

**THE ACUTE EFFECTS OF PHYSICAL ACTIVITY ON THE
STIFFNESS OF THE PLANTAR SKIN OF PEOPLE WITH AND
WITHOUT DIABETES**

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The Academic Faculty

by

Deborah Michael Wendland

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Approved by:

Dr. Stephen H. Sprigle, Advisor
School of Applied Physiology
Georgia Institute of Technology

Dr. Mindy Millard-Stafford
School of Applied Physiology
Georgia Institute of Technology

Dr. Sharon E. Sonenblum
School of Applied Physiology
Georgia Institute of Technology

Dr. Teresa Snow
School of Applied Physiology
Georgia Institute of Technology

Dr. Michael Mueller
Program in Physical Therapy
*Washington University School of
Medicine*

Date Approved: 11-15-2013

This thesis is dedicated to those with and without diabetes who willingly participated in this research to advance our understanding of how exercise affects the skin of people with diabetes.

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LIST OF SYMBOLS AND ABBREVIATIONS

AGEs	advanced glycosylated end products
ROS	reactive oxygen species
PN	peripheral neuropathy
ANS	autonomic nervous system
DM	diabetes mellitus
C	Celsius
RH	relative humidity
TID	tissue interrogation device
EC	environmental chamber
N	Newton
m	meter
kg	kilogram
SD	standard deviation
CV	coefficient of variance
k	stiffness
NWB	non-weight-bearing
TM	treadmill

SUMMARY

Diabetes affects 25.8 million Americans. The complications related to this growing disease impact public health. One secondary complication of diabetes is changes in skin that can contribute to an increased risk for ulceration. The skin of people with diabetes has not been characterized over time nor has the skin's acute response to exercise been assessed. The objective of this project was to establish the changes in skin properties over time, within different ambient environments, and after acute exercise. This objective sought to address the central hypothesis that skin will demonstrate a decrease in stiffness and increased elasticity as a result of acute physical activity. Skin stiffness, compliance, and thickness measurements of the plantar foot were compared across time and environment. Skin stiffness and compliance were also compared before and after treadmill walking.

1 Introduction

1.1 Background and Significance

Diabetes is a disease that affects 25.8 million Americans.(1) With its growing prevalence, the complications of this disease impact public health.(1) One way it does this is through changes that occur to the skin and the body's ability to respond to such changes. These changes can contribute to an increased risk of ulceration among those with diabetes. (2) The purpose of this chapter is to make a case for examining the impact of physical activity on the plantar skin. Multiple steps will be taken to complete this task. This chapter will examine the common complications of diabetes that affect the skin and will look at the significance of exercise in relation to diabetes. Next, the skin's response to stress will be examined with particular emphasis on the skin in people with diabetes. Finally, it will describe the equipment to be utilized along with its supporting research. Within this context, missing pieces in the literature will be highlighted so that the need for further research will be made evident. In particular, it will clarify the need to investigate the acute effects of exercise on plantar skin.

To illustrate the factors impacting skin and foot health related to diabetes, the broad complications of diabetes will first be described. The mechanisms behind diabetes that may lead to these complications will also be presented. Further, the factors and behaviors necessary to limit the complications that may occur in the disease process will be considered. Such a connection is imperative to paint a broad picture of how to minimize diabetes-related health concerns. Moreover, the relationship between the diabetes complication and the skin manifestation will be emphasized. Together, these will clarify diabetes' impact on the skin of the plantar foot.

Diabetes and poor control of blood glucose are linked with many subsequent complications. Broadly, complications of diabetes include both those of the micro- and macro-vasculature, which affect the eyes, kidneys, nervous system, circulation, and skin.

The mechanism by which these complications occur is not specifically known, but there are several possible mechanisms contributing to these problems.

The proposed pathways to diabetic complications are related to how the body handles hyperglycemia. One such mechanism that has been proposed to lead to chronic complications involves the formation of advanced glycosylated end products (AGEs) via nonenzymatic glycosylation of intra- and extra-cellular proteins. The AGEs then cause proteins to cross-link. Included among these proteins is collagen.(3, 4) Raised serum levels of AGEs are associated with individuals with hyperglycemia compared to individuals without high blood glucose levels.(5)

Other pathways have been proposed to contribute to complications associated with diabetes as well. One of these pathways involves an increase in glucose metabolism via the sorbitol pathway that generates reactive oxygen species (ROS) and likely leads to cellular dysfunction. This pathway, in particular, with its accumulation of sorbitol and fructose contributes to changes in the nervous system.(6) Alternatively, hyperglycemia may increase the formation of diacylglycerol leading to the activation of protein kinase C, which changes the transcription of genes for fibronectin. Finally, hyperglycemia has been proposed to act by increasing flux through the hexosamine pathway, which could change the function by glycosylation of proteins.(6)

Because these mechanisms are tightly associated with hyperglycemia, it is necessary to work to prevent these diabetic complications through both improved management of blood glucose and with prevention specific to each complication. Good blood glucose control has been associated with fewer complications from diabetes. In particular, several large-scale studies (Diabetes Control and Complications Trial (DCCT)(7-9) and United Kingdom Prospective Diabetes Study (UKPDS)(10) have found that those with more tightly controlled blood glucose levels had fewer complications.

In agreement with the findings of these studies, the Standards of Medical Care in Diabetes' recommendations call for tight control of blood sugars such that A1C is less

than 7% for non-pregnant adults.(11) To control blood glucose, particularly with type 2 diabetes, a first line of defense is to control blood sugars utilizing diet and exercise.(11) When these techniques are being utilized, physical activity is an important component to monitor. Exercise, both resistance training and endurance training (stationary bicycle), increases the ability of muscle tissue to uptake glucose via the movement of GLUT4 transporters as well as by increased insulin sensitivity, thereby assisting with blood glucose management. (12-15)

Despite the positive effects of exercise on diabetes control, areas of high pressure, potentially increased during exercise, have been associated with increased risk of skin breakdown. Therefore historically, consideration was made to the type of exercise encouraged or discouraged based on the diabetes complications that were present. For example, for those with diabetes and “severe” peripheral neuropathy (PN), previous guidelines for physical activity suggested non-weight-bearing exercise to meet the recommendations for 150 minutes of exercise per week.(16) More recently it has been shown that with walking exercise, the skin’s risk for breakdown does not seem to increase despite the accumulation of stresses.(17-20) Originally, it is likely that this weight-bearing concern was reflective of the risk for re-ulceration that can occur with return to walking that is not tightly controlled and slowly increased. This issue can still be seen with documentation of re-ulceration or ulceration that occurred with exercise that was variable rather than exercise that was progressively increased.(19, 21)

How does skin respond to the stress of exercise? Generally, tissue that experiences a large magnitude of physical stress over a short time or a lesser stress over a longer time could develop an ulcer, particularly if other factors contribute to the risk.(22) This “stress” includes any pressures that are imposed on the skin including compressive, tensile, torsional, and shear forces which may occur while walking or doing other activities. While it is clear that off-loading a sore is important during recovery following injury, it seems that skin which regularly receives more stress, including standing and

walking, is actually more tolerant to stress.(17, 18, 20) This is in agreement with the physical stress theory of Mueller and Maluf.(18) Since it was published in 2002, other studies have supported the concept.(17-20) Maluf and Mueller found that, in a population with diabetes and peripheral neuropathy, those with a history of ulcers were less active, taking fewer steps each day and applying less stress to their feet.(20) LeMaster et al. found that with an activity intervention, there was no increase in wound rate.(18) Previously LeMaster et al. had found that those who spent more time on their feet (7.5 hours vs. 4.5 hours) were less likely to re-ulcerate.(17) Similarly, Armstrong et al. found in their study that people who developed ulcers had a lower mean activity level but a higher degree of activity variability. This variability increased further two weeks before ulceration.(19) Together these studies point that a chronic response to increased stress is an increased tolerance to subsequent stress.

However, the study by Armstrong et al. highlights the need to also consider the acute effects of exercise. From this study, it is clear that the mode of physical activity or exercise prescription (frequency, intensity, duration) is potentially critical. With the relationship drawn between increased activity variance and ulceration,(19) one must consider how a single bout of physical activity affects the skin on the plantar surface of the foot. Further research is important to more thoroughly understand the response of skin to physical activity, such as walking, relative to the dosage and timing of the activity. It is important to look at these changes not only chronically, as was done with much of the previous work,(17-20) but also acutely.

When glycemic control is not well maintained, many systemic complications related to diabetes can develop. While the complications are systemic, this chapter focuses on the complications that affect the skin. These skin complications may consequently elevate one's risk for plantar ulceration and subsequent amputation.(2) The changes to skin and its properties are typically described as being multi-factorial. Included among these multi-factorial changes are alterations to the nervous tissue and

system along with direct structural modifications to collagen and subsequently tissue.(3, 4, 6) Figure 1.1 summarizes the multi-factorial contribution to a person's ulcer risk.

