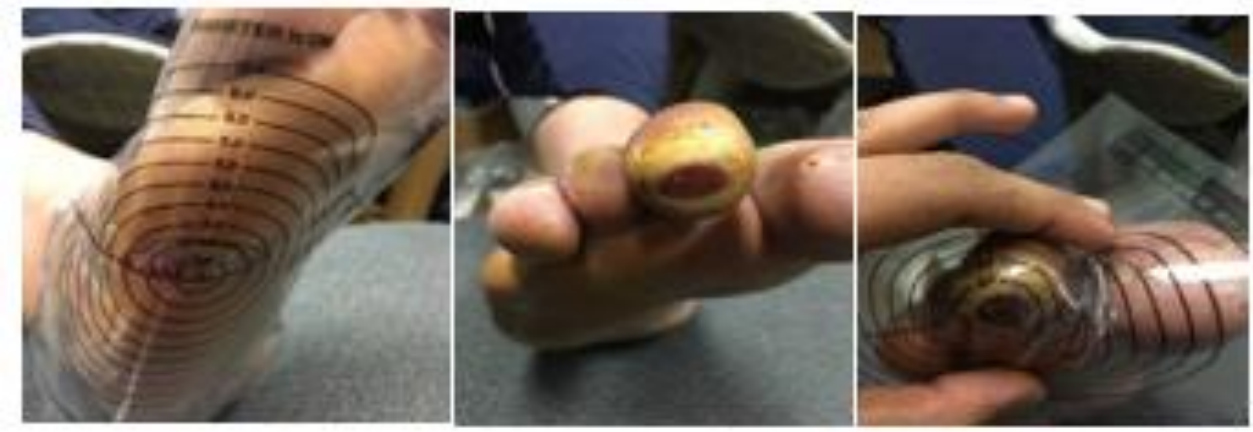


Figure 11. Wound measured by bull eye transparent film

For the first part of the study, the two hypothesis that were tested, included the microvascular response parameters to pressure which were; Normalized peak skin blood flow (NpSBF), Perfusion Area (aSBF), Time to peak (TpSBF) and similar heat that included baseline skin blood flow (bSBF), peak skin blood flow (pSBF) and % perfusion(%SBF).

4.3 REACTIVE HYPEREMIC RESPONSE

Figure 12**.** Demonstrates the baseline skin blood flow response for all the participants at the great toe and heel, before the 60mmHg pressure application. ***At the Great Toe***: The subject with the DFU history and one of the healthy individuals (G5_10) showed the highest baseline skin blood flow, followed by a diabetic (G4_01) and the subject with DFU. One of the healthy individuals (G5_01) and a diabetic (G4_03) had the least baseline skin blood flow ***At the Heel:*** Similarly, the

subject with the DFU history had the highest baseline skin blood flow compared to all the other subjects. On the other hand, the baseline in the subject with DFU (G1_04), diabetic (G4_03) and healthy (G5_01) individual had almost similar baseline values. Surprisingly, one of the healthy subjects (G5_10) had the least baseline skin blood flow at the heel. Figure 13**.** Demonstrates the Normalized peak (Np) skin blood flow response for all the participants at the great toe and heel, after the 60mmHg pressure release. ***At the Great Toe***: The peak for the subject with DFU was the highest, followed by the subject with DFU history. Conversely, the peak for one healthy individual (G5_01) was observed to be the least. However, the other healthy subject (G5_10) had greater peak compared to the diabetic subjects. ***At the Heel:*** The peak at the heel was higher at the heel of the subject with DFU history and was observed least in one of the diabetic subjects (G4_03). A better response of the peak was observed at the heel for the diabetic subject (G4_01). Moreover, the peak observed for both healthy subjects did not show major differences compared to one of the diabetic subjects (G4_03).

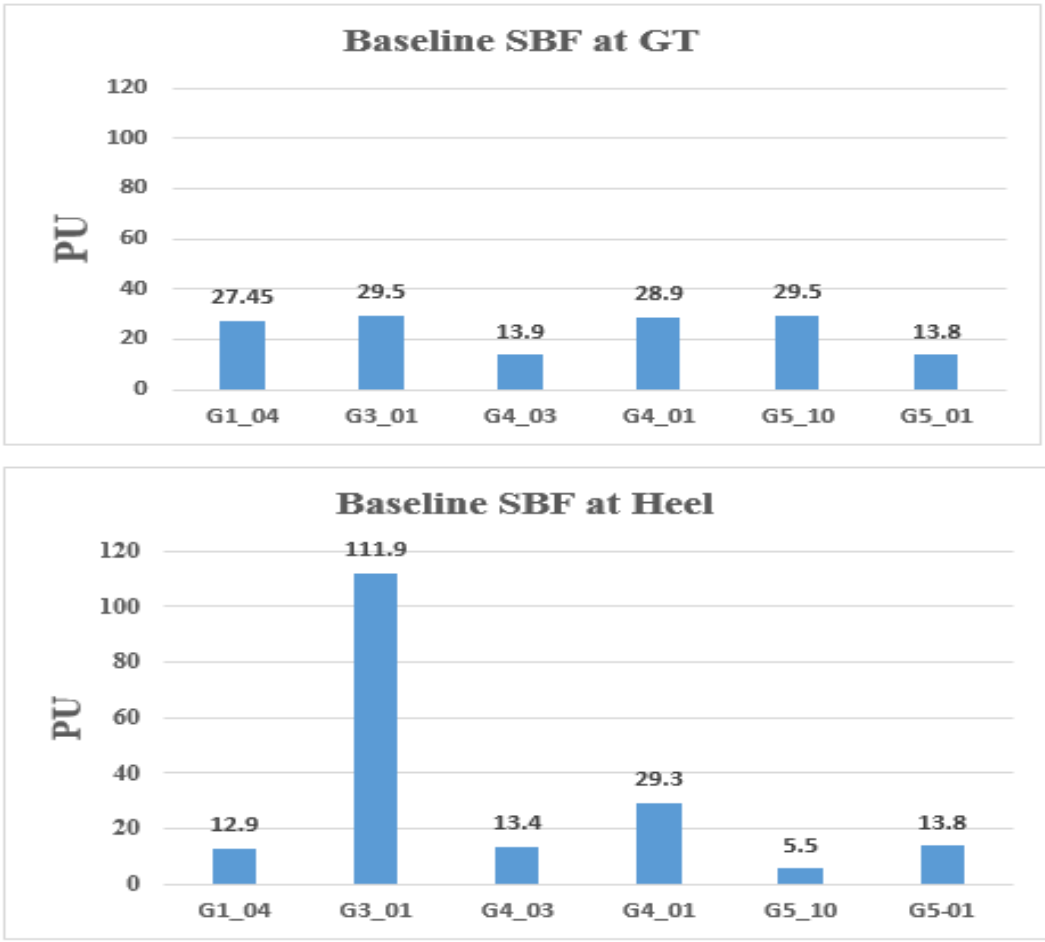


Figure 12. bSBF at Great toe and heel during RH

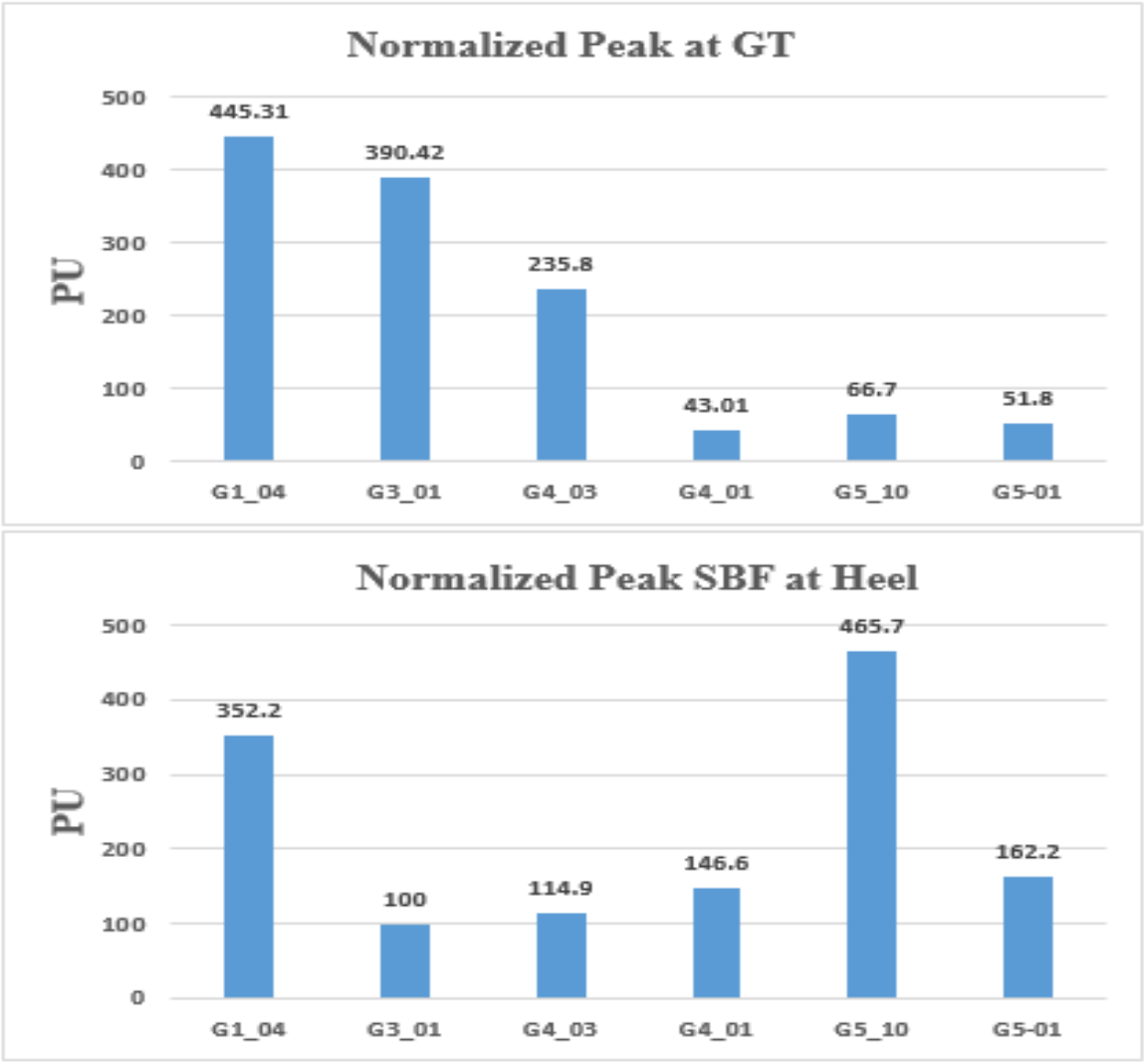


Figure 13. Npeak at great toe and heel during RH

Figure 14. Demonstrates the time to peak of skin blood flow response for all the participants at the great toe and heel, after the 60mmHg pressure release. ***At the Great Toe***: The time taken to reach the peak of skin blood flow at the great toe was observed to be the longest in the subject with DFU. The subject with DFU history had a longer time to peak than diabetic subjects. However, a major difference in the time to peak was not found in between the diabetic and healthy individuals, but a descending pattern of time to peak was observed considering the diabetic status of diabetes. ***At the***

heel: All the participants showed a reduced time to peak compared to the great toe. Similar to the response observed at the great toe, the subject with DFU had the longest time to peak. The time taken for the peak was shorter in the subject with the DFU history and the diabetic subjects. Time to peak was the shortest in the healthy subject (G5_10).