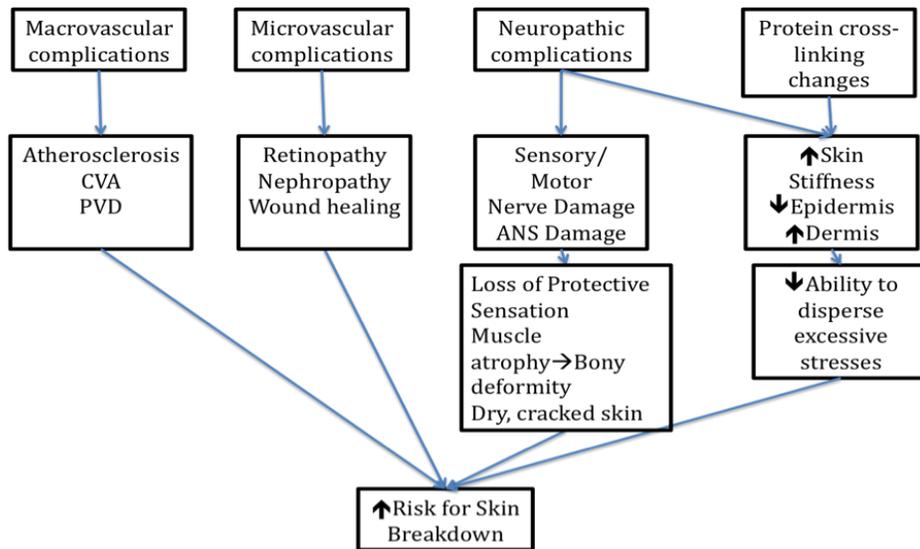


Figure 1.1 Summary of increased risk for ulceration as related to diabetes mellitus

First, one of the more prevalent complications is neuropathy, which can include changes to the autonomic nervous system (ANS)(6, 23, 24) and somatic nervous system.(6, 23-25) ANS complications can involve changes in the skin related to temperature control(26) and tissue moisture.(24) Somatic nervous system changes, on the other hand, can include changes to motor and sensory nerves including peripheral neuropathy. Sensory neuropathy symptoms affect between 30 and 40% of those with Type 1 or Type 2 diabetes.(27) Changes associated with neuropathy that may occur include decreased moisture to tissue via sweating, increased difficulty with temperature regulation, skin cracking and fissures secondary to decreased sweating,(24) changes in motor nerve function leading to muscle weakness followed by structural deformity,(25, 28) and changes to sensory nerves leading to a loss of protective sensation(27, 29, 30) and proprioception.(23, 25) All of these changes can lead to a change in gait pattern(31-36) as well as an increased risk for plantar ulceration.(30) Plantar ulceration may progress

to non-traumatic amputation. In fact, more than 60% of non-traumatic amputations occur in people who have diabetes.(1) For this reason, it is important to continue looking for ways to prevent such complications through improved management of blood glucose as well as through better preventive foot care.

Neuropathy can be especially problematic when it comes to the skin. When the sensory nerves are affected, the result is a decrease in protective sensation. It is well documented that if a person is unable to feel the 10 grams of pressure applied with a 5.07 Semmes Weinstein monofilament, then that person has an increased risk for plantar ulceration.(29, 30, 37) Similarly, with damage to the motor nerves, one can see a change in the musculature most noticeably in the intrinsic muscles of the feet and the hands. Such changes in the muscles may lead to deformities that can increase pressure on certain areas of the feet.(38) These increased areas of pressure may increase one's risk for skin breakdown.(39)

When computed tomography was utilized to look at changes in the structure of the feet in people with diabetes, Robertson et al. found that the mean density of plantar muscle under the mid-metatarsals was significantly less in people with diabetes compared to matched controls.(28) In the same study, they also found that people with diabetes were more likely to maintain foot posture in extension. There were significant differences for the extension noted in the first and third toes in people with diabetes compared to those without.(28) These changes may all contribute to the increased rate of breakdown that is seen among people with diabetes compared to those without the disease.

The prevalence of skin lesions is another important factor to consider in people with diabetes. A group of 238 people with type 1 diabetes for more than 5 years were compared to a group of 122 healthy controls to determine the prevalence of skin lesions in people with diabetes. The investigators found that skin problems were much more common in the group with diabetes, namely, "ichthyosiform skin changes..., scleroderma-like skin changes, tinea pedis, and dry scaly palms." (40) In a more recent

study looking at both type 1 and type 2 diabetes, Farshchian et al. (2010) found that 71% of their subjects had some skin lesion “associated with DM.”(41) They found that infectious lesions were more typical in this population.(41) This group did not look at healthy controls along with the group with diabetes, making it difficult to determine whether the same skin lesions were typical in a healthy population as well.

Skin manifestations that have been related to these diabetes complications include alterations in its structural and mechanical properties. Some groups have looked at specific effects of diabetes on the mechanism of skin changes. Many of the studies point to the advanced glycosylation end products (AGEs) that are produced when glycemic control is poor. AGEs have been associated with changes in collagen formation in tissue; particularly, more cross-linking has been seen.(3, 4) Collagen, along with elastin, are largely responsible for much of the tensile properties of skin tissue.(42) Diabetes-associated changes in collagen have been tested utilizing rat models. Oxlund and Andreassen applied a load to the tail tendons of rats and measured the speed at which the tendons broke. They found that the rats not given aminoguanidine, a substance that inhibits the alteration of the properties of collagen associated with diabetes, had tail tendons that took longer to break. These increased times likely demonstrate the increased cross-linking of proteins seen with diabetes.(43) AGEs have also been found to be more prevalent in the skin of people with higher levels of diabetes complications.(44)

These structural changes are accompanied by measurable changes in the skin properties. Most notably, changes in skin stiffness and thickness can be seen in those with diabetes when compared to those without the disease.(4, 45-49) First, changes in skin stiffness relative to location and disease severity will be addressed.

Multiple investigators have noted that skin stiffness increases in a population with diabetes.(45-47) In these studies, skin stiffness was measured by several mechanisms including an indenter, a tissue ultrasound palpation system, and indentation associated with the use of a MRI.(45-47) In the study by Klaesner et al., the authors report

measuring stiffness utilizing an indenter while measuring foot locations with the subject in prone. With their indenter, pressure was applied orthogonally while position and force were measured simultaneously to give a stiffness value.(45) The increased stiffness they found was variable according to foot location.(45) While stiffness of the metatarsals measured (1st, 3rd, and 5th) varied between the groups with and without diabetes, heel stiffness in people with diabetes did not vary from those without the disease.(45)

Similarly, orthogonal force was applied and removed with the tissue ultrasound palpation system in the study by Sun et al. In this system, an ultrasound transducer at the end of the indenter enabled visual monitoring of compression and decompression of plantar tissue. Custom software allowed the calculation of stiffness.(46) These investigators found increased stiffness at the great toe, metatarsal heads (1st and 2nd), and heel among the group with diabetes and peripheral neuropathy compared to people without diabetes.(46) Also using technology, Gefen et al. used spherical indentation and MRI to find stiffness and shear. This group found increased effective shear and elastic moduli at the area between the first two metatarsal heads.(47) Together, these studies using orthogonal pressure, found increased stiffness in people with diabetes compared to those without in some of the plantar locations tested, but not all locations. These findings should be considered when making the selection of locations to be tested experimentally.

Skin stiffness also varies with the level of diabetes complication. In the study by Chao, Zheng, and Cheing (2011), stiffness increased across subjects with diabetes compared to those without it.(50) Sun, Cheng, Zheng, et al. (2011) found that neuropathic changes were accompanied by increased stiffness of the foot.(46) Other studies have also reported increased stiffness in people with diabetes, particularly in those with complications.(45, 47) The study by Gefen et al., which used an MRI to look at load and deformation, unfortunately compared elderly people to diabetes with normal, young subjects.(47) By making such a study design decision, these investigators confounded their results such that one cannot dissociate age from disease process so that differences

in stiffness cannot be assigned causally to one or the other. Klaesner et al. found that those people with diabetes and peripheral neuropathy and other complications were more profoundly stiff than those without complications.(45) An earlier study found similar results using a different measurement device. The device used, the durometer, was held, perpendicularly to the tissue with only the weight of gravity. In this study, the authors found that plantar skin tissue hardness was increased with more severe neuropathy. (51)

Stiffness in people with diabetes also has been studied relative to compressive stresses. Hsu et al. (2009) looked at stiffness of the heel in those with and without diabetes. They found that changes related to diabetes may result in a poorer ability to cushion at the heel.(52) Similarly, Pai and Ledoux (2010) looked at the mechanical compressive properties of skin in people who had diabetes versus those who did not. They too found those with diabetes had stiffer tissue and therefore probably had increased difficulty dissipating stresses to the skin as well.(49) This study looked at skin from cadaveric subjects with the skin removed.(49) Thus, caution should be used regarding interpretation of the data because of the possibility of secondary changes to the structure of the tissue caused by either removal from the body or by death of the tissue.

Other changes to skin also occur in the presence of diabetes. Multiple studies have looked at the change in skin thickness as well as a change in soft tissue thickness associated with diabetes.(4, 46, 48, 50) Skin thickness findings of these groups varied. Chao, Zheng, and Cheing(50) found that, compared to a control group without diabetes, people with type 2 diabetes without neuropathic complications had an increased thickness of the epidermis. Those with neuropathy and/or ulceration (present or past) had decreased epidermal thickness. In a similar study, but involving people with earlier neuropathic changes (inability to feel a 4.31 Semmes Weinstein monofilament instead of a 5.07 monofilament), Sun, Cheng, Zheng, et al. did not find a difference in the soft tissue thickness on the plantar foot.(46) Hanna et al. found those with diabetes had increased skin thickness in the dermis rather than the epidermis. This group found that, although

some with diabetes were described as having “thick skin,” all of the people tested with diabetes had skin that was twice as thick as the control subjects.(4) Additionally, the morphology of the biopsies was also viewed and those with diabetes were found to have “hyalinized and disorganized” collagen with mostly large fibers which differed from the controls or those with scleroderma.(4) A valuable point made by this study is that some of the changes that can be measured in a population with diabetes cannot outwardly be seen as changes to the skin tissue.(4) When Huntley et al. (1990) tried to define a relationship between the thickness of skin in those with diabetes and age, they found that diabetes type, level of glycosylated hemoglobin (diabetes control), or presence of other complications could not be correlated. This group did find that, in those with diabetes, skin thickness was increased on the hands and feet, but not on the back.(48) Together, these studies show the wide variability of skin thickness in people with diabetes. Despite the variability in thickness, it is clear that skin morphology changes in people with diabetes. This evident variability makes it obvious that testing needs to be included which helps to quantify the extent of neuropathic involvement in those with diabetes. Such quantification may help explain differences that may be seen with further investigation.

There are certainly other disease and non-disease specific reasons that skin changes. There is evidence that the properties of skin, such as thickness, stiffness, viscoelasticity, and echogenicity, differ with age,(53, 54) gender,(55) time of day,(56) level of hydration,(57-60) and disease.(4) In addition to these causes for differences, skin properties have been shown to change with interventions such as cortisone.(61, 62)

Skin thickness is typically measured noninvasively using ultrasound (US). US can identify thickness of tissue layers and presence of subcutaneous structures. US displays the echogenicity of tissue, meaning how much the tissue reflects the ultrasound waves. Something with high echogenicity would appear bright white while something with low echogenicity would appear darker because of minimal reflection. The level of echogenicity helps the investigator determine the thickness of the tissue being measured.

In a review study, Waller and Maibach report that whole skin changes throughout the lifespan are a bit controversial (2005). (63) They convey that some of the differences depend on whether or not tissue has been exposed to the environment.(63) Additionally they report a “subepidermal low echogenic band that thickens with age.”(63) Age-related skin changes primarily occur early and later in life. (53) According to Escoffier, et al. (1989),(53) there is a thickening of skin up until the third decade, but overall, there are few changes that happen to skin from age 15 until 65. After 65, the skin reportedly thins.(53) Laurent et al. found that there was little difference in skin thickness in the subjects they tested who were between the ages of 18 and 70.(64) Similarly, de Rigal, et al. (1989) also report that thinner skin is present among the young and the elderly.(54) Additionally, there is evidence that skin thickness may increase or decrease with age based on the location of the skin.(56) For example, the investigators report that facial skin thickens with age while the skin of the forearm thins when measured in the morning.(56)

Changes in fluid levels associated with the time of day also seem to impact the properties of skin. For instance, Tsukahara et al. found that as the day progressed, the tissue thickness increased in the lower extremities but decreased in the upper extremity and face.(56) Not only were changes seen with skin thickness, but echogenicity also increased as skin thickness decreased.(56) In the same study, elasticity decreased in the lower extremities while it increased in the face and the forearm. (56) Seemingly, these diurnal changes may be related to the amount and distribution of fluid that is present in the limb during the day. This fluid shifts relative to gravity during the day. (56)

Similarly, one can see a change in the tissue thickness when an edematous limb was compared to an unaffected limb (unpublished data). In a different study looking at the effects of altitude on skin, the authors found skin thickness changed depending on fluid distribution and related to altitude.(65) To our knowledge, plantar skin was not tested in these studies looking at diurnal variations. With this available literature, it seems that, when designing a study looking at skin, it would be prudent to measure the skin at

similar times of the day to avoid confounding skin property results with natural diurnal variation. However, given the locations of skin tested in the previous literature, it would also be helpful to look at the plantar skin at different times of the day to add to the body of knowledge regarding possible diurnal variation within this particular skin area.

A few studies have more directly looked at the level of hydration by studying the epidermis. (57-59, 66) While these studies attempted to look at the mechanical properties of skin relative to the hydration levels, the investigators used skin that had been hydrated via external mechanisms. For example, rather than have the subjects drink appropriate amounts of fluids to be considered euhydrated and then test either their urine or blood for markers of hydration, the investigators soaked the subjects' skin in water, (66) applied water directly to the skin, (58) or applied moisturizers to the skin,(59) thus secondarily hydrating the tissue by minimizing evaporation. Klaus et al., on the other hand, found a strong relationship between skin thickness and the amounts of fluid replacement during a surgical procedure. (67)

Whether the mechanism by which the skin achieves a status of hydration affects the mechanical properties is unknown. What is known is that by the mechanisms studied, hydration does seem to affect the mechanical properties of tissues.(57-59, 66) Specifically, an increase in hydration was accompanied by an increase in the friction coefficient. (58, 59) The coefficient of friction is the ratio of the frictional force between the instrument and the skin divided by the normal interface force. Increased hydration was also accompanied by a change in thickness. (67) When viscoelastic properties of skin are viewed in the presence of hydration, Christensen, et al. found that hydration increased the amount of hysteresis that occurred with mechanical testing (1977).(57) Jemec, et al. in 1990, similarly described this phenomenon.(66) While hydration levels are likely to affect skin behavior, hydration level may not be affected by diabetes. (68) But, in people with diabetes, Seirafi, et al. found diminished activity of the sebaceous gland resulting in a change in sebum content along with lowered skin elasticity in skin regions.(68) Given

the possibility of the hydration effects on skin mechanical properties, it is important to consider an individual's level of hydration to see if those levels can be correlated to skin properties. The literature does not assess dietary hydration's effect on skin tissue.

Other factors have also been described that influence the response of skin to pressure and shear forces. Jagoda et al. (1981) illustrate that, depending on skin color, their subjects responded differently to blister prevention techniques. Those with fair skin actually developed fewer blisters if using an underlying nylon sock instead of the white athletic sock that was more beneficial for those with a darker complexion.(69) Skin environment also seems to impact how the skin responds. Whitfield (1932) reported that callus is more likely to form in a dry environment whereas a blister is more likely to form in a wet environment.(70)

Skin has been shown to change in response to certain medical treatments such as topical steroid use. One double-blind, controlled study using hydrocortisone cream found that some of the thinning of the skin that occurred over six weeks could actually be seen in as little as two days. Interestingly, removal of the treatment resulted in a recovery of the loss in thickness to 91 to 96% of the original thickness of the skin.(62) These measurements were determined using ultrasound techniques. (62) This interventional study shows that skin has the capacity to change quickly and acutely.

As detailed, research has documented a wide variety of changes in skin and tissue properties, but gaps in knowledge still persist. Few studies have considered the natural variation of skin properties within the same individual over time or across different environments. To our knowledge, no studies have measured the properties of plantar skin in an environment similar to the one in which physical activity occurs, the shoe. Given these gaps in our knowledge, research is needed to document the stability of skin properties over time and within varying environments. This knowledge will then allow more informed research into skin and tissue changes that occur after activity and other therapeutic interventions.

People with diabetes are prescribed many types of therapeutic interventions including physical activity and foot orthotic devices that promote an improved gait pattern or alter the loading on aspects of the foot. The impacts of these interventions on skin and skin breakdown must be evaluated.

Ultimately, the effects of these interventions on skin and overall health must be assessed in a longitudinal manner. Only then can the effectiveness and cost efficiency be measured. However, prior to establishing a long-term approach to testing, we must close the existing knowledge gaps about variations and changes in skin properties.

The objective of this project is to establish the changes in skin properties over time and after acute exercise. This objective seeks to address the *central hypothesis* that skin will demonstrate a decrease in stiffness and increased compliance as a result of a 10-minute bout of walking. To meet this objective, three Specific Aims are proposed:

1.2 Specific Aim #1: Validate the instrumentation necessary to characterize skin tissue.

This aim addresses the need for validated tools for assessing plantar skin. We will be utilizing three devices to characterize the properties of tissue. Two of these devices have not been used on plantar skin so each requires reliability and validity assessment. Additionally, we plan to utilize this equipment under two ambient environments, typical room condition as well as in high temperature and high humidity (32 degrees C and 66% relative humidity). We will evaluate the equipment in the two environments to insure that the equipment is not accountable for any variation in measurement. Finally, to assess activity, namely walking, we must make sure that we can accurately monitor this activity in the presence of altered gait patterns.

1.3 Specific Aim #2: Characterize the skin tissue of the foot across environmental conditions and time.

This aim will address the need to understand how the skin of people with and without diabetes behaves under certain conditions and across different time periods. This

will be tested by utilizing US, a myotonometer, and a tissue interrogation device (TID) to measure skin in room ambient conditions as well as within an environmental chamber mimicking the environment of the foot within a shoe. Multiple measurements will be taken within a single day (morning and afternoon testing), within a week, and within a month. Because we will look at groups of people with and without diabetes, the study will reveal the stability of skin properties but will also expose the differences in properties based on the environment and time. The findings will help to inform the timing of the methods of future studies.

1.4 Specific Aim #3: Identify the impact of a 10-minute bout of walking on skin tissue.

This aim will address the hypothesis that skin will change in response to acute physical activity imposed on an individual. To accomplish this task, we will utilize similar skin property measurements as in Specific Aim #2. Individuals will walk at similar speeds on a treadmill. Throughout their activity, we will collect temperature and relative humidity data within the shoe as a measure of ANS response. Following activity, the skin property measurements will examine how the skin changes with activity.

These studies lay important foundational groundwork to extend what is already known about diabetes and tissue. First, the validation of instrumentation that is capable of measuring skin properties of the plantar foot is crucial for distinguishing changes to the plantar skin with different interventional studies. The natural variation of skin relative to environment and physical activity is also critical for future interventional studies including those further examining various modes, intensities, and durations of exercise. Also, this background is necessary to better look at the impact of foot orthotic devices and their timing for the prevention of plantar ulceration and subsequent lower extremity amputation.

2 Validation of the Instrumentation Required to Characterize Skin Tissue

2.1 Specific Aim #1: Validate the instrumentation necessary to characterize skin tissue

Many instruments and devices have been used to assess skin properties, including xerography/ xeroradiography,(71, 72) ultrasound (US),(48, 50, 52, 56, 73, 74) various indentation devices(45, 50, 52) including sub-Metatarsal Pad Elasticity Acquisition Instrument (MPEAI),(75) suction cup technique,(76) computed tomography,(28, 77) OCT imaging,(78) digital measuring screw,(79) magnetic resonance imaging (MRI) (47) biopsies,(80, 81) wavelet transform,(82) Dermalab USB equipment,(83) infrared scanner,(83) and durometer testing.(51) These techniques address different facets of skin including thickness and stiffness. Thickness and stiffness, in particular, are addressed most commonly using US and indentors, respectively.

The measurement of skin thickness using US (48, 50, 52, 56, 62, 67, 73) has been found to be reliable and valid.(62, 71, 72) The type of US device that was to be used for the upcoming experiments was the Longport Episcan. Kong et al. described this specific device as being able to measure thickness with a coefficient of variation of less than 1% and a resolution of approximately 0.01 mm for a 5 mm thickness.(84) The Longport has also been previously used to measure skin thickness and depth of structure in a variety of studies.(85-87)

Stiffness, which Sun et al. relate to affecting the tissue's ability to dissipate stress,(46) has largely been measured with a variety of indentors.(45, 50, 52) Indentors typically measure the normal force or pressure required to induce displacement. This orthogonal measurement reflects the combined stiffness of all tissues under the indenter. In addition to measuring the normal force applied to skin and the tissue's response to this force, the skin also responds to a traction force across its surface that occurs with

movement. The tissue's response to this force can be measured using a tissue interrogation device (TID) developed at the Georgia Institute of Technology and McGill University. This prototype tool needed to be validated prior to use on the plantar skin.