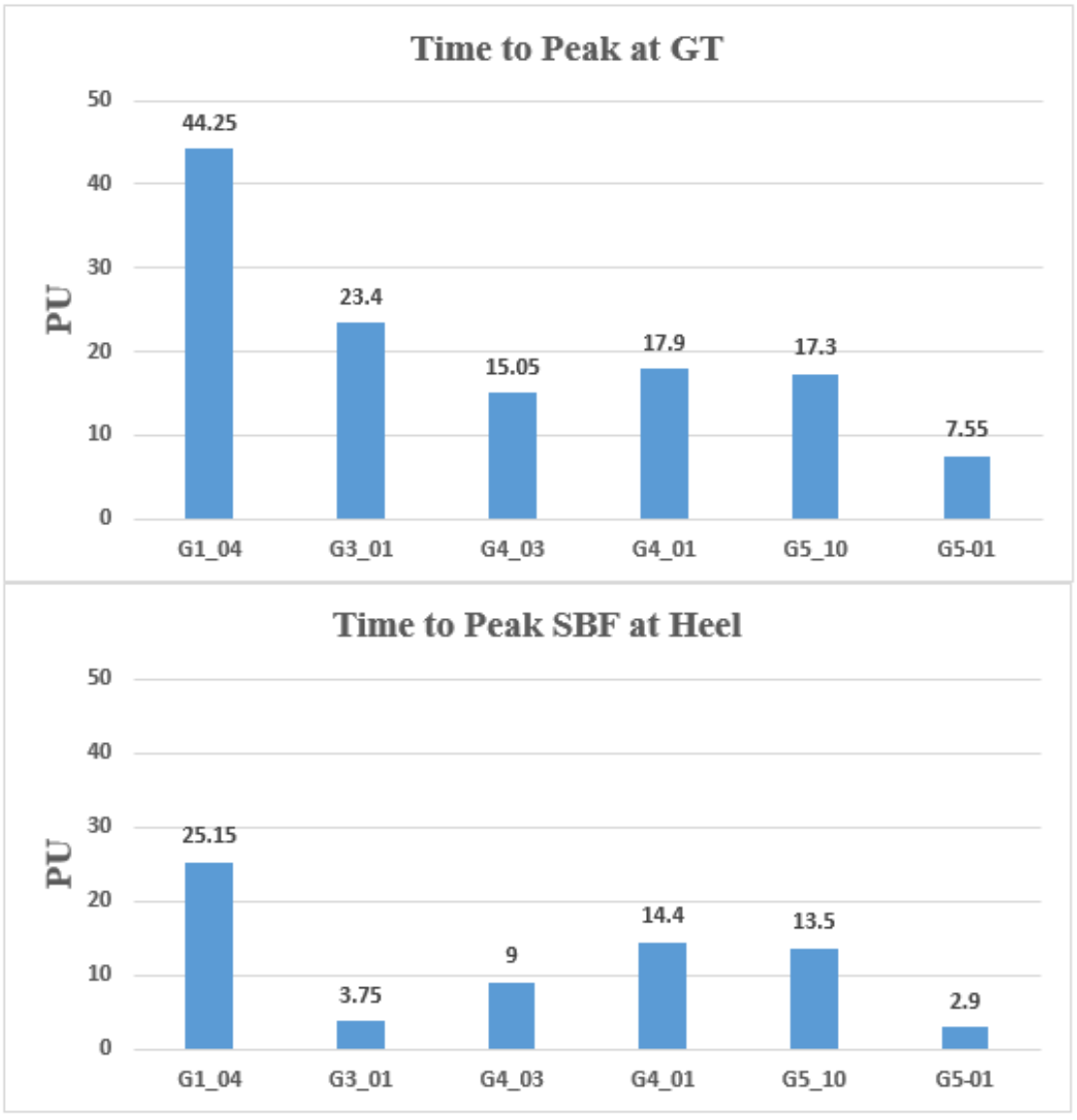


Figure 14. Tpeak at great toe and heel during RH

shows the perfusion area of skin blood flow at the great toe and heel in all the participants after the release of the 60mmHg pressure. ***At the great toe:*** The perfusion area of skin blood flow responses at great toe was greatest in the subject with DFU and least in the healthy subjects. A considerable difference in the perfusion area was observed between the the subject with DFU, history of DFU and only diabetes.There was not much difference observed between the diabetic and healthy perfusion area. ***At the heel*** :Similar to the great toe, the perfusion at the heel was the highest compared to other participants, but lesser compared to at the great toe. The perfusion area at the heel was greater in the both diabetic subjects and one of the healthy subject(G5_10). The response at the heel was the least compared to all participants and also lesser compared to response observed at the great toe (Figure 15)

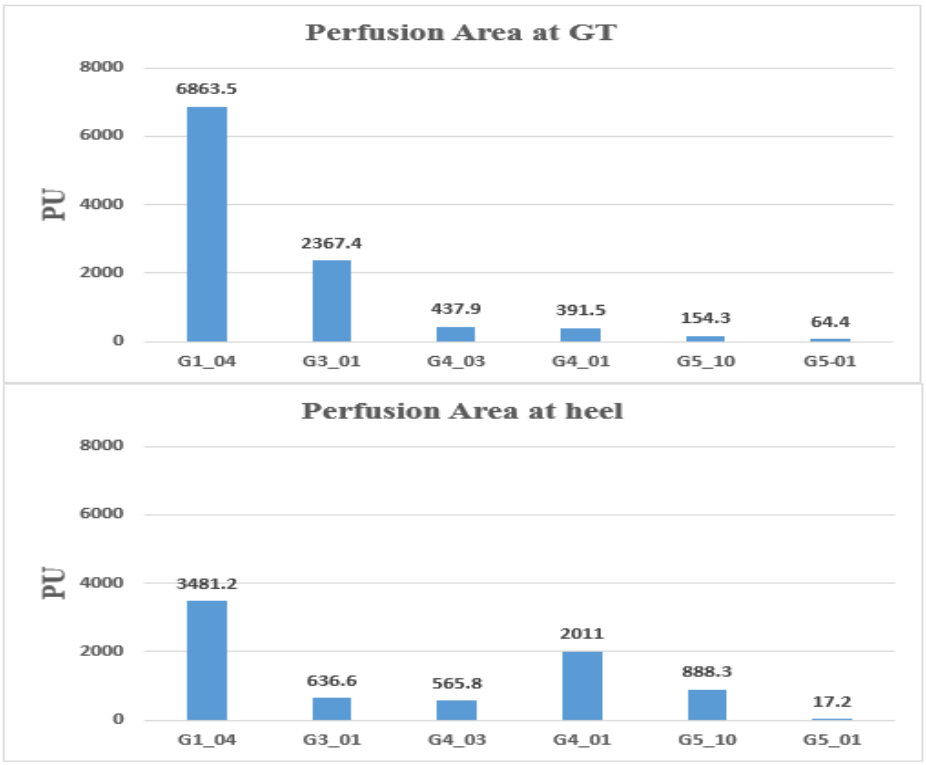


Figure 15. Asbf at great toe and heel

4.4 HEAT HYPEREMIC RESPONSE

Figure 16. Represents Baseline skin blood flow in all the participants at the great toe and heel before the application of heat at 41°C. ***At the great toe:*** The baseline skin blood flow at great toe was observed to be the greatest at in the subject with DFU subject. These responses were considerably reduced in the diabetic (G4_01) and healthy (G5_01), but greater than the responses in the other participants. The SBF response was the least in the healthy individual (G5_10). ***At the Heel:*** All the responses at the heel were reduced compared to baseline observed at the great toe. Although the responses observed at the heel were greater in the healthy individual, diabetic and subject with DFU history and the least response was obtained in the healthy individual. Figure 17. demonstrates the peak during the 41°C heat application at the great toe and heel. ***At the Great Toe:*** The highest peak was obtained at the great toe in the subject with DFU history followed by the diabetic subejct(G4_03).However, the other diabetic patient (G4_01) obtained the lowest peak amongst all the participants.***At the heel***: The peak SBF response obtained at the heel was highest in the healthy subject (G5_01) and a little lesser in the subject with DFU history, however the difference between both responses was not appreciable.The diabetic individual (G4_03) showed the least response in the peak.

Figure 18. shows the perfusion % of skin blood flow after the decrease in the temperature from 41°C.***At the Great Toe:*** The highest perfusion % was observed in the healthy subjects compare to other diabetic subjects. The least perfusion % was observed in the diabetic subject (G4_01) which was considerably less compared to other diabetic subjects. However, the perfusion % between the DFU subject and the diabetic subject did not have major differences. ***At the heel:*** Similar responses were observed at the heel. The perfusion % was greatest in the healthy subject compared to other

subjects. There was also a major difference in the perfusion % amongst both the healthy subjects. The least perfusion % was observed in the diabetic subject than the subject with DFU and the subject with DFU history.