The purpose of this aim was to address the need for validated tools for assessing plantar skin properties. Equipment was to be utilized that had not been used on the plantar surface of the foot or in a population with altered gait. For this reason, it was important that the equipment used to monitor physical activity as well as the equipment used to examine any changes in the stiffness of skin on the plantar foot be validated. With the resultant validation, one could be reasonably sure that changes measured experimentally were related to actual changes in the skin rather than measurement error associated with a device.

This specific aim was accomplished through several experiments. First, a valid mechanism was needed to measure physical activity in a group of people who may have altered gait patterns. This component was necessary so that physical activity could be quantified using step count, regardless of the speed at which an individual walked. The StepWatch activity-monitoring device was intended for this purpose. Thus, the StepWatch activity-monitoring device was validated. Next, testing elastomeric phantoms with known stiffness checked the reliability of the Tissue Interrogation Device (TID v1). During experimentation, a second iteration of the TID (TID v2) was completed. Therefore, the TID v2 was also checked for reliability. Finally, the myotonometer, an indenter device, was tested for its consistency between typical room temperature and warm, humid environments (Environmental Chamber (EC)). Together, validation of this equipment enabled the remaining specific aims to be addressed.

2.2 StepWatch Validation:

The StepWatch is a device designed to measure activity levels. It has been used and/or validated across many populations including people who have experienced spinal cord injury,(88, 89) stroke,(90-92) and amputation(93). It has also been used among the

elderly(94-96) and those with diseases such as diabetes(20, 97, 98). While these studies reported that some of the participants used assistive devices, the StepWatch had not been specifically validated on a population who used assistive devices. Given that people with diabetes often experience a change in gait pattern related to their disease process(31-36) and sometimes need an assistive device, it was necessary to perform a validation study to enable use of the StepWatch in people with diabetes who may use an assistive device. This study was completed and has been published. It can be read in its entirety in Appendix A.(99)

2.3 Tissue Interrogation Device (TID) Validation:

Stiffness of skin, and the plantar skin in particular, has been measured in previous studies.(45-47, 50) The mechanism by which the stiffness of skin has traditionally been calculated utilizes indenter devices.(45, 50, 52) Stiffness is generally defined as the resistance to deflection or deformation. Indenter devices quantify stiffness by applying an orthogonal force to the surface of the skin and measuring the amount of skin displacement. When indentors are used *in vivo*, the measured tissue stiffness is reflective of the combination of skin and the subcutaneous tissues. Plantar skin stiffness as measured by an indenter mimics the normal loading on skin during stance.

However, people move. During the gait cycle, the forefoot moves into extension as a person moves from mid-stance to push-off. This movement creates a stretching of the skin, which is resisted by tensile forces. Characterizing this type of skin stiffness is more appropriately measured by applying tangential forces on the skin. The measurement of tangential stiffness would be valuable because it would provide a more complete picture of the foot's stiffness, and by extension, its risk for breakdown. While the Cutometer® may be able to address measuring this type of stiffness as it looks at

different depths of the skin, depending on the size of the probe,(100) the contours of the foot and size of the regions of interest would make use of this device difficult.

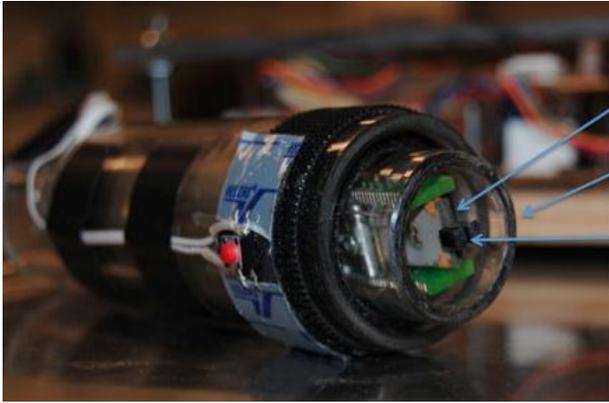
The TID (Figure 2.1a and b), developed at Georgia Tech and McGill University, was designed to measure localized tangential stiffness. Briefly, the device contacts the skin at two points with a small tweezers-like probe and applies tangential force to the tissue. Piezoelectric benders drive these contact points laterally with a gentle traction force to the skin at a frequency of five hertz (Hz). The device measures both force and displacement and provides outputs of skin stiffness and viscosity. With the traction force, the measure of stiffness focuses on the more superficial tissues compared to the devices that apply a strictly normal, compressive load. Preliminary testing demonstrated that the device was able to measure differences between the skin at different anatomical sites. (Wang et al, 2006).(101) Each trial with this device lasted a total of ten seconds. A normal force was applied with the device to the skin to provide adequate friction to load the skin in traction. A constant force spring, aligned with the plastic housing, decreased variation in the normal force applied to the skin. The force used to maintain the TID positioning was limited to approximately 1.5 N. To eliminate the risk of electric shock and to prevent slipping, two, textured delrin boots were adhered to the tips of the piezoelectric benders.

Validation of this prototype instrumentation was critical to ensure the reliability of the TID for measuring the stiffness and viscosity of the plantar skin. Also, this reliability was essential to help determine how many TID measurements were needed for accuracy in future experiments. During the course of the entire project, the next

generation of TID was completed, resulting in the need for additional reliability tests. The two separate sets of TID prototype reliability follow.

2.3.1 TID v1 Prototype Reliability:

First, the TID v1 (Figure 2.1) was tested using elastomeric phantoms of a known stiffness. The testing utilized both a mechanical jig (Figure 2.2) and a handheld technique (Figure 2.3). The testing demonstrated the ability of the TID v1 to distinguish surfaces of varying stiffnesses. It was also intended to expose any differences between the use of a jig, which maintained consistent positioning, and the handheld technique. Following the initial phantom testing, the TID v1 was also tested in typical room temperatures as well as a warm, humid environment. This environmental comparison testing was designed to ensure consistency in device output between environments. Following all of the phantom testing, the TID v1 was utilized on skin.

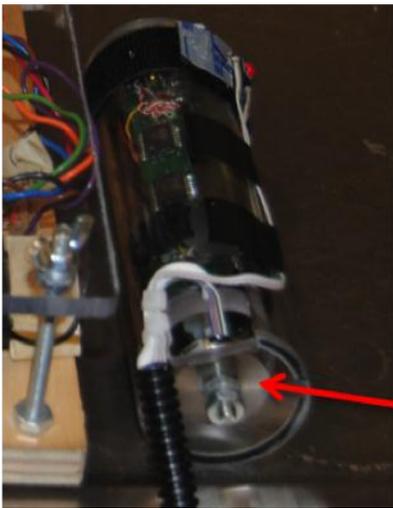


Piezoelectric
Benders

Plastic housing
aligned with spring

Delrin boots

a. Anterior view

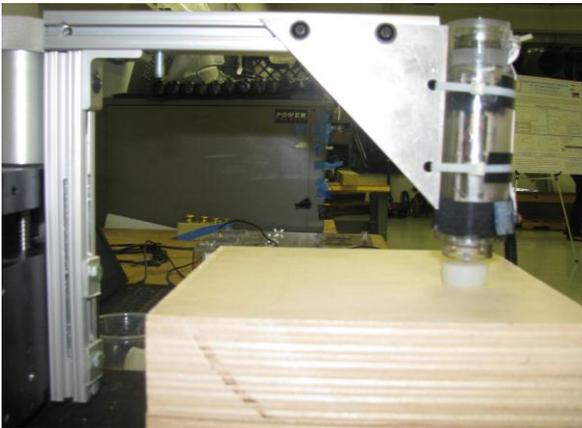


Load
Cell

b. Posterior view
Fig 2.1 TID v1



a. Front view



b. Side view

Fig. 2.2 Mechanical jig



Fig. 2.3 Handheld technique

2.3.1.1 Methods

Samples: Elastomeric phantoms made of Dragon Skin FX-Pro ® (Smooth-On, Inc., Easton, PA), a silicone rubber that was combined with various amounts of thinner to make the substance more elastic. The phantoms used were made with 30% thinner and 80% thinner. Stiffness of the elastomeric phantom decreased with the addition of a larger percentage of thinner. Therefore, the phantom with 30% thinner was relatively stiffer than the phantom containing 80% thinner. The phantoms were selected to bracket the ranges of skin properties so that the reliability of the TID v1 device for testing skin across its surface could be determined. After fabrication, the density and Young’s modulus of elasticity were measured using a high precision multi-tone resonance technique (Table 2.1)(102)

Table 2.1. Description of phantoms

	Young’s modulus (kPa)	Density (kg/m ³)
30% phantom (Stiffer)	9.55	990
80% phantom (Softer)	3.32	974

Equipment: The TID v1 prototype characterized stiffness in N/m and viscosity in Ns/m². The TID v1 was mounted to a jig that enabled precise height adjustment using a precision screw (Figure 2.2). This jig enabled a consistent normal force of 1.5 N to be applied to the phantom for measurement.

Environment: Initial testing for reliability was performed in typical room temperature conditions using both handheld and jig techniques. The typical room conditions ranged from approximately 20 to 24 degrees Celsius and 35-50% relative humidity. The device was also tested in an environmental chamber (EC) set to 32 degrees Celsius and 66% relative humidity.

Protocol: For repeatability testing, TID v1 measurements were taken approximately 15 times per phantom, and technique. Measurement sessions were repeated with each

session considered as independent measurements with respect to data acquisition. Finally, to compare environments, the handheld technique was used in each environment over two measurement sessions.

For each trial, the TID v1 was first allowed to acclimate to the testing environment for at least 10 minutes. Elastomeric phantoms were maintained in the testing room environment for testing completed under typical room conditions. In the case of EC conditions, the phantoms were stored in the typical room environment until TID v1 testing was imminent. This procedure limited the effects that temperature and relative humidity had on the phantoms. Approximately 15 measurements were taken using the TID v1 per phantom for all trial sessions. Testing procedures for loading the elastomeric phantoms were consistent across trials and proceeded as follows. First, TID v1 measurements were performed in an unloaded position. Then, measurements were taken with the TID v1 applying 1.5 N of force to the phantom. The TID v1's benders were programmed to oscillate at five Hz. Measurements were taken for five seconds of oscillations each, both unloaded and loaded. The device outputs were stiffness (N/m) and viscosity (Ns/m²).