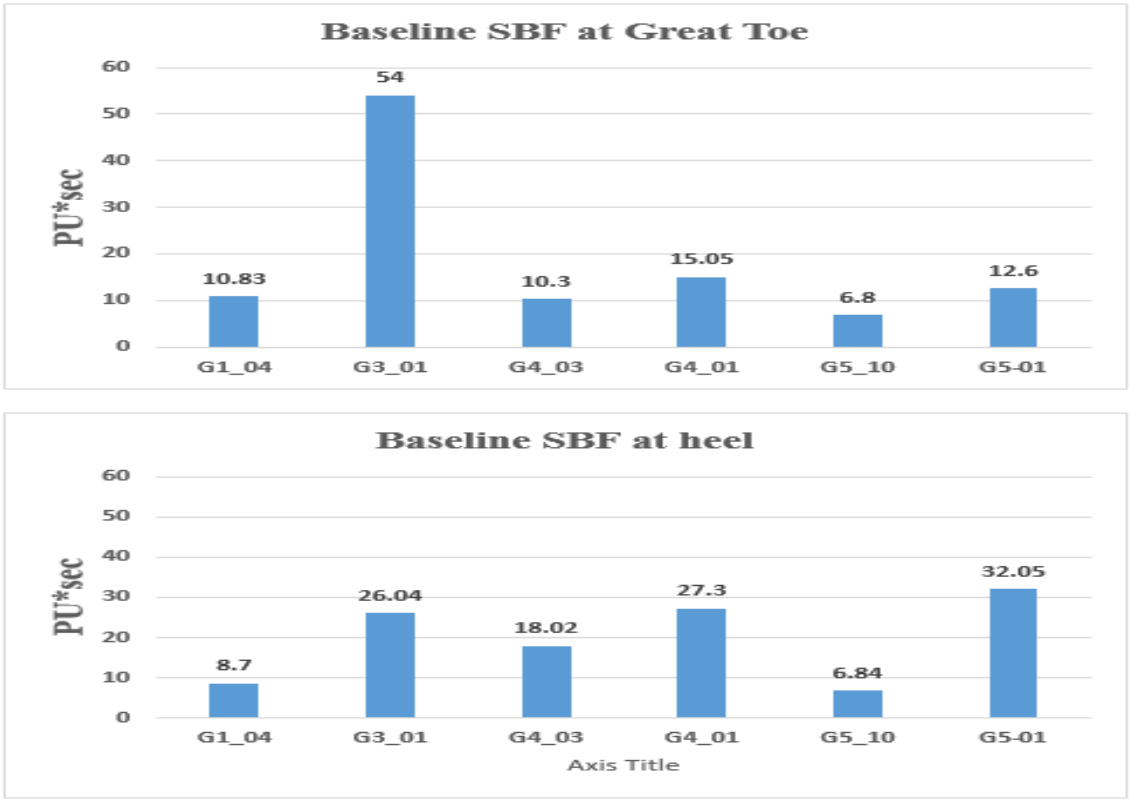


Figure 16. Bsbf at great toe and heel during HH

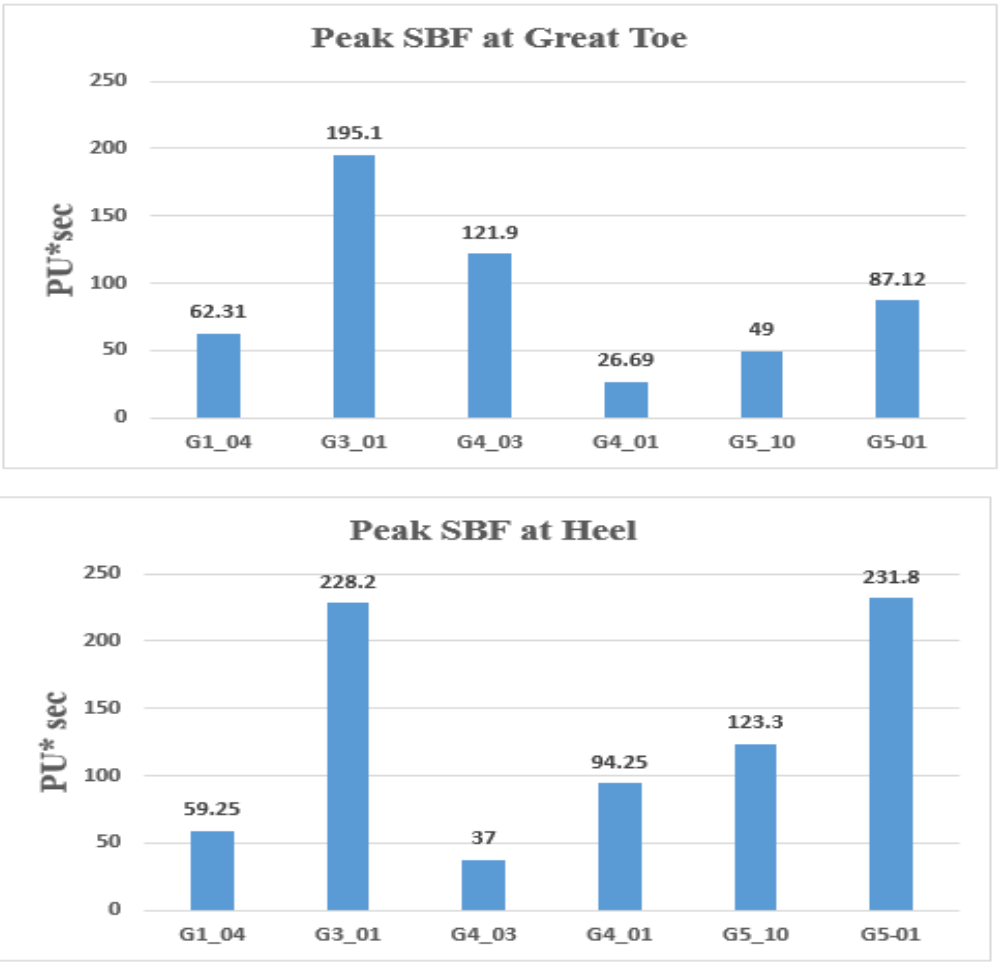


Figure 17. Peak SBF at great toe and heel during HH

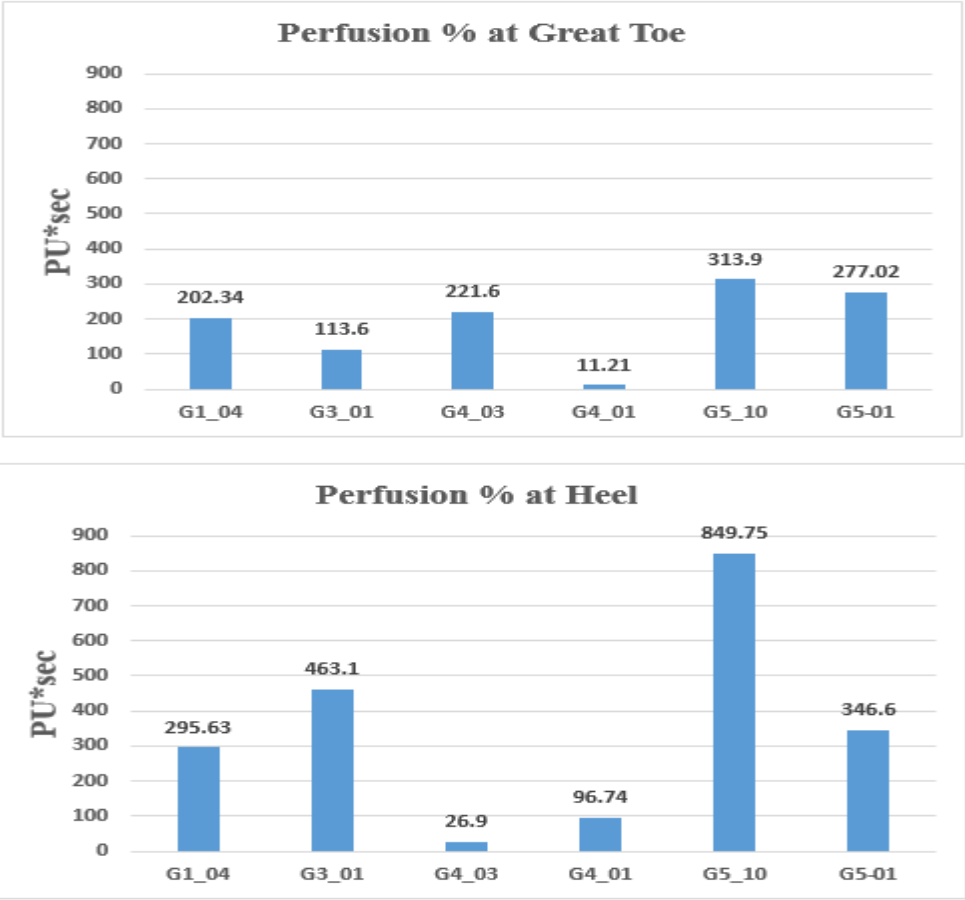


Figure 18. Perfusion % at great toe and heel during HH

Peak power testing

All the subjects except the one with diabetic foot ulcer, were successful in completing the 12-week exercise program. Power used during the 12-week exercise increased in all the subjects except the subject with history of foot ulcer and a diabetic subject. The characteristics pre and post exercise is listed in the Table 5 below.

Table 5

ID	Pre-exercise peak(watts)	50% Pre-exercise peak(watts)	Post-exercise peak(watts)	50%Post-exercise peak(watts)
G5_01	25	13	30	15
G5_10	25	13	35	18
G4_01	15	8	25	13
G4_03	20	10	15	8
G3_01	30	15	30	15

4.5 REACTIVE HYPEREMIC RESPONSE POST EXERCISE

Following are the figures that describe the reactive hyperemic responses to pressure at the great toe and heel post 12 week exercise training.

Figure 19. describes the baseline SBF post exercise.***At Great Toe:*** There was a major difference in the baseline SBF at the great toe post exercise only for one healthy subject(G5_01).Post exercise baseline SBF decreased for majority of the subjects.***At the heel :*** The baseline SBF decreased at the heel in all the subjects except for the one healthy subject(G5_10). However, the increase in the

baseline SBF was small in the healthy subject. Figure.20. corresponds to the Peak SBF post exercise.***At the Great Toe:*** There was an increase in the peak SBF at the great toe only in 3 subjects(G5_01, G4_01, G3_01) post exercise. However, only healthy and diabetic subject demonstrated a greater difference, whereas the subject with DFU history showed a small increase in the peak compared to week 1. ***At the Heel:*** Also, at the heel, there was increment in the Peak SBF in both diabetic and one healthy subject(G5_01). Although, the increase was small compared to the peak at week 1. Figure 21. represents the time to peak post exercise at great toe and heel.***At great toe:*** Although the decrease in the time to peak was small post exercise, it reduced in all subjects except in one diabetic subejct(G4_03). ***At the heel :*** The time to peak reduced in only 2 subjects, however the difference observed was greater in the diabetic compared to in the healthy subject. Figure 22. demonstrates the perfusion area of SBF at the great toe and heel post exercise.Variable responses of perfusion area were observed at great toe and heel. Only G5_10 observed a decrease in the perfusion area at the great toe and heel. In the other subjects, some showed a decrease in the perfusion area at the great toe and increase in the perfusion at the heel and some showed a vice versa response in the perfusion area

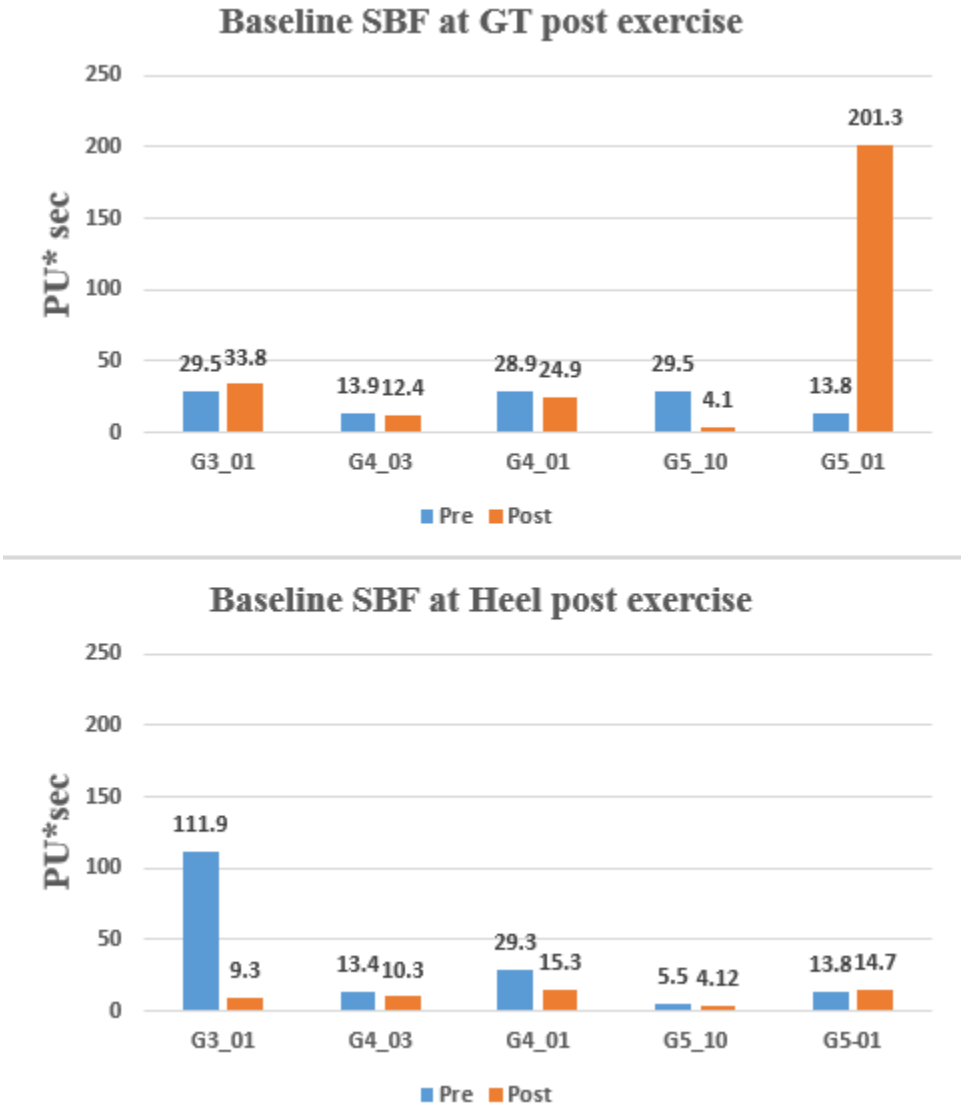


Figure 19. Bsbf at great toe and heel during RH post exercise

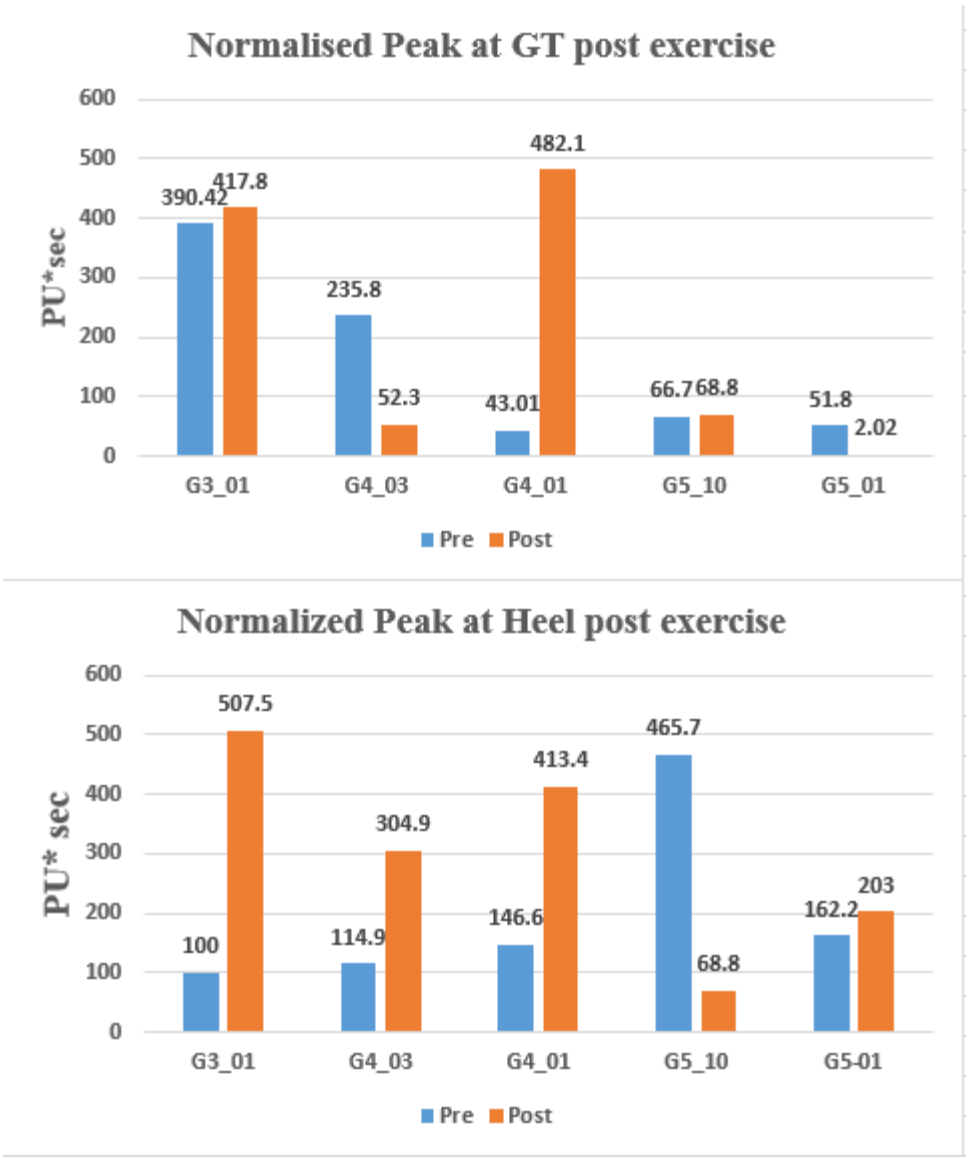


Figure.20 Npsbf at great toe and heel during RH post exercise.

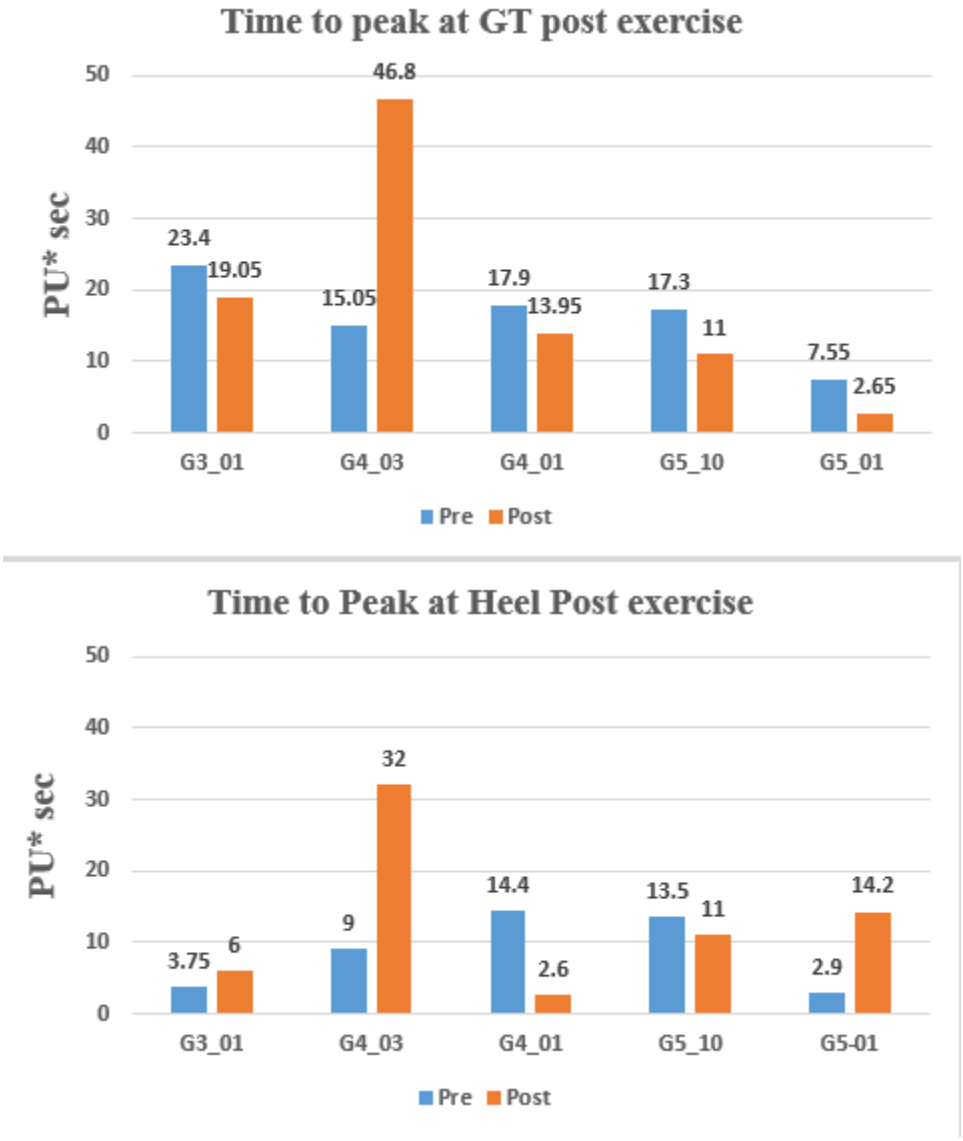


Figure 21. Tpeak at great toe and heel during RH post exercise

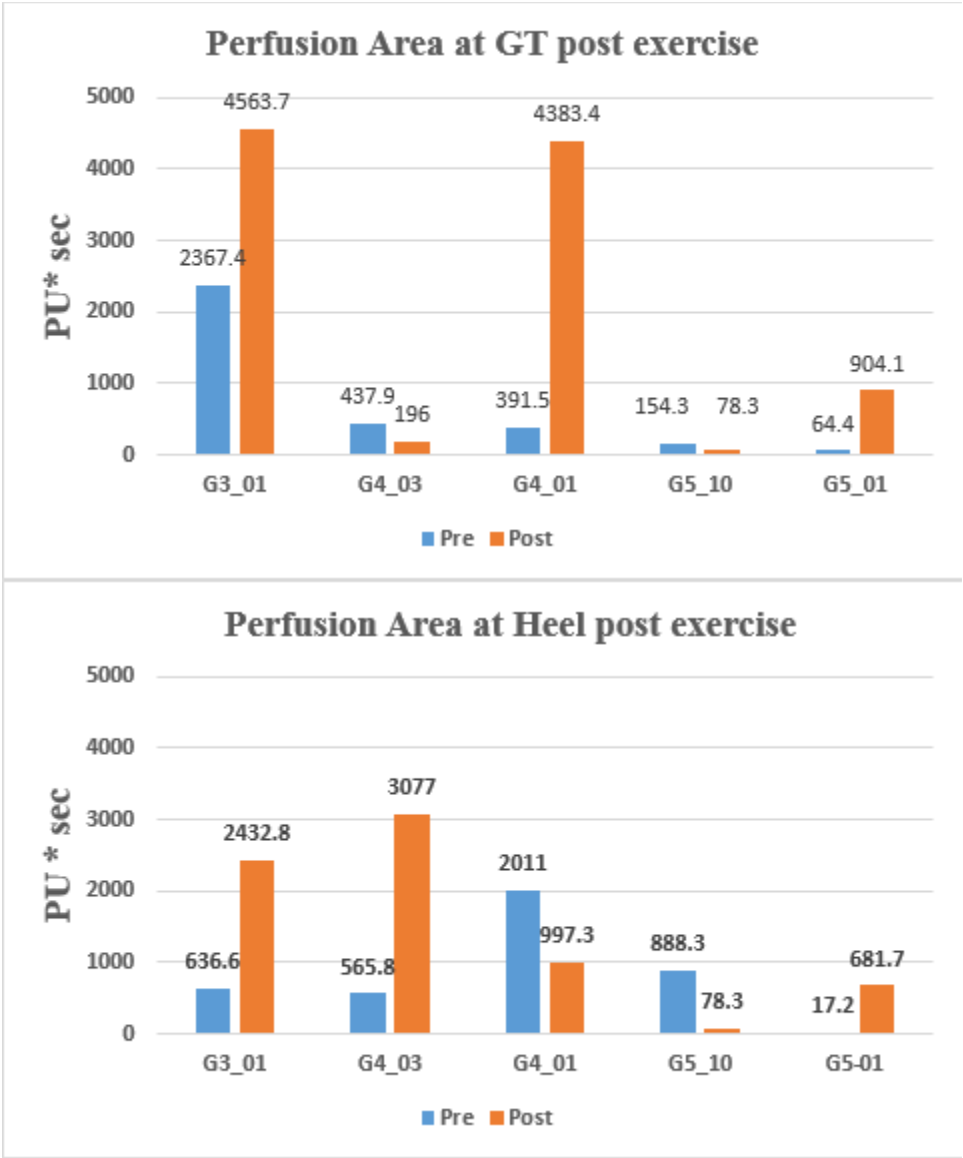


Figure 22. Asbf at great toe and heel during RH post exercise

4.6 HEAT HYPEREMIC RESPONSE POST EXERCISE

Figure 23, Figure 24& Figure 25 represents the parameters of heat hyperemic response; baseline, peak and perfusion %, pre and post exercise at the great toe and heel in all subjects, respectively. The baseline SBF increased post exercise at the the diabetic and healthy subjects and did not increase in the subject with DFU history at great toe.Baseline SBF decreased at the heel in all the subjects post exercise compared to week 1. The peak SBF at the great toe increased considerably in both the healthy subjetcs and diabetic subject (G4_01) post exercise compared to week 1 and the peak at the heel increased only in the diabetic subjects at the heel post exercise.The perfusion % post exercise at the great toe increased in both healthy and diabetic(G4_01) at the great toe, where as the perfusion % at the heel considerably increased at the heel in both the diabetic subjects and healthy subject.However, the difference in the perfusion % in the healthy suebject was small.

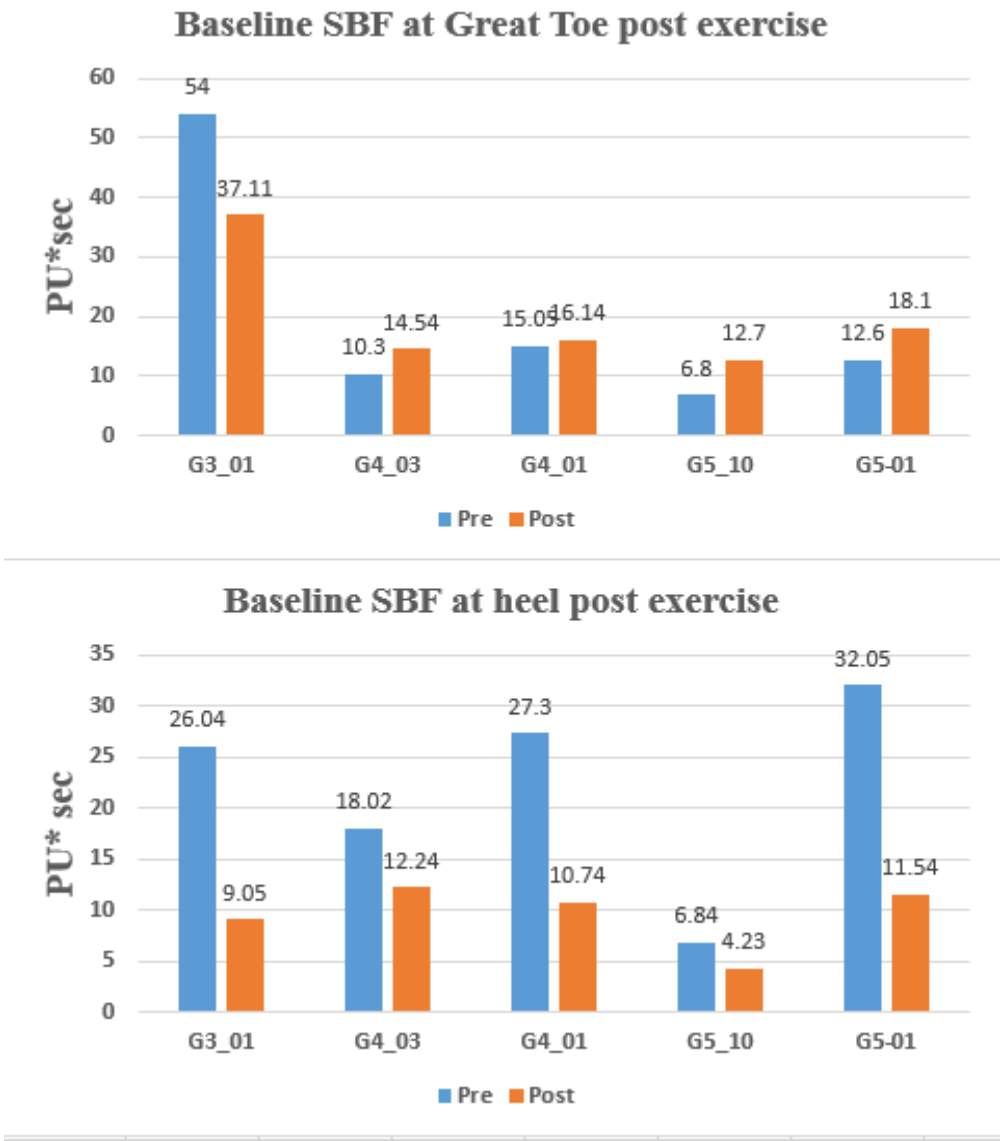


Figure 23. bsbf at great toe and heel during HH post exercise.