Performing this experiment in two environments enabled comparison of the effects of these environments on the equipment. If the environments had an effect, the interaction between the equipment and the environment would potentially confound the results. Such a situation would necessitate the development of a mechanism to account for any differences that were noted. This procedure minimized the risk that the equipment would be a confounding factor for the study.

Data Analysis: Independent sample t-tests were utilized to compare means for stiffness between the two techniques for testing elastomeric phantoms. This comparison was intended to expose any effects that the lowering technique had on the accuracy of

measurement. Additionally, independent sample t-tests were utilized to compare stiffness means between the 30% thinner (stiffer) and 80% thinner (softer) phantoms. This comparison determined if the TID v1 was able to detect differences between phantoms of different densities. Similarly, independent sample t-tests were used to compare means of stiffness measured within each environment (typical room conditions versus EC) to expose any effects that the environment had on the device. Descriptive statistics were used to report the findings of all of the techniques. Coefficients of variation were utilized to demonstrate the variability of the data. Finally, intra-class coefficients were used to demonstrate reliability using measurements taken during two independent measurement sessions.

2.3.1.2 Results

Independent sample t-tests found no differences in stiffness measured using the two techniques (stiffer phantom, $p=0.660$ with equal variance not assumed; softer phantom, $p=0.125$ with equal variance assumed). When viscosity was compared between techniques, again there was no difference (stiffer phantom, $p=0.622$ with equal variance assumed; softer phantom, $p=0.311$ with equal variance assumed). Independent sample t-tests also demonstrated that the TID v1, regardless of technique, was able to differentiate between the stiffer and softer elastomeric phantoms ($p=0.000$ with equal variances assumed in all cases). The precision of the instrument to find the true mean was described using the 95% confidence intervals (Tables 2.2 and 2.3). For stiffness, these intervals represented a precision of instrument measurement that was within 10% of the mean stiffness. The intra-class coefficient for the stiffness measurements taken in two measurement handheld sessions within typical room conditions was 0.982. The viscosity data for this device demonstrated large variations as can be seen in the coefficients of variation (CVs), which were between 45 and 82% (Table 2.3). The intra-class coefficient for the viscosity measurements taken from the same sessions as the above stiffness measurements was 0.610.

When the device was tested within different environments, the results were similar. Two sessions were completed on different days and combined into one dataset. In the typical room environment, a total of 54 trials was performed with 27 trials for both the stiffer and softer phantoms. Fifty-three trials were performed within the EC with 28 trials for the stiffer phantom and 25 for the softer phantom. (Table 2.4) Again, independent sample t-tests confirmed no differences in stiffness values between the two environments (stiffer phantom, $p=0.775$ with equal variances assumed; softer phantom, $p=0.354$ with equal variances assumed). The 95% CI of the handheld measurement sessions taken when comparing typical room and EC conditions represents 10.3% or less of mean stiffness of the same trials. Several trials during the mechanical lowering were lost yielding differences between the number of trials for the stiffer and softer phantom.

Table 2.2. Stiffness measurements for Mechanical jig vs. Handheld techniques. StDev=Standard Deviation; CI=Confidence Interval; CV=coefficient of variation; SEM=standard error of the mean

	Phantom	N	Mean	StDev	Min	Max	CV (%)	SEM	95% CI
Mechanical jig	Stiffer	16	258.8	10.39	241.8	288.3	4.02	2.6	253.69, 263.87
	Softer	13	155.6	14.69	132.0	185.4	9.44	4.07	147.65, 163.62
Handheld	Stiffer	28	261.0	22.42	229.4	303.7	8.59	4.24	252.68, 269.28
	Softer	28	146.8	17.53	112.6	180.1	11.94	3.31	140.35, 153.33

Table 2.3. Viscosity measurement for Mechanical jig and Handheld techniques.
 StDev=Standard Deviation; CI=Confidence Interval; CV=coefficient of variation;
 SEM=standard error of the mean

	Phantom	N	Mean	StDev	Min	Max	CV (%)	SEM	95% CI
Mech- anical jig	Stiffer	13	-1.693	0.88	-3.046	-0.434	52.0	0.22	-2.13, -1.26
	Softer	16	-0.084	0.69	-2.246	0.298	81.9	0.19	-1.21, -0.46
Hand- held	Stiffer	28	-1.825	0.83	-3.617	0.451	45.3	0.16	-2.13, -1.52
	Softer	28	-1.105	0.81	-2.862	0.298	73.5	0.15	-1.41, -0.80

Table 2.4. Stiffness values taken under typical room conditions versus EC with the
 handheld technique. StDev=Standard Deviation; CI=Confidence Interval; CV=coefficient
 of variation; SEM=standard error of the mean

	Phantom	N	Mean	StDev	Min	Max	CV (%)	SEM	95% CI
Room conditions	Stiffer	27	247.44	24.74	200.00	288.24	10.00	4.76	238.11, 256.77
	Softer	27	140.52	18.57	107.97	174.18	13.21	3.57	133.52, 147.52
EC	Stiffer	28	245.21	30.36	209.88	334.89	12.38	6.07	233.31, 257.11
	Softer	25	135.78	18.99	97.15	187.05	13.98	3.59	128.75, 142.81

2.3.1.3 Discussion

The results indicated that the TID v1 demonstrated high reliability in measurements taken on two different elastomeric phantoms, under different ambient conditions (room versus EC) and using different techniques (handheld versus jig). These findings enabled several subsequent decisions to be made about using the TID v1 for measurement of plantar tissues.

The ability of the TID v1 to identify differences in stiffness in a reliable manner provided a realistic expectation that the device could be utilized effectively to measure stiffness at several locations on the plantar foot. Because there was no statistically significant difference between jig and handheld techniques, a decision was made to utilize the handheld technique to collect the stiffness data on the plantar foot. It was also determined that the data could be trusted to be accurate whether measurements were taken within the EC or under typical room conditions. Finally, high correlation of stiffness measurements between the sessions ($r=0.982$) offered assurance that the handheld technique could be utilized to compare stiffness across different measurement sessions.

Viscosity measurements showed wide variability, leading to the decision not to utilize the TID v1 for viscosity measurements in the study of plantar tissue.

2.3.2 TID v2 Reliability:

Deficits in TID v1 motivated an iterative process to develop an improved device. This next generation of the TID (TID v2) (Figure 2.4) was completed prior to the initiation of Specific Aim #3. Changes were made to the electronics and improved fabrication techniques. These changes resulted in a better signal-to-noise ratio of the acquired data and a more reliable operation. Because of these changes and improvements, the TID v2 device also needed to undergo reliability testing. This testing again compared

two elastomeric phantoms (Table 2.1), two measurement techniques (handheld and mechanical jig), and two environmental conditions. Finally, the multiple measurement sessions were utilized to characterize how the TID v2 measured stiffness and viscosity.

2.3.2.1 Methods

Samples: Elastomeric phantoms, like those used for the TID v1 prototype testing, were used to test the reliability of the TID v2. A stiffer phantom (30% thinner) and a softer phantom (80% thinner) were again utilized for testing stiffness and viscosity across the surface of the phantoms.

Equipment: The TID v2 was utilized to gather the stiffness and viscosity data of the elastomeric phantoms. The TID v2 was mounted to a rig utilizing a precision screw that permitted precise height control to apply a 1.5 N orthogonal force to the phantom in advance of applying the tangential forces.

Environment: Testing was performed within typical room conditions (between 20 and 24 degrees Celsius and 35-50% relative humidity) and within an EC set to 32 degrees Celsius and 66% relative humidity.

Protocol: Initial testing was performed to assess the differences between the mechanical jig and handheld measurement techniques. Subsequent testing utilized the mechanical jig technique to allow the most precise control of the forces employed. Testing procedures for loading the elastomeric phantoms were consistent across trials and proceeded as follows. First, measurements were performed in an unloaded position. Then, orthogonal pressure by the device to the phantom was applied to 1.5 N during the measurement period to maintain the position of the device. The probe moved at a frequency of five Hz. Measurements were taken for five seconds of oscillations each, both unloaded and

loaded. The device outputs were stiffness (N/m) and viscosity (Ns/m²). Test sessions were considered to be independent.

Testing was performed in sessions of 22 trials each. Sessions were conducted in the lab at the Center for Assistive Technology and Environmental Access (CATEA), the exercise physiology laboratory (EPL), and in the EC within the EPL. For measurement technique comparisons, three sessions were performed on each elastomeric phantom (30% thinner (stiffer) and 80% thinner (softer)) using both techniques. Next, to assess differences in environmental conditions, test sessions were repeated under typical room conditions and within the EC. Finally, the first two sessions for each phantom measured in each location (CATEA, EPL, and EC) were utilized to measure day-to-day reliability for the TID v2 device.

Data Analysis: Comparison of means testing was performed using independent sample t-tests. These were employed for the following comparisons: measurement techniques, phantom stiffness, and consistency of measurement within different environmental conditions. All independent sample t-tests were performed separately for stiffness and viscosity outputs. For reliability, descriptive statistics were used to describe findings of the techniques. Coefficients of variation were used to describe variability of data. Additionally, intra-class correlation coefficients were used to demonstrate day-to-day reliability.

Finally, statistical analysis was used to determine how many trials should be used experimentally when measuring plantar tissues. This analysis assessed using three and five repeated measures using the softer phantom data collected with the handheld technique because of its greater variance. Sixty-four points of stiffness and viscosity were

used in the analysis. A set of five data points was randomly identified using www.random.org. This random selection was chosen because no ordering effect was noted for trials within a session. This selection process was repeated five times. After each randomization, the first three points were placed in the “3 Trials” dataset and the five points were placed into the “5 Trials” dataset. There were a total of five sets of both “3 Trials” and “5 Trials” data that were representative of the initial 64-point dataset. The mean and median were calculated and compared to the overall mean of the 64 points.

2.3.2.2 Results

Differentiation of measurement techniques: Using an independent sample t-test to compare means for the same phantoms using different lowering techniques, there was no difference between the two techniques when measuring the stiffness ($p=0.672$ with equal variances not assumed) or viscosity ($p=0.845$ with equal variances assumed) of the stiffer elastomeric phantom (30% thinner). However, when the two techniques were compared when testing the stiffness of the softer phantom (80% thinner), there was a difference ($p=0.013$ with equal variances not assumed) between the two. The viscosity of the 80% thinner phantom did not show a difference between the techniques ($p=0.507$ with equal variances assumed).