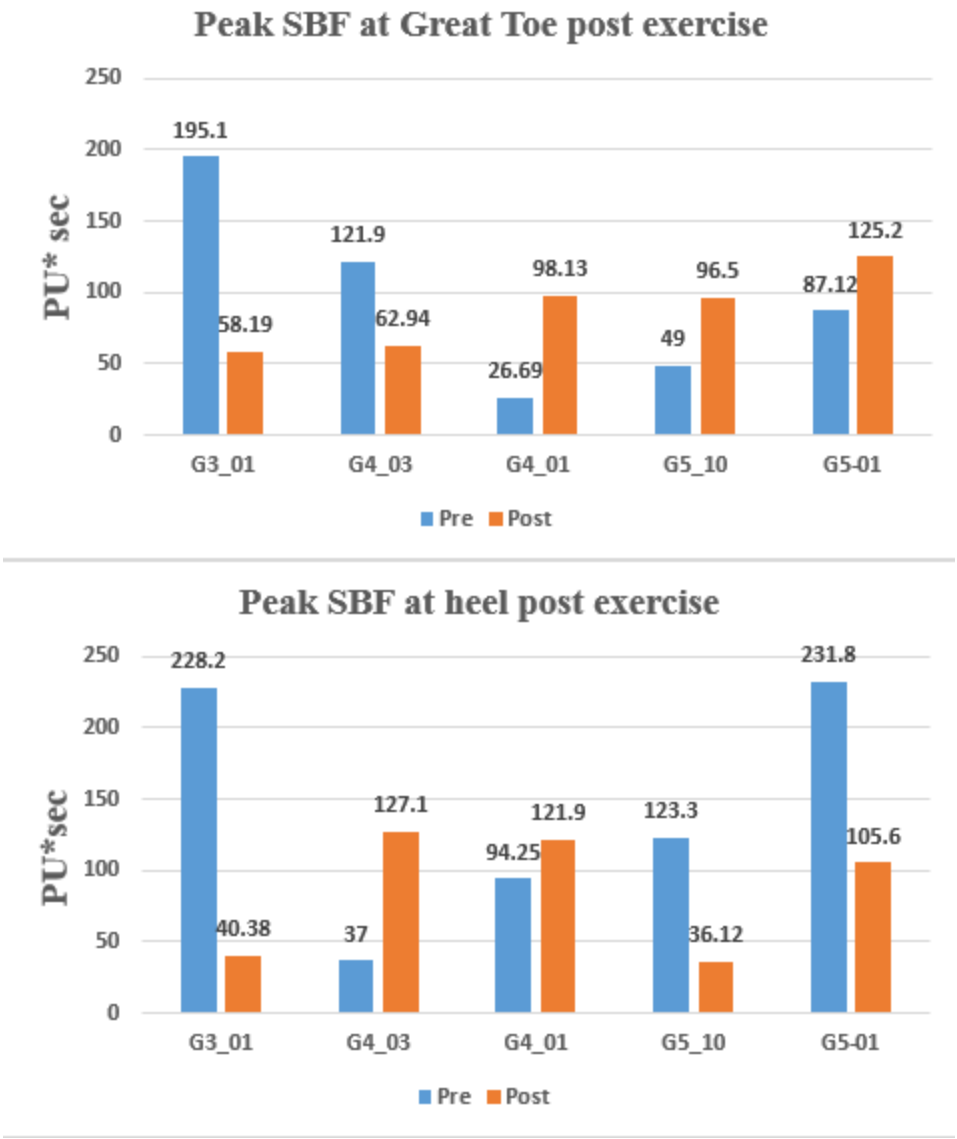


Figure 24. Peak sbf at great toe and heel during HH post exercise.

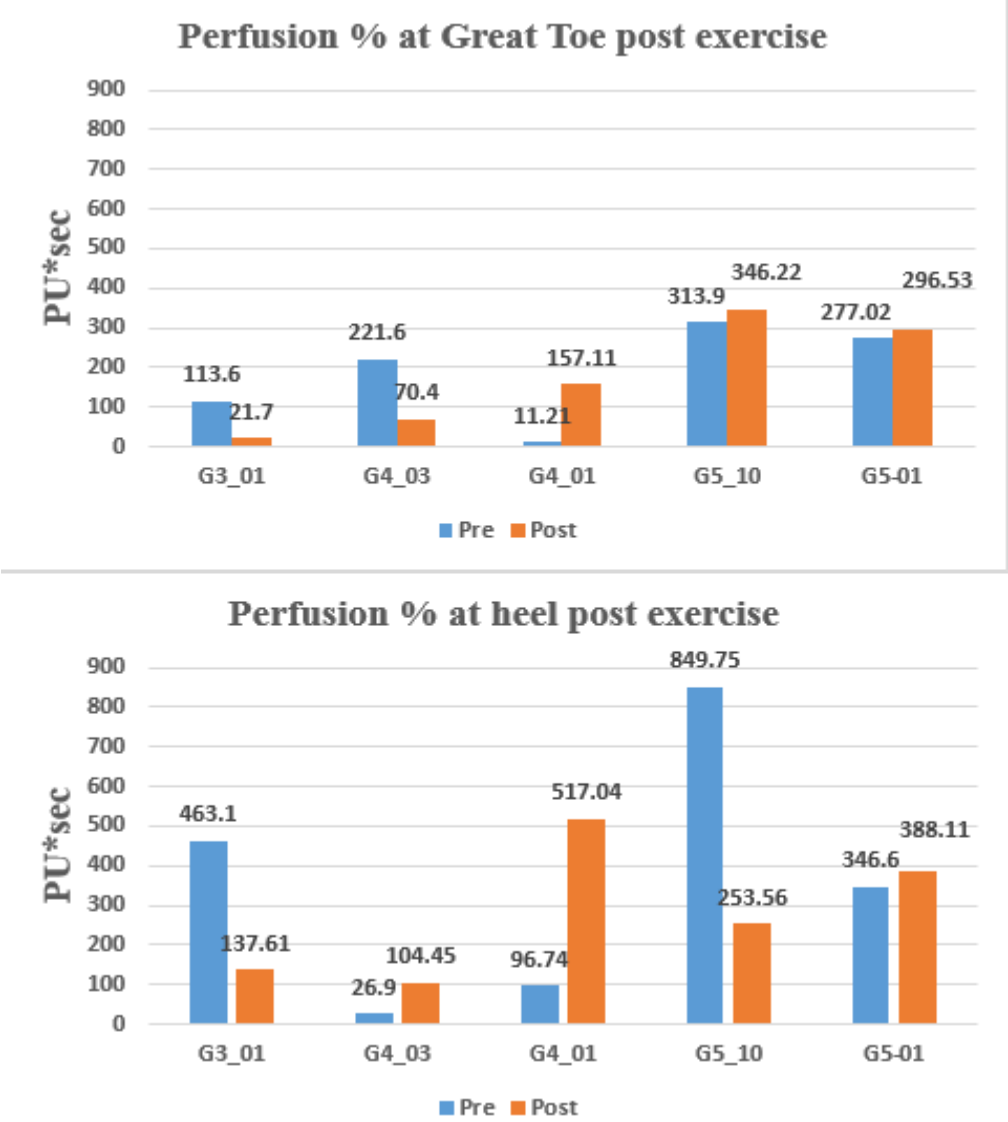
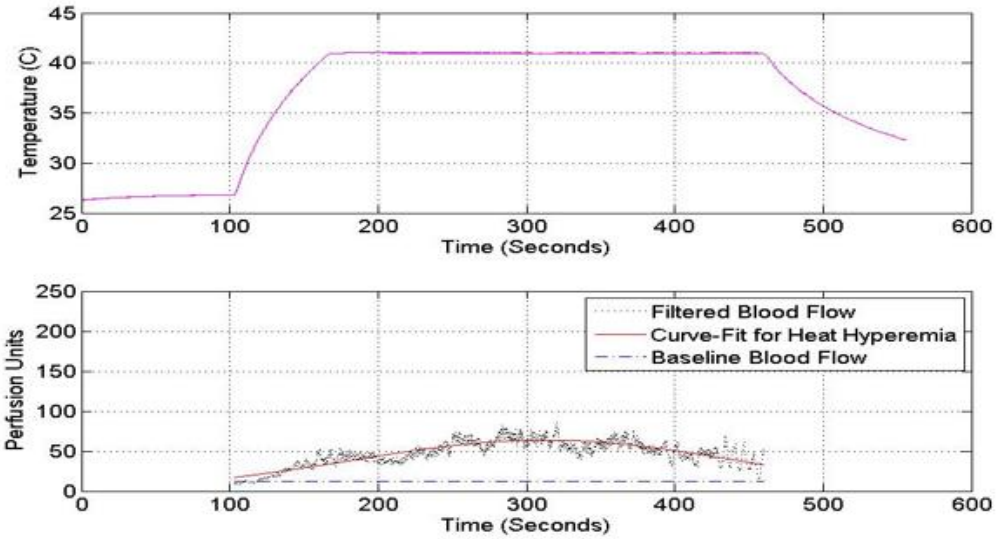


Figure 25. Perfusion % at great toe and heel during HH post exercise.

Following figures represent the plots of the heat hypremic response in G5_01, G5_10, G4_01 and G3_01 pre and post exercise. Healthy subject, , G5_01 demonstrated a monophasic response before exercise and an appearance of a bi-phasic nature in the heat hyperemic response post exercise only at the heel This response was not observed at the great toe. A bi-phasic response in the heat hyperemia was observed in G5_10 at the great toe before exercise with an appearance of an early initial peak followed by nadir and the plateau phase. Post exercise, also a bi-phasic

repsonse was observed at the great toe, with a delay in the initial peak compared to pre exercise state. Also, Heat hyperemia at the heel showed a monophasic response pre and post exercise. All the other participants showed a monophasic reesponse to heat hyperemia before and after exercise at the great toe and heel.