These testing sessions were further examined for measurement reliability by calculating CVs for both stiffness and viscosity. CVs for stiffness measurements of individual sessions utilizing the jig were less than 1% for both phantoms. Aggregating data from all sessions slightly raised the CVs but they remained less than 2%. The CVs for individual sessions using the handheld technique were also low, with a range of 2.1% to 5.3%. Aggregate values for stiffness CVs using the handheld technique were 2.9% for the stiffer phantom and 5.1% for the softer phantom (Tables 2.5 and 2.6). CVs for viscosity measurements utilizing the mechanical jig for individual sessions were less than 8% as were the aggregated values (Table 2.7). For individual sessions, the handheld

technique exhibited CVs that ranged from 4.6% to 10.3%. Aggregate values for handheld viscosity CVs were less than 10% (Table 2.8). In general, variations in both stiffness and viscosity were greater with the softer material and when using the handheld measurement technique. The precision of the instrument to find the true mean for both stiffness and viscosity can be seen with the 95% confidence intervals (CI) also listed in the same Tables. The 95% CI using the mechanically jig sessions represented 1.5% or less of the mean stiffness values and 6.5% or less of the mean viscosity values.

Differentiation of phantom densities: Using independent sample t-tests to compare the overall means of both measurement techniques together as well as each separately, the device was able to detect differences in both stiffness and viscosity between the phantoms ($p=0.000$ with equal variances not assumed for stiffness and equal variances assumed for viscosity).

Differentiation of environmental conditions: When the device was tested within different environments, results were dependent on material and situation (Tables 2.9 and 2.10). Again, independent sample t-tests were used to confirm any differences between the two environments. With the softer phantom (80% thinner), stiffness had $p=0.054$ (equal variances assumed) whereas viscosity had $p=0.058$ (equal variances not assumed) so no differences were present. For the stiffer phantom (30% thinner), on the other hand, an event during the testing affected the outcome. During the first session of measuring the stiffer phantoms in the EC, the Delrin boot on one of the piezoelectric benders broke requiring its reattachment. When all the sessions' trials were combined, no differences in stiffness ($p=0.233$ with equal variances not assumed) or viscosity ($p=0.792$ with equal variances assumed) were found. But, if the EC trials prior to the break were thrown out, there was a difference between environmental conditions for both stiffness ($p=0.042$ with equal variances not assumed) and viscosity ($p=0.025$ assuming equal variances).

Day-to-Day Reliability: Using intra-class correlation coefficients, the day-to-day reliability for stiffness was moderate to high as it varied between 0.802 for all the

conditions measured in the EPL (room and EC environments) and 0.932 for all of the typical room conditions. Also, viscosity values varied between 0.996 (room and EC in the EPL) and 0.855 (for all typical room conditions) (Tables 2.11 and 2.12).

Trial Number: When the differences between the mean and median of the random data sets and that of the entire dataset (Table 2.13) are reviewed, the “3 Trials” and “5 Trials” reveal equivalent results. Stiffness values of the randomized sets were within 3.5% of the overall mean of 22 trials, whereas viscosity values were within 9%. These values are consistent with the CVs that were found within the sessions as well as the aggregate values, which suggest that, either three or five trials is an appropriate number to be used experimentally.

Table 2.5 Stiffness Measurements with Mechanical jig
 N=number of trials; StDev=Standard Deviation; Min=minimum; Max=maximum;
 CV=coefficient of variation; SEM=standard error of the mean; Agg=aggregate; CI=95%
 Confidence interval

Phantom	Session	N	Mean	StDev	Min	Max	CV (%)	SEM	CI
Stiffer	1	22	547.1	2.44	541.26	550.38	0.45	0.520	546.08, 548.12
	2	22	548.4	2.25	543.14	551.34	0.41	0.480	547.49, 549.37
	3	22	535.7	2.63	527.11	539.06	0.49	0.562	534.58, 536.78
	Agg	66	543.7	6.25	527.11	551.34	1.15	0.769	542.23, 545.25
Softer	1	22	477.9	4.30	464.38	487.53	0.90	0.917	476.1, 479.71
	2	22	486.0	8.79	462.98	498.30	1.81	1.875	482.33, 489.67
	3	22	459.1	6.25	444.76	468.16	1.36	1.332	456.47, 461.69
	Agg	66	474.3	13.143	444.76	498.30	2.77	1.618	471.16, 477.50

Table 2.6 Stiffness Measurements with Handheld Lowering

N=number of trials; StDev=Standard Deviation; Min=minimum; Max=maximum; CV=coefficient of variation; SEM=standard error of the mean; Agg=aggregate; CI=95% Confidence interval

Phantom	Session	N	Mean	StDev	Min	Max	CV (%)	SEM	CI
Stiffer	1	21	552.9	11.94	516.59	567.44	2.16	2.606	547.83, 558.05
	2	22	536.1	16.5	481.77	552.58	3.08	3.518	529.22, 543.00
	3	22	540.0	12.85	516.41	561.9	2.38	2.740	534.61, 545.35
	Agg	65	542.9	15.49	481.77	567.44	2.85	1.921	539.09, 546.63
Softer	1	20	481.7	25.45	424.63	514.26	5.28	5.691	470.52, 492.82
	2	22	451.7	17.2	425.99	480.85	3.81	3.667	444.48, 458.86
	3	22	466.0	18.46	439.03	505.14	3.96	3.936	458.33, 473.75
	Agg	64	465.8	23.73	424.63	514.26	5.09	2.966	459.98, 471.60

Table 2.7. Viscosity Measurements with Mechanical jig
 N=number of trials; StDev=Standard Deviation; Min=minimum; Max=maximum;
 CV=coefficient of variation; SEM=standard error of the mean; Agg=aggregate; CI=95%
 Confidence interval

Phantom	Session	N	Mean	StDev	Min	Max	CV (%)	SEM	CI
Stiffer	1	22	5.782	0.348	5.415	6.751	6.02	0.0742	5.637, 5.927
	2	22	5.616	0.308	4.813	6.580	5.48	0.0656	5.487, 5.745
	3	22	5.588	0.193	5.214	5.999	3.46	0.0412	5.507,5.669
	Agg	66	5.662	0.299	4.813	6.751	1.15	0.0368	5.590, 5.734
Softer	1	22	4.992	0.320	4.141	5.552	6.41	0.0682	4.858, 5.126
	2	22	5.164	0.402	4.460	6.080	7.79	0.0858	4.996, 5.332
	3	22	4.923	0.364	4.440	5.834	7.39	0.0776	4.771, 5.075
	Agg	66	5.027	0.372	4.141	6.080	7.41	0.0458	4.937, 5.117

Table 2.8 Viscosity Measurements with Handheld Lowering
 N=number of trials; StDev=Standard Deviation; Min=minimum; Max=maximum;
 CV=coefficient of variation; SEM=standard error of the mean; Agg=aggregate; CI=95%
 Confidence interval

Phantom	Session	N	Mean	StDev	Min	Max	CV (%)	SEM	CI
Stiffer	1	21	5.804	0.471	5.081	6.853	8.12	0.1028	5.603, 6.005
	2	22	5.515	0.311	4.843	6.308	5.63	0.0662	5.385, 6.645
	3	22	5.709	0.349	4.887	6.274	6.12	0.0745	5.563, 5.855
	Agg	65	5.674	0.395	4.843	6.853	6.95	0.0489	5.578, 5.770
Softer	1	20	5.19	0.535	4.606	6.545	10.31	0.1196	4.956, 5.424
	2	22	4.977	0.481	4.348	6.303	9.66	0.1026	4.776, 5.178
	3	22	4.787	0.221	4.446	5.214	4.61	0.0471	4.695, 4.880
	Agg	64	4.978	0.455	4.348	6.546	9.13	0.0568	4.867, 5.090

Table 2.9. Ambient versus EC (32° C, 66% relative humidity) descriptive data for stiffness (Aggregate from 2 sessions each). StDev=Standard Deviation; CI=Confidence Interval; CV=coefficient of variation; SEM=standard error of the mean

Condition	Phantom	N	Mean	StDev	Min	Max	CV (%)	SEM	95% CI
Ambient	Stiffer	44	542.11	10.88	514.7	561.0	2.0	1.64	538.90, 545.33
	Softer	44	467.33	21.17	433.0	517.8	4.5	3.19	461.08, 473.59
EC	Stiffer	44	540.18	6.30	531.7	545.8	0.7	0.56	537.72, 539.92
	Softer	44	475.29	16.71	455.6	502.0	3.5	2.52	470.35, 480.23

Table 2.10. Ambient versus EC (32° C, 66% relative humidity) descriptive data for viscosity (Aggregate from two sessions each). StDev=Standard Deviation; CI=Confidence Interval; CV=coefficient of variation; SEM=standard error of the mean

Condition	Phantom	N	Mean	StDev	Min	Max	CV	SEM	95% CI
Ambient	Stiffer	44	5.620	0.219	5.131	5.985	0.039	0.0330	5.555, 5.684
	Softer	44	5.057	0.416	4.349	6.164	0.082	0.0628	4.934, 5.180
EC	Stiffer	44	5.749	0.304	5.292	6.771	0.055	0.0473	5.625, 5.810
	Softer	44	4.913	0.270	4.235	5.533	0.055	0.0406	4.833, 4.993

Table 2.11. Stiffness reliability for combined stiffer and softer phantoms.

	Intra-class r
All locations/sessions/environments	0.850
Lab room and EC	0.802
All room conditions	0.932
EC	0.860

Table 2.12. Viscosity reliability for combined stiffer and softer phantoms.

	Intra-class r
All locations/sessions/environments	0.919
Lab room and EC	0.996
All room conditions	0.855
EC	0.957

Table 2.13 Determination of trial number

Stiffness					
<i>3 Trials</i>	A	B	C	D	E
mean	0.51%	-0.03%	-1.53%	-0.77%	2.38%
median	-3.23%	1.09%	-3.45%	-1.47%	3.31%
<i>5 Trials</i>					
mean	0.69%	0.54%	0.99%	-2.09%	2.52%
median	0.80%	1.09%	0.80%	-1.47%	3.31%
Viscosity					
<i>3 Trials</i>	A	B	C	D	E
mean	-8.62%	1.05%	-0.41%	1.98%	2.00%
median	-7.47%	-5.80%	-0.63%	-1.05%	4.74%
<i>5 Trials</i>					
mean	-7.11%	0.66%	-3.40%	-1.20%	4.39%
median	-7.47%	-2.84%	-5.99%	-3.46%	4.74%

2.3.2.3 Discussion

The TID v2 was tested for reliability using two elastomeric phantom densities (30% thinner and 80% thinner). Statistically significant differences were noted between the mechanical and handheld lowering techniques. Statistically significant differences

were also noted when comparing ambient versus EC environment testing, depending on which values were utilized. These findings resulted in several decisions.

First, it was determined that using the mechanical jig resulted in smaller CVs and thus measurements that were more repeatable within a testing session. Because of this finding, the subsequent use of the mechanical jig was chosen to improve the precision of measurement. No further handheld measurements were conducted with this device. This finding also suggested that development of a mechanism to mechanically control the lowering of the device for future experimental usage was important.

Differences were found between the environmental condition measurements only in the case of the stiffer phantom when the trials from a more similar device were thrown out. This “more similar device” was described as such because the boot location was identical between the trials until the boot detached. With such an equipment failure, repair was unlikely to have resulted in an exactly identical placement of the boot as was previously employed. Despite this, to minimize the effects of the heat and humidity on the device, it was planned that the actual device be maintained outside of the chamber during testing. The EC comes equipped with a hole in the wall through which the probe can be passed for use in the chamber. While it is not being used, the probe can rest within the wall at a temperature closer to the ambient conditions of the laboratory. However, when one considers the expectation that all experimentation associated with Specific Aim 3 will take place in the EC, then, it is more important to look at the reliability within a single condition.

The TID v2 demonstrated that it was able to detect differences in stiffness where differences existed. With this determination, it can be expected that this device has appropriate precision and reliability so that differences in stiffness and viscosity can be detected on the skin of the plantar foot. Based on the dataset from the three sessions of 22

trials, especially looking at the CV and standard error of the mean, the measurement accuracy was expected to be $\pm 5\%$ for stiffness and $\pm 10\%$ for viscosity.

It should be noted that with many of the independent sample t-tests equal variance could not be assumed. Additionally, there was a sizable variance between ICC values, depending on what sessions were combined. It is clear that the sessions that occurred under more similar conditions also had higher correlations and smaller variance. With the high precision of this instrument, it is likely that its immediate environment, to some degree, affects the TID v2. For instance, some trials, particularly at CATEA had various amounts of air disturbance present during testing. This included large outside doors opening and closing as well as air conditioning coming on or turning off. Such changes in the immediate airflow, could have affected the consistency of the measures. With the possibility that variances in the environment, including differences in temperature and relative humidity, affect precision, it is important that one considers the location where testing is completed. It is suggested that testing is not performed directly under air vents and it is encouraged to test in a consistent environment when comparisons are to be made.

The results for the sampling guidelines for three or five trial sessions are consistent with the mean and median results from larger samplings. This result suggests that either a sampling of three measures or a sampling of five measures would be equally appropriate to perform.

2.4 Myotonometer Reliability in Typical Room versus Environmental Chamber Conditions:

Stress to feet occurs with both static and dynamic positioning. For this reason, it is still important to measure stiffness in a normal direction. Stiffness measured by applying

a normal force, often with an indenter, is the traditional way to evaluate a tissue's response to stress. As is typical of indentors, the myotonometer (Neurogenic Technologies Inc.) can be used to measure compliance when a given force is applied orthogonally. While this device was initially designed to measure muscle tone,(103) it was previously used on the skin of the buttock in patients with spinal cord injury.(104) Previous measurement had been performed in a typical room temperature environment. Because multiple environmental conditions were anticipated, it was important to assess the ability of the device to be consistent across conditions, including one that was warm and humid.

2.4.1 Methods

“Samples”: Elastomeric phantoms, like those used for the testing of both TIDs, were used to test the reliability of the myotonometer. A stiffer phantom (30% thinner) and a softer phantom (80% thinner) were utilized for testing stiffness.

Equipment: The myotonometer (Neurogenic Technologies Inc.) was utilized to measure the compliance (displacement/force) of the elastomeric phantoms with a given force. The force that was provided was 1.5 kg. The myotonometer measured displacement at the following force application points (kg): 0.25, 0.43, 0.61, 0.79, 0.96, 1.14, 1.32, and 1.5. The manufacturer selected these forces to allow for an adequate range of force-displacement measurements.

Environment: Testing was performed in a typical room temperature condition as well as in a warm, humid condition within an EC. The room condition was between 20 and 24 degrees Celsius and 35-50% relative humidity. For environmental comparison testing, the EC was set to 32 degrees Celsius and 66% relative humidity.

Protocol: For each session, the myotonometer was first allowed to acclimate to the testing environment for at least 10 minutes. The elastomeric phantoms were maintained in the room environment for testing completed under typical room conditions. In the case of the EC condition, the phantoms were maintained in the typical room condition until testing was imminent. This procedure limited the effects that the temperature and relative humidity had on the phantoms. Performing this experiment in two environments allowed for analysis to compare environmental effects on the equipment.

Each trial consisted of eight repetitions of an orthogonal force of 1.5 kg being applied to the phantom. Each repetition took between one and two seconds. The myotonometer software combined the repetitions for a single output per trial. Six trials were performed for each phantom in each condition.

Data Analysis: Independent sample t-tests were utilized to compare the displacement means of the stiffer and the softer phantoms both together and separately between the two environments. Comparisons were made at each of the eight points of force where measurements were taken (0.25, 0.43, 0.61, 0.79, 0.96, 1.14, 1.32, and 1.5 kg).

2.4.2 Results

When the stiffer and softer phantoms were compared together for the separate environmental conditions, there were no differences (p values ranged from 0.641 to 0.991). When the stiffer phantom was compared separately between the two conditions, again no differences were found between the typical room and EC conditions (p values ranged from 0.130 to 0.892). In each of these cases, there was no violation of homogeneity of variances. Finally, when the softer phantom was compared separately between the two conditions, a difference was found only at the initial displacement point (0.25 kg) with a p value of 0.043. Additionally, violations of homogeneity of variance occurred at the displacements occurring when 1.14, 1.32, and 1.5 kg of force were applied.

2.4.3 Discussion

When the myotonometer outputs were compared between the typical room and EC environments, there was minimal inconsistency. Overall, there were not differences in the way the device measured between conditions. This was the case when both phantoms were combined and when the stiffer phantom was reviewed separately. However, for the softer phantom, a difference at the first displacement point was made evident. All other points showed no differences. Given these findings, caution should be taken when analyzing data with this device. A possible solution may include selecting displacement points to use for further analysis that focus on the central displacements. Thus, the initial displacement point would be avoided to minimize possible measurement error.

3 Characterization of Plantar Skin across Environmental Conditions and Time

3.1 Specific Aim #2: Characterize the skin tissue of the foot across environmental conditions and time.

Multiple studies have investigated skin stiffness and thickness in people at a single point in time.(45, 46, 50, 51, 105, 106) Other studies have looked at skin at different times within the same day to better understand diurnal variations.(56) Studies looking across days are very limited.(107) Overall, stiffness and thickness findings from these studies have been variable depending, in part, on location and level of neuropathy.(45, 46, 51, 106) Little is known, however, about the key attributes of skin stiffness and thickness relative to how they behave across time or between environments. Studies that have examined the stiffness and thickness of plantar skin have made little effort to evaluate the foot within an environment consistent with its typical surroundings, the shoe. Additionally, stiffness measured tangentially across the skin tissue, as opposed to perpendicularly, has not been evaluated in the plantar feet.

Specific aim #2 was designed to address the need to understand and characterize how the skin of people behaves under different environmental conditions and across time. This characterization is an important step to provide foundational knowledge of typical skin behavior. The resultant information is critical for interventional study design, especially that which requires repeated measures.

The purpose of this study was to characterize the plantar skin across environmental condition and time. The null hypothesis was that plantar skin properties do not change across environment and time. Specifically, answers to the following questions were sought:

1. Do plantar tissue properties such as stiffness, compliance, and thickness change within a day and across a few weeks of time?
2. Do plantar tissue properties such as stiffness, compliance, and thickness behave differently with respect to different environments (temperature and relative humidity)?

Also, the question “do stiffness, compliance, and thickness of the plantar skin differ between people with and without diabetes” was considered. Because this question is not central to Specific Aim #2, the answer was addressed in Appendix B.

3.2 Determination of Shoe Environment:

First, it was necessary to determine the environment of a foot within a shoe. The quantification of such an environment was important to allow the determination of a realistic and clinically relevant comparison in which to evaluate skin tissue across conditions. The aim of this experiment was to measure the temperature and relative humidity (RH) of the foot within a shoe environment as well as the temperature and RH of the shoe itself. Sensors that measured the temperature as well as the RH were utilized to determine the appropriate environmental conditions that mimic the conditions of the foot inside of a shoe.

3.2.1 Methods

Subjects: People with and without diabetes participated. Each subject had protective sensation to the plantar foot as determined by sensation testing using a 5.07 Semmes Weinstein monofilament.

Equipment: Temperature and RH sensors (MSR Electronics GmbH, Seuzach, Switzerland) (Figure 3.1) were utilized on the foot and shoe. These devices were

integrated with loggers (MSR) that recorded the temperature and RH as long as the devices were in place. Modular Signal Recorder (MSR) software was used to download the data. Accuracy for the temperature sensors within the range of temperatures used was $\pm 0.1^\circ$ Celsius (C). Accuracy for RH sensors was $\pm 4\%$ RH at this range of temperatures.