Heat Hyperemia at GT–Week 1



Heat Hyperemia at GT–Week 11

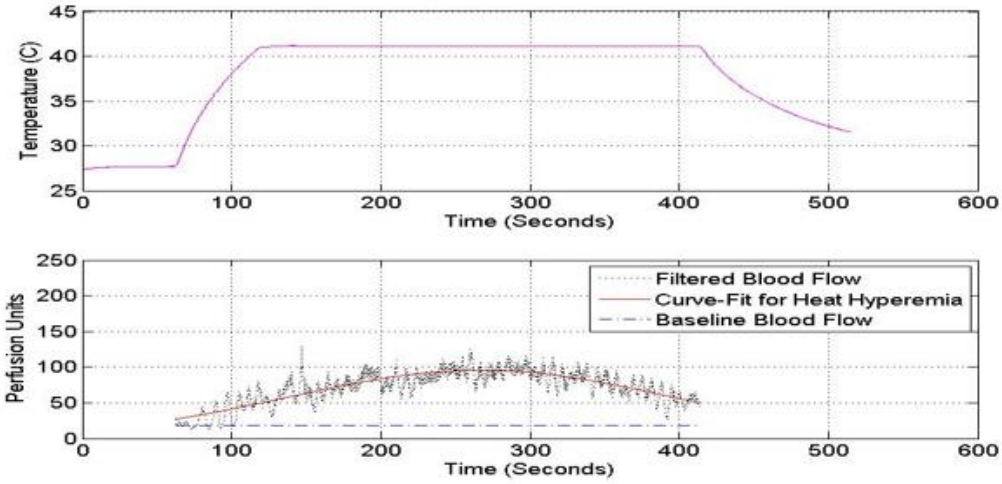


Figure 26. Heat Hyperemia in G5_01 at Great Toe

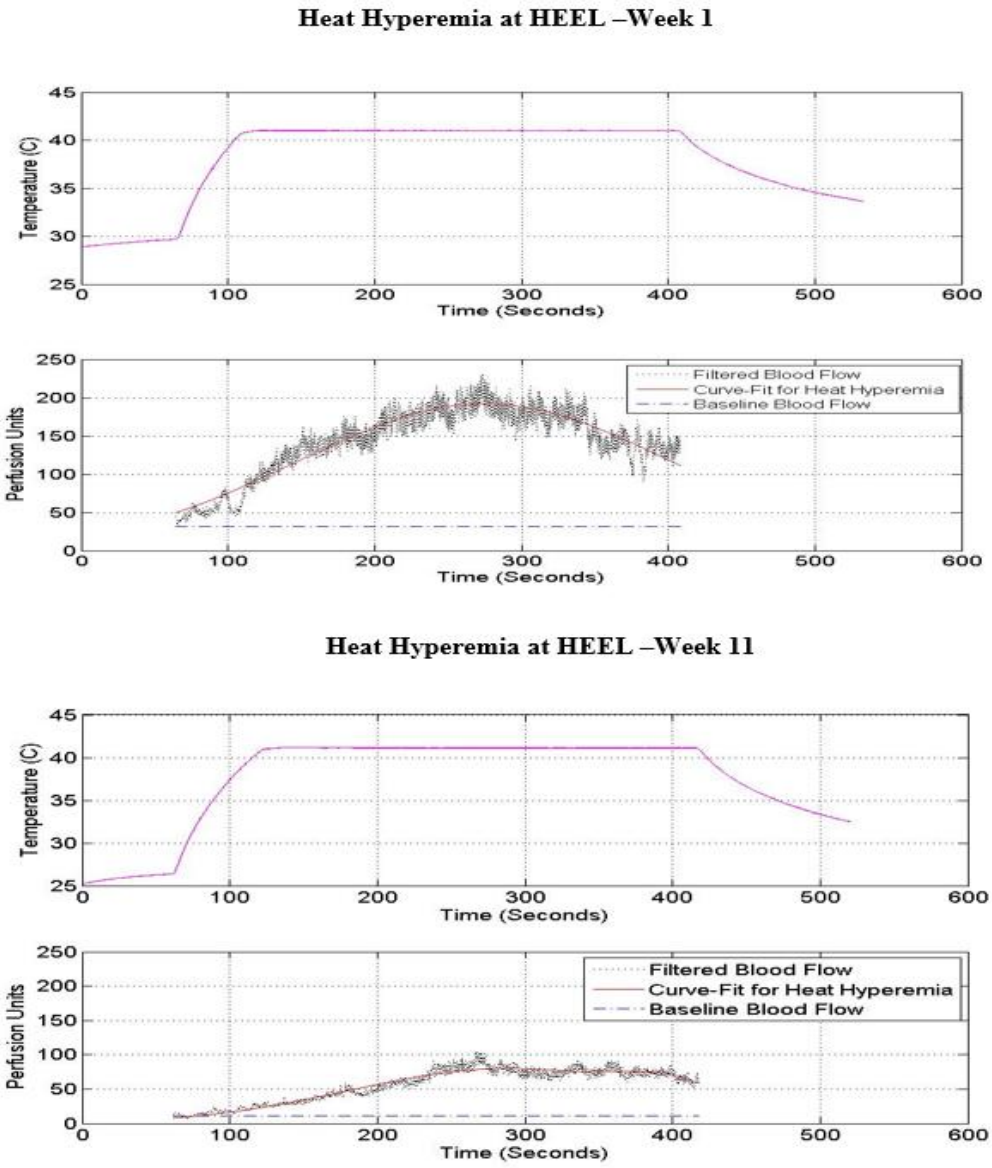


Figure 27. Heat Hyperemia at Heel in G5_01

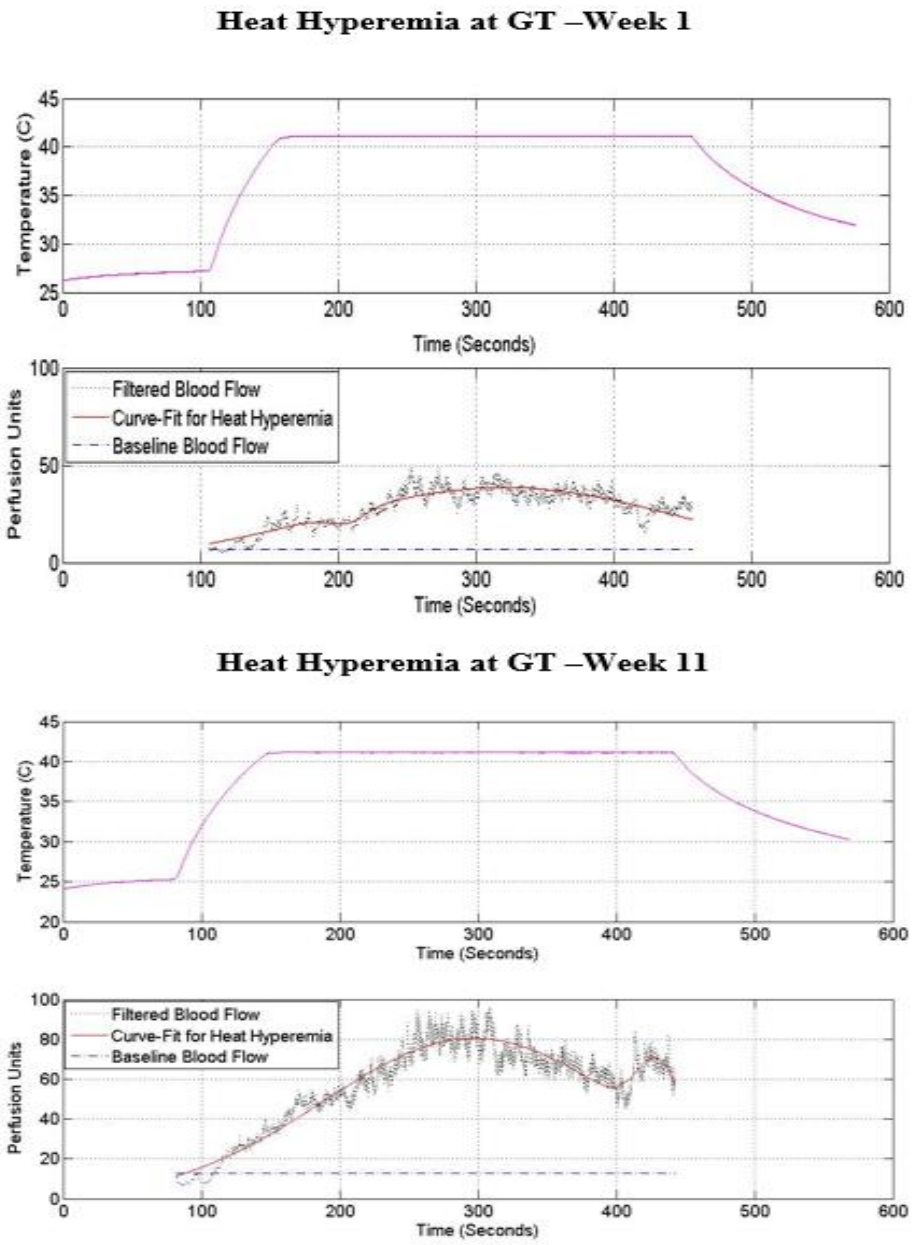


Figure 28. Heat Hyperemia at Great Toe in G5_10

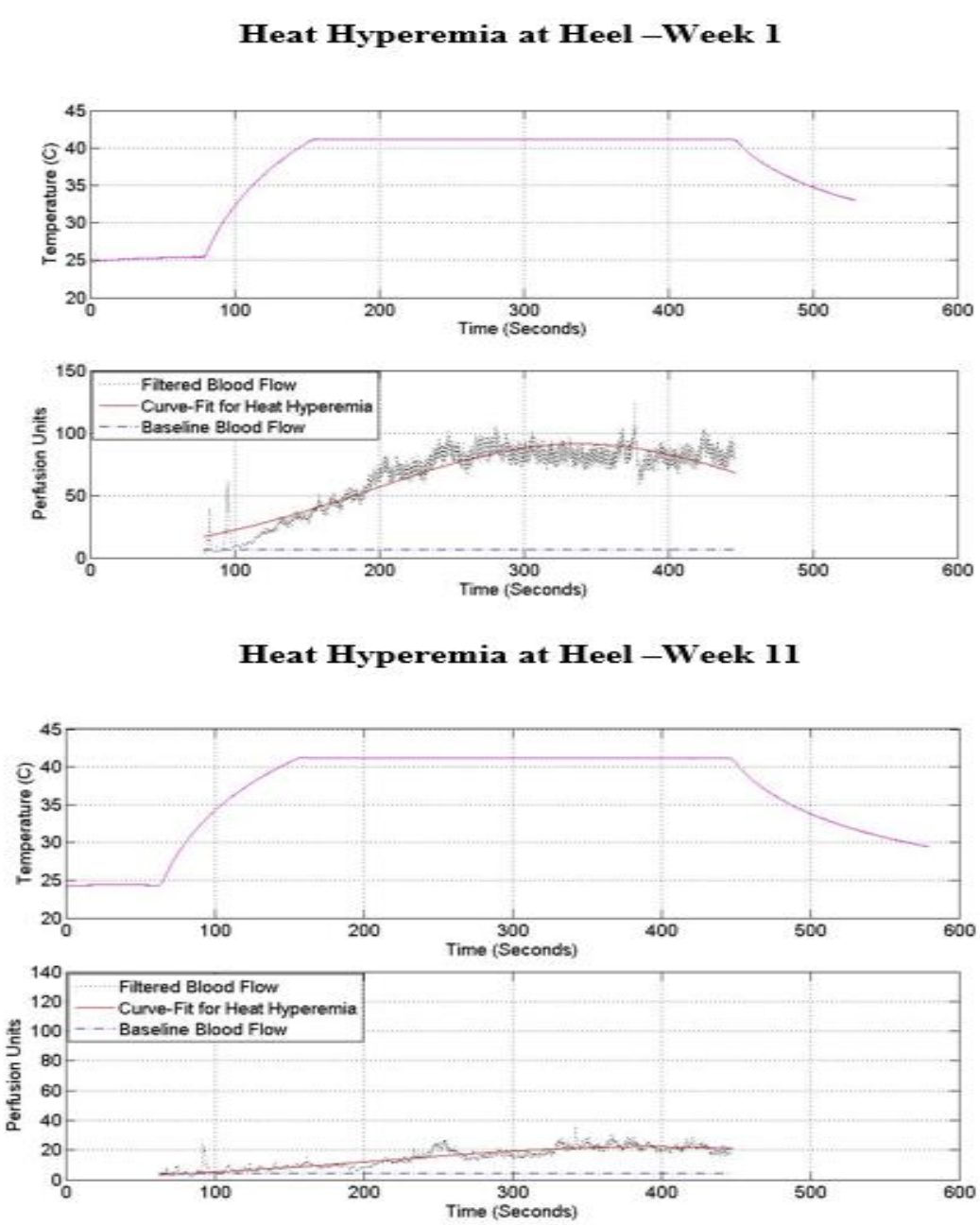


Figure 29. Heat Hyperemia at HEEL in G5_10

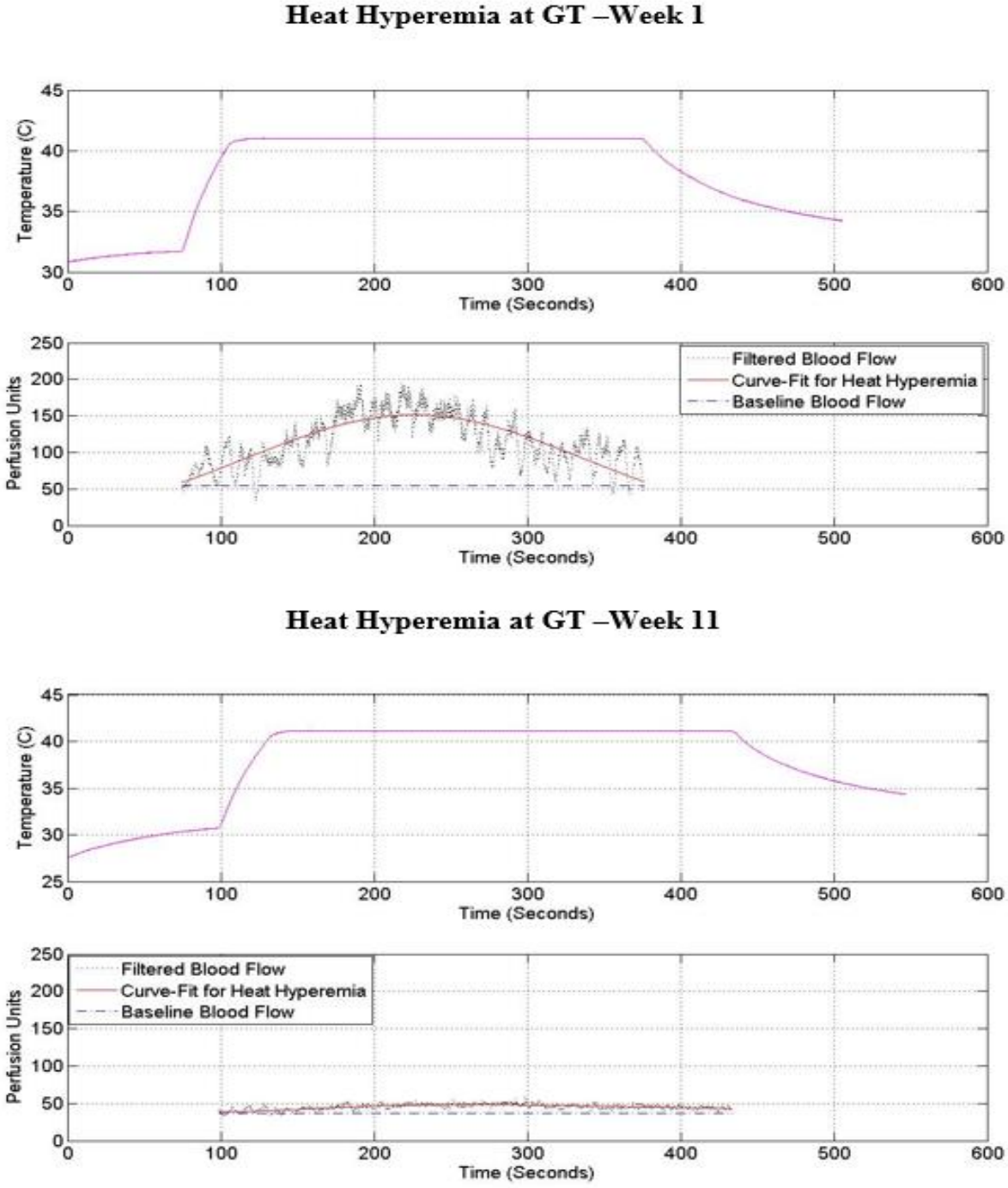


Figure 30. Heat Hyperemia at Great Toe in G3_01

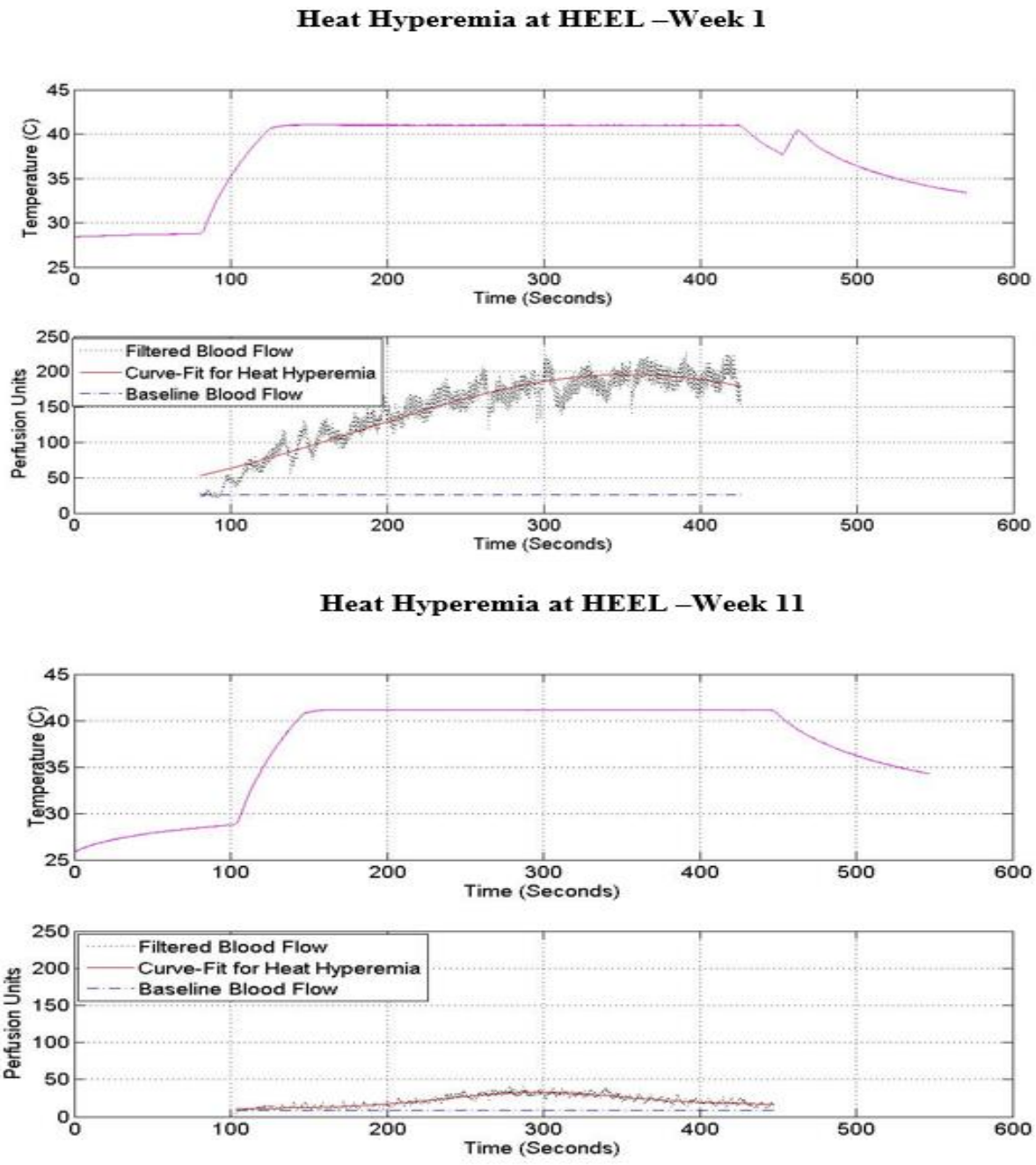


Figure.31 Heat Hyperemia at Heel in G3_01

RELATIONSHIP BETWEEN MICROVASCULAR FUNCTION AND DIABETIC CHARACTERISTICS

In addition to understanding the relationship between diabetic characteristics and microvascular function, Spearman Rho correlation coefficient tests were performed. The results were demonstrated in

Table. 7,

Table 8 &Table. 9.

RH at Great Toe: The PSBF and NpSBF was highly positively correlated in the pre exercise status in subjects with impaired foot sensation, higher ABI, foot conditions and medical history including hypertension and cholesterol, but did not have any significant change post exercise. TpSBF was highly positively correlated with impaired foot sensation before exercise, however there was no significant change in the TpSBF post exercise. Similarly, ASBF was highly positively correlated with higher diabetic duration, foot sensation, foot history, ABI& medical history. The baseline SBF responses were not significant amongst all subjects pre and post exercise.

RH at Heel: At the heel, the ASBF were highly correlated with diabetic duration and medical history post exercise.

HH at Great Toe and heel: All of the responses of heat hyperemia were significant only after exercise. Baseline SBF was highly correlated with foot ulcer/amputation history. PSBF was

highly negatively correlated with foot ulcer/amputation history and ABI. Percentage perfusion was highly correlated with diabetic duration, foot history, medical history, and ABI. The heat hyperemic responses at the heel were not significant in any of the parameters pre or post exercise.

Table.6 Correlation between RH response and diabetic characteristics at heel pre and post exercise

Diabetic Characteristics	N	Exercise	BSBF		PSBF		NpSBF		TpSBF		ASBF	
			r	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Diabetic Duration	6	Pre	0.08	0.86	0.7	0.117	0.8	0.059	0.7	0.117	**0.9**	**0.001****
	5	Post	0.0	1.000	0.0	1.000	0.3	0.604	**.9**	**0.014****	0.8	0.111
	5	Post-pre	0.1	0.800	-0.1	0.80	0.0	1.000	0.8	0.111	0.0	1.000
Foot sensation	6	Pre	0.4	0.414	**.8**	**0.042***	**.8**	**0.042***	**.8**	**0.042***	**0.8**	**0.042***
	5	Post	0.3	0.559	0.3	0.559	0.3	0.559	0.3	0.559	0.7	0.182
	5	Post-pre	0.3	0.559	0.0	1.000	0.3	0.559	0.0	1.000	0.3	0.559
ABI	6	Pre	0.5	0.288	**.8**	**0.036***	**.8**	**0.036***	0.7	0.084	**0.8**	**0.036***
	5	Post	0.2	0.741	-0.2	0.741	0.4	0.493	0.8	0.054	0.8	0.089
	5	Post-pre	-0.0	0.935	-0.4	0.434	0.1	0.870	0.4	0.434	-0.0	0.935
Current ulcer history	6	Pre	0.1	0.805	0.6	0.158	0.6	0.158	0.6	0.158	0.6	0.158
	5	Post	-	-	-	-	-	-	-	-	-	-
	5	Post-pre	-	-	-	-	-	-	-	-	-	-
Previous ulcer history	6	Pre	0.6	0.158	0.4	0.441	0.4	0.441	0.4	0.441	0.4	0.441
	5	Post	0.3	0.559	0.3	0.559	0.3	0.559	0.3	0.559	0.7	0.182
	5	Post-pre	0.3	0.559	0.0	1.000	0.3	0.559	0.0	1.000	0.3	0.559
Previous ulcer location	6	Pre	0.6	0.158	0.4	0.441	0.4	0.441	0.4	0.441	0.4	0.441
	5	Post	0.3	0.559	0.3	0.559	0.3	0.559	0.3	0.559	0.7	0.182
	5	Post-pre	0.3	0.559	0.0	1.000	0.3	0.559	0.0	1.000	0.3	0.559

Foot History/Amputation	6	Pre	0.1	0.774	**.8**	**0.046***	**.9**	**0.005****	0.7	0.123	**0.9**	**0.005****
	5	Post	0.1	0.858	0.1	0.858	0.1	0.858	0.8	0.118	0.6	0.215
	5	Post-pre	0.3	0.581	-0.2	0.718	-0.1	0.858	0.4	0.450	-0.1	0.858
Medical History	6	Pre	0.2	0.700	0.7	0.084	**.8**	**0.050***	0.8	0.066	**0.9**	**0.000****
	5	Post	0.1	0.870	0.1	0.870	0.4	0.493	0.8	0.054	0.8	0.054
	5	Post-pre	0.2	0.741	-0.1	0.870	0.1	0.805	0.6	0.219	0.1	0.805

[2]

Table. 7 Correlation between RH response and diabetic characteristics at heel pre and post exercise

Diabetic Characteristics	N	Exercise	BSBF		PSBF		NPSBF		TPSBF		ASBF	
			r	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Diabetic Duration	6	Pre	0.1	0.868	0.2	0.612	-0.3	0.492	0.4	0.381	0.4	0.381
	5	Post	0.0	1.000	0.3	0.604	0.8	0.111	0.0	1.000	**0.9**	**0.014****
	5	Post-pre	-0.8	0.111	0.0	1.000	0.8	0.111	0.3	0.604	0.6	0.252
Foot Sensation	6	Pre	0.2	0.694	0.6	0.188	-0.2	0.694	0.2	0.694	0.4	0.414
	5	Post	-0.3	0.559	0.3	0.559	0.7	0.182	-0.3	0.559	0.3	0.559
	5	Post-pre	-0.7	0.182	-0.7	0.182	0.7	0.182	0.0	1.000	0.3	0.559
ABI	6	Pre	0.2	0.700	0.4	0.425	-0.5	0.354	0.2	0.658	0.3	0.499
	5	Post	-0.4	0.493	0.1	0.870	0.6	0.219	-0.1	0.870	0.7	0.172
	5	Post-pre	-0.8	0.089	-0.3	0.553	0.6	0.219	0.1	0.805	0.5	0.322
Current Ulcer History	6	Pre	-0.4	0.441	0.1	0.805	0.4	0.441	0.6	0.158	0.6	0.158
	5	Post	-	-	-	-	-	-	-	-	-	-
	5	Post-pre	-	-	-	-	-	-	-	-	-	-
Previous Ulcer History	6	Pre	0.6	0.158	0.6	0.158	-0.6	0.158	-0.4	0.441	0.1	0.805
	5	Post	-0.3	0.559	0.3	0.559	0.7	0.182	-0.3	0.559	0.3	0.559
	5	Post-pre	-0.7	0.182	-0.7	0.182	0.7	0.182	0.0	1.000	0.3	0.559
Previous Ulcer Location	6	Pre	0.6	0.158	0.6	0.158	-0.6	0.158	-0.4	0.441	0.1	0.805
	5	Post	-0.3	0.559	0.3	0.559	0.7	0.182	-0.3	0.559	0.3	0.559

[2] *. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

	5	Post-pre	-0.7	0.182	-0.7	0.182	0.7	0.182	0.0	1.000	0.354	0.559
Foot History	6	Pre	0.0	0.954	0.2	0.600	-0.2	0.600	0.2	0.600	0.3	0.518
	5	Post	-0.3	0.581	0.1	0.858	0.6	0.215	0.1	0.858	0.7	0.118
	5	Post-pre	-0.6	0.215	-0.2	0.718	0.6	0.215	0.4	0.450	0.7	0.118
Medical History	6	Pre	0.1	0.742	0.4	0.425	-0.3	0.461	0.4	0.425	0.4	0.354
	5	Post	-0.1	0.935	0.4	0.493	0.8	0.054	-0.1	0.805	**0.8**	**0.054***
	5	Post-pre	-0.8	0.054	-0.2	0.741	0.8	0.054	0.2	0.741	0.5	0.322

Table 8. Correlation between HH response and diabetic characteristics at Great toe pre and post exercise

Diabetic Characteristics	**N**	**Exercise**	**BSBF**		**PSBF**		**% SBF**	
			r	***p***	***r***	***p***	***r***	***p***
Diabetic Duration	6	Pre	0.1	0.738	0.3	0.492	-0.5	0.280
	5	Post	0.8	0.111	-0.8	0.111	**-.9**	**0.014****
	5	Post-pre	-0.8	0.111	-0.6	0.252	-0.6	0.252
Foot Sensation	6	Pre	0.4	0.414	0.4	0.414	-0.4	0.414
	5	Post	0.7	0.182	-0.7	0.182	-0.7	0.182
	5	Post-pre	-0.7	0.182	-0.7	0.182	-0.3	0.559
ABI	6	Pre	0.2	0.618	0.5	0.288	-0.4	0.354
	5	Post	0.6	0.219	**-.9**	**0.005****	-0.8	0.089
	5	Post-pre	-0.6	0.219	-0.6	0.219	-0.5	0.322
Current Ulcer History	6	Pre	-0.1	0.805	-0.1	0.805	-0.1	0.805
	5	Post	-	-	-	-	-	-
	5	Post-pre	-	-	-	-	-	-
Previous Ulcer History	6	Pre	0.6	0.158	0.6	0.158	-0.4	0.441
	5	Post	0.7	0.182	-0.7	0.182	-0.7	0.182
	5	Post-pre	-0.7	0.182	-0.7	0.182	-0.3	0.559
Previous Ulcer Location	6	Pre	0.6	0.158	0.6	0.158	-0.4	0.441
	5	Post	0.7	0.182	-0.7	0.182	-0.7	0.182

	5	Post-pre	-0.7	0.182	-0.7	0.182	-0.3	0.559
Foot History	6	Pre	0.1	0.774	0.5	0.295	-0.3	0.518
	5	Post	**.8**	**0.041***	**-.8**	**0.041***	**-.8**	**0.041***
	5	Post-pre	-0.7	0.215	**-.8**	**0.041***	-0.8	0.118
Medical History	6	Pre	0.3	0.577	0.3	0.461	-0.5	0.228
	5	Post	0.8	0.089	-0.8	0.089	**-.9**	**0.005****
	5	Post-pre	-0.8	0.054	-0.6	0.219	-0.5	0.322

Table. 9 Correlation between HH response and diabetic characteristics at Heel pre and post exercise

Diabetic Characteristics	**N**	**Exercise**	**BSBF**		**PSBF**		**% SBF**	
			r	***p***	***r***	***p***	***r***	***p***
Diabetic Duration	6	Pre	-0.2	0.612	-0.6	0.191	-0.441	0.381
	5	Post	0.3	0.604	0.4	0.420	-0.632	0.252
	5	Post-pre	0.0	1.000	0.1	0.800	0.316	0.604
Foot Sensation	6	Pre	-0.2	0.694	0.0	1.000	0.207	0.694
	5	Post	-0.3	0.559	-0.3	0.559	-0.354	0.559
	5	Post-pre	-0.3	0.559	-0.7	0.182	-0.354	0.559
Abi	6	Pre	-0.3	0.461	-0.3	0.499	-0.029	0.957
	5	Post	-0.1	0.870	0.0	0.935	-0.718	0.172
	5	Post-pre	0.2	0.741	-0.0	0.935	-0.103	0.870
Current Ulcer History	6	Pre	-0.4	0.441	-0.4	0.441	-0.131	0.805
	5	Post	-	-	-	-	-	-
	5	Post-pre	-	-	-	-	-	-
Previous Ulcer History	6	Pre	0.1	0.805	0.4	0.441	0.393	0.441
	5	Post	-0.3	0.559	-0.3	0.559	-0.354	0.559
	5	Post-pre	-0.3	0.559	-0.7	0.182	-0.354	0.559
Previous Ulcer Location	6	Pre	0.1	0.805	0.4	0.441	0.393	0.441
	5	Post	-0.3	0.559	-0.3	0.559	-0.354	0.559

	5	Post-pre	-0.3	0.559	-0.7	0.182	-0.354	0.559
Foot History	6	Pre	-0.3	0.518	-0.4	0.439	-0.152	0.774
	5	Post	0.1	0.858	0.112	0.858	-0.783	0.118
	5	Post-pre	-0.1	0.858	-0.2	0.718	-0.112	0.858
Medical History	6	Pre	-0.2	0.658	-0.5	0.321	-0.319	0.538
	5	Post	0.1	0.805	0.3	0.614	-0.564	0.322
	5	Post-pre	-0.1	0.870	-0.0	0.935	0.205	0.741

5.0 DISCUSSION

Based on the screening exam, patient who had a present DFU or history of DFU showed abnormal foot sensation (impaired sensation at >8 sites at the foot). In addition, these individuals also demonstrated higher BMI (30-35) as compared to diabetic only and healthy individuals. Similar results of subject characteristics have been confirmed by several studies (E.Tur et al. 1991). For the subject with current DFU, despite the long history of diabetes (14 years) and higher BMI, he also demonstrated Charcot foot deformity, which is one of the major contributors of developing a foot ulcer (Rogers et al. 2011). These observations are consistent with the clinical manifestation of DFU.

5.1 REACTIVE HYPEREMIC RESPONSE

For the first part of this study that compared the reactive hyperemic response parameters amongst the six subjects before exercise training, we found that the baseline SBF at the great toe and heel was similar among all subjects except a much higher level at the heel in the subject with history

of DFU. Baseline skin blood flow may not be very comparable as it could vary from person to person due to its nature at that specific time point. Hence it becomes challenging to determine baseline skin blood flow for such a population. However, this still requires further investigation and it is also difficult to determine due to the interaction of various factors. The Npeak represents the peak flux normalized to the baseline. Decreased response of the Npeak can be explained due to concurrence of failure of maximum vasodilation at the capillary level, sympathetic denervation, and nutrition to cutaneous circulation, and change in the shape of red blood cells due to glycosylation reducing capillary blood flow, and deficiency of prostaglandins in the arterial wall in the diabetic patients (E.Tur et al. 1991). Although this study, demonstrated a decrease in the healthy individuals and increase in the Npeak in diabetic subjects, another speculation for such a response should be considered. Few studies such as Edmonds et al and Ward et al have suggested that an increased in the peripheral blood flow due to arteriovenous shunting due to rigid vessels with association of severe neuropathy, including some subjects with Charcot foot. This is also supported by our correlation analyses, which demonstrated that the more impaired foot sensation, history of ulcer/ amputation, higher and medical history, ABI before exercise, the greater is the Npeak response. This theory can also explain the presence of a higher ABI in the subject with history of DFU compared to other diabetic subjects in this study. Tpeak refers to the time needed for blood flow to reach the peak after pressure relief. This study demonstrated a longest Tpeak in subjects with DFU and history with DFU followed by subjects with only diabetes. Increased time taken to produce this response can be described due to the impaired neurogenic vasodilator release involving small sensory nerves in the periphery which is most important for healing after an injury (Lanting et al. 2017). The same study also demonstrated a strong association of increased Tpeak in subjects with peripheral neuropathy plus history of previous foot complication and has stated

Tpeak as an important PORH predictor of CVD in older population. This was confirmed by the correlational analyses that demonstrated, higher Tpeak with more impaired foot sensation in diabetic subjects. Perfusion area represents the amount of blood flow post pressure relief, is another reactive hyperemic response parameter which has been studied in this study, however no other researcher has investigated this property in this population. In our study, the aSBF was tremendously greater compared to other individuals and observed the least in the healthy individuals. This extreme increase in the skin blood flow in the subject with DFU and DFU history could be postulated due to the arteriovenous shunting mechanism in the diabetic vessels. Also, a decreased perfusion post pressure relief in healthy individuals can be estimated due to amount of pressure application. Other studies that have explored the parameters of the skin reactive hyperemic response have employed the application of pressures higher than 200mmHg pressure with pressure cuff (E.Tur et al. 1991, Lanting et al. 2017, Y.K Jan et al. 2013), whereas our study applied 60 mmHg pressure, which is the minimum pressure which is sufficient to cause a pressure ulcer (Tzen et al. 2010). It can be hypothesized that, the application of greater pressure has reproduced a greater peak in healthy subjects compared to diabetic subjects (Tur et al. 1991). Hence, a small pressure equivalent to 60mmHg pressure application would unlikely be able to produce a greater perfusion area owing to the healthy nature of vessels. This result can be supported by the correlational analyses performed which showed that, the higher diabetic duration, impaired foot sensation, higher ABI, foot history and medical history before exercise. The need for higher perfusion can also be presumed due to need for compensating the ischemic condition induced due to pressure occlusion in diabetic subjects. However, further investigation is required in this area of microvascular properties.

5.2 HEAT HYPEREMIC RESPONSE

Heat hyperemic response parameters namely; initial peak and % perfusion and a bi-phasic response were suggested by previous investigators and it has been explored and compared in our study amongst diabetic and healthy subject at the great toe and heel for the first time. Baseline SBF before the application of heat were variable in subjects with diabetes and healthy subject. Higher baseline SBF in diabetes, can again be explained due to diabetic neuropathy. The autonomic factor of the peripheral nervous system impairs skin microcirculation due to affection to the normal sympathetic tone causing arteriovenous shunting (Charkoudian. 2003). However, this component needs further investigation in a larger sample for a more standardization of data. The peak response in heat hyperemia is axon-reflex mediated (Minson et al. 2001, Ciplak et al. 2009, Sorenson et al. 2016). Reduced peak response according to the above statement would indicate an impaired axon-mediated reflex, although an impairment or absence in this mechanism has not been explained, it may need detailed analysis between various sub groups of diabetes and healthy population. Also, since, the occurrence of axon mediated reflex is an independent mechanism, the presence of nitric oxide (NO) which is responsible for primary vasodilation would not be responsible for a greater peak. In this study, most of the subjects did not demonstrate a bi phasic pattern of heat hyperemic response. In addition, it also be noted that subjects in which, an initial peak was not demonstrated irrespective of the presence of neuropathic condition. Therefore, due to this the mechanism of axon mediated reflex remains questionable. The perfusion % is controlled by endothelial NO, which is responsible for producing primary vasodilation (Vinik et al. 2001). These results are supported by our correlational analyses which showed that a decreased % perfusion and peak was found in subjects with foot history and higher ABI. Reduced peak and % perfusion in diabetic subjects

could indicate impaired microvascular function to heat hyperemic response due to decreased vasodilatory mechanism. However, these responses were observed significantly at the great toe only. Also, the presence of a higher BMI could be a possibility of a lesser perfusion in individuals with or without a compromised vascular condition (E. Tur et al. 1991). Moreover, a reduced perfusion at the heel could be presumed due to the glabrous nature of the skin at the heel is subjected to a more severe microvascular dysfunction than dorsal skin (non-glabrous) of the foot (Vinik et al. 2001, Y K Jan et al. 2013). Therefore, although majority of the subjects did not demonstrate a bi-phasic heat hyperemic response, this response requires exploration on a considerably larger sample population.

5.3 SKIN BLOOD FLOW PARAMETERS POST EXERCISE

In the second part of the study, the effect of this exercise model on the skin blood flow response to pressure and heat were variable. It should be considered that although this model has been used previously by past researches to study leg blood flow during exercise (Hoelting et al. 2001, Osada et al. 2012), this study is the first to employ this exercise model in exploring microvascular skin blood flow parameters. Majority of the subjects showed an increase in the SBF parameters; baseline, peak and perfusion area and a decrease in the time to peak post 12- week exercise training, which was helpful in proving our hypothesis. Time to peak has been reported to be an indicator of cutaneous vascular tone (Yvonne-Tee et al. 2005). A study that was done by Jong et al, stated an improvement of vascular tone with moderate –intensity aerobic exercise which may be useful in explaining a shorter time to peak post exercise. Vascular tone is regulated by vascular

endothelium which is an important factor in causing vasodilation by the release or the bioactivity of NO. Increased perfusion has been known to escalate these responses with aerobic exercise (JS Wang et al). Moreover, exercise has long been proposed to control hyperglycemia by increasing perfusion through the synthesis of vasodilator NO (Nwanko et al. 2014, JS Wang et al. 2004). Along with the possibility of arteriovenous shunting in producing a reduction in the peak and perfusion area, muscle fatigue (Sjogaard et al. 1985) could also be one of factors that could probably answer this. Muscle fatigue due to the repeated isometric contractions of the quadriceps muscle could have led to a decrease in the blood flow. However, this exercise involves submaximal muscle contractions which has the capability to sustain contractions even after the onset of muscle fatigue (Enoka et al. 2008). Apart from increased perfusion, another positive effect due to exercise is its anti-inflammatory properties in reducing the deleterious effects of diabetes and aging (Petersen et al. 2005). Previous literatures have assessed different kinds of aerobic exercise training effects in older adults, diabetic subjects and older mice that have shown improved healing condition due to reduction in the pro-inflammatory properties. (Emery et al. 2005, Nwanko et al. 2014, Keylock et al.2007, Petersen et al. 2005). Hence, the idea of preferring whole body or arm-leg exercise to one leg exercise in this study, was to test the feasibility in utilizing this exercise model as an intervention in the treatment in diabetic foot ulcers in the future. The mechanism behind this idea was found in a study by Saltin and Andersen that stated the quadriceps femoris is a large enough muscle to induce a marked elevation in the whole body O_2 uptake. However, detailed understanding in the skin blood flow responses with exercise is necessary to deduce a more standardized result in a larger sample size.

LIMITATIONS

There were some important limitations in this study. A really small number of subjects in the study can be explained due to the complex nature of the diabetic condition that could interfere with vascular insufficiency, makes it challenging to obtain subjects only with diabetes within a short period of time. The duration of the training program was really long due to which few of the subjects presented inconsistency in attending the training program, which could have caused detraining changes in between the exercise program. Also, although debatable, the difficulty in maintaining the ambient temperature is important to produce reliable results in assessing vascular properties. A cultural bias in this group of subjects expects, could have interfered with the skin blood flow properties, which demands more diverse subjects to obtain standardization of data. In the methodology section, the pressure induced in our study; 60mmHg might be less to cause the reactive hyperemic response was not sufficient to induce the response in some subjects due to which the RH parameters were difficult to determine. Moreover, skin blood flow signals in some subjects contained more noise due to movement of the subject, which could have hampered true blood flow characteristics.

RECOMMENDATIONS

The findings of this study recommends the employment of evaluating skin blood flow responses in studying the microvascular nature in chronic diabetes and healthy adults and exercise to manage diabetic condition in a larger sample size. Studying the same characteristics and biochemical properties of skin blood flow on a larger sample size is warranted.

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