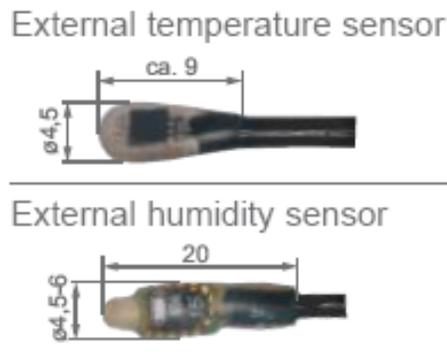


Figure 3.1 Temperature and RH sensors

The StepWatch Activity Monitor (Orthocare Innovations, Oklahoma City, OK), a user-worn sensor, and a United States government FDA cleared class II device designed for long-term assessment of community walking function, was used to monitor activity. The device was 75 x 50 x 20 mm and weighed 38 grams.(108)

Protocol: Following informed consent, initial testing was performed to determine if a subject had intact protective sensation. Protective sensation was defined as the ability to feel a 5.07 Semmes Weinstein monofilament (10 g). Subjects then completed a short questionnaire including a basic history and level of physical activity. Next, temperature and RH were measured using small probes (Figure 3.1) placed on the surface of the plantar skin and within an individual's shoe. A temperature sensor and a RH sensor were placed at the medial longitudinal arch of the foot and, similarly, temperature and RH

sensors were also placed at the medial wall of the shoe (Figure 3.2). Sensors were adjusted so that they did not impose uncomfortable pressure on the area or affect a subject's gait. To monitor physical activity for the study, the StepWatch was placed just above the lateral malleolus. The StepWatch was calibrated according to the manufacturer's instructions using short bouts of gait. Following set-up, the sensors remained on a subject for approximately six hours of one day. The subjects were asked to go about their day as they normally would and record their activities on a log form. The sensors collected data the entire time they were positioned. The subjects returned after wearing the sensors for the allotted time to have the loggers/sensors removed.

Descriptive statistical analyses were utilized to find the mean temperature and RH of the foot within a shoe as well as the shoe itself.



Figure 3.2 Temperature and RH sensor placement a. foot, b. shoe

3.2.2 Results

Nineteen subjects with and without diabetes participated in this study. The mean (\pm standard deviation) temperature within the shoe was 32 ± 1.6 ° C with a range of 28° C to 34° C. The mean (\pm standard deviation) RH was $66 \pm 14.1\%$ with a range of 37% to 95%. The mean (\pm standard deviation) temperature as measured on the shoe wall itself was 29 ± 1.9 ° C with a range of 25 to 32° C. The mean (\pm standard deviation) RH was measured on the shoe wall to be $65 \pm 13.6\%$ with a range of 41% to 89%. The mean number of strides (\pm standard deviation) walked across subjects was 2662 ± 1825 . This amount of wear-time activity certainly varied across individuals with a range of 328 strides to 6894 strides. Similarly, durations of time that sensors were worn also varied across people from four to seven hours with most wearing the sensors six hours.

3.2.3 Discussion

The average temperature and RH of the foot within the shoe was 32° C and 66% RH. These were selected for the condition within the environmental chamber (EC) to mimic the environment within a shoe. While these averages were utilized, there was a certain amount of variability between people as was noted within the resultant temperature and RH ranges. The variability for temperature was relatively limited with an actual range of seven degrees among those participating. The relative humidity, however, had a larger variability with a range of 37% to 95%.

Differences in hosiery, sock and footwear design can impact air exchange and therefore, in-shoe temperature and humidity. This study chose to measure conditions while subjects wore a variety of clothing and footwear in an attempt to identify environmental conditions that reflect such variation.

3.3 Characterization of Skin across Time and Environmental Condition:

Once the environment of the foot was determined, the behavior of skin properties across time and environmental conditions was compared. The subsequent characterization study was designed to fully address Specific Aim #2 and its related questions. The null hypothesis was that tissue properties do not change across environment and time.

3.3.1 Methods:

Subjects: The subjects included people with and without diabetes who were between 18- and 85-years-old. People who were unable to assume testing positions were excluded. A broad range of subjects was included to give a more comprehensive picture of skin.

Equipment: Tissue Interrogation Device (TID v1): This non-commercial, research tool measures the tensile stiffness of tissue using a small tweezers-like probe that applies gentle traction force to the skin at frequencies of five Hz. Each trial with this device lasts five seconds each for an unloaded condition as well as a loaded condition. A 1.5 N normal force was applied to give adequate friction to load the skin in traction. A constant force spring aligned with the plastic housing reduced variability of the normal force applied.

The myotonometer (Neurogenic Technologies, Inc.) measures tissue compliance by recording displacement in response to orthogonal forces. Tissue compliance was measured at the following forces: 0.25 kg, 0.43 kg, 0.61 kg, 0.79 kg, 0.96 kg, 1.14 kg, 1.32 kg, and 1.5 kg. These forces were manufacturer-selected to allow for an adequate range of force-displacement measurements.

An US (Longport Episcan I-100) was utilized to measure skin thickness. This 20 MHz unit measured the skin utilizing a B-scan technique. A blinded researcher, not otherwise associated with the project, measured thicknesses of the epidermis and dermis using custom LabView software.

To assess hydration status, a hand refractometer (Atago USA, Inc., Bellevue, WA) was utilized to measure the urine specific gravity (USG) of the urine sample provided.

Additionally, an osmometer (Precision Systems micro-Osmette, Natick, MA) was utilized to determine urine osmolality (Uosm). Finally, a color chart was used to help establish the level of hydration of each subject as well. For Uosm, three measurements were averaged together to determine the overall urine osmolality. Together, USG and Uosm, along with urine color, were utilized to quantify individual hydration levels.

Environment: Testing was performed in typical room temperature conditions where the temperature ranged from approximately 20 to 24 degrees C and 35-50% relative humidity. An EC set to 32 degrees C and 66% RH, as previously determined, was used as the second testing environment so that the environment of a foot within a shoe would be approximated.

Procedures: Following a study explanation and prior to any participation, subjects signed an informed consent to participate in this study. Testing was performed over four visits to better understand and characterize skin over time. The first two visits occurred on the same day so that diurnal variation could be evaluated. Visit one was completed in the morning and visit two was completed in the afternoon. Several hours separated these visits. The third visit occurred within the same week as the initial two visits. It was either in the morning or afternoon at the same time of day as either visit one or visit two. The

final visit was at least one week after the third visit and within a month of initial testing. The visit was scheduled at the same time of day as the third visit. Three visits were scheduled at the same time to enable consistent comparison across days, regardless of the variation that may occur within a single day.

On the first visit, subjects were asked to fill out a demographic survey (Appendix C). Height was also measured. Protective sensation on the plantar feet was tested on the initial visit using a 5.07 Semmes Weinstein monofilament (10 g). These tests were not repeated on each subsequent visit. An individual's protective sensation and height were assumed to be stable within a month.

On each of the four visits, subjects were weighed and were asked to urinate into a specimen container. Hydration level was determined by testing the urine specimen for urine color using a color chart, USG using a hand refractometer (Atago USA, Inc., Bellevue, WA), and Uosm using an osmometer (Precision Systems micro-Osmette, Natick, MA). The hydrated state was defined as having urine color of less than four, USG of 1.020 or less(109), and Uosm of less than or equal to 700 mOsm/L.(109)

At each visit, testing was performed in two environmental conditions. Within each testing condition, measurements were taken using the TID v1, the myotonometer, and the US. Measurements were taken at the great toe, first metatarsal head, third metatarsal head, lateral midfoot, and heel. Some of these locations were chosen because they are sites where skin breakdown is common (primarily at the forefoot). The sites were also chosen because of the differing forces to which they are subjected during weight-bearing and gait. Finally, they were chosen for their differing anatomy. Measurement locations were palpated and marked using the outline of the myotonometer

as well as its central plunger. By marking these sites, measurements within a session could be taken at consistent locations. Additionally, on the first day, the markings were not removed so that testing done at visit one and visit two occurred at the same locations. Slight variations in measurement location were likely between the other test sessions because the site markings typically wore off and had to be re-palpated. Location order and the order of testing device utilization were randomized. The same location testing order was used across devices within a single visit. Environmental condition was blocked so that each subject had two sessions beginning in the EC and two sessions beginning in a typical room condition. All possibilities of order were utilized. Testing took approximately one hour in each environmental condition per session.

For device measurements, the following specific procedures were performed. Throughout all procedures, a customized positioning device was used to assist the examiner in maintaining the foot in a neutral position of dorsiflexion/ plantarflexion (Figures 3.2, 3.3, 3.4).

The TID v1 was used to test skin stiffness by applying a tangential force across the skin. While the TID does not measure friction, it requires a consistent normal load to provide adequate friction so that it can measure the tangential stiffness without the occurrence of slippage. To test, the device was lowered by hand such that approximately 1.5 N of orthogonal force were applied (Figure 3.3). This force provided enough friction so that the piezoelectric benders/delrin boots did not slip when the traction force was applied. In the case when the subject's skin did not have the requisite friction, a thin layer of hypoallergenic body glue (It Stays, Sigvaris Inc., Peachtree City, Georgia) was rolled onto the skin to prevent slippage. The glue was not used if the subject had an allergy to

adhesives. Each site was tested five times. The skin area was wiped clean of any body glue following measurement.



Figure 3.3 TID v1 measurements using positioner

Tissue compliance was measured at each site using the myotonometer. (Figure 3.4). Orthogonal force was applied with the central plunger component of the probe, progressively to 1.5 kg over the course of one to two seconds. This was repeated eight times at each site. The myotonometer software calculated the load and amount of tissue displacement. Tissue compliance was recorded by measuring displacement at 0.25 kg, 0.43 kg, 0.61 kg, 0.79 kg, 0.96 kg, 1.14 kg, 1.32 kg, and 1.5 kg.



Figure 3.4 Myotonometer measurement using positioner

For US measurements, a water-based gel was used at each site to enable the transmission of the US waves. The US was applied perpendicularly to the skin at each marked site (Figure 3.5). US images were taken when the scanned images appeared clearly on the computer monitor in real time. Five images were taken per testing site per condition.



Figure 3.5 US data collection using positioner

Statistical analyses utilized descriptive statistics as well as repeated measures ANOVA. Independent variables included time (visits), condition (EC/Typical room environment), and anatomical location. Dependent variables included tissue stiffness, tissue compliance, and skin thickness. Independent t-tests were utilized to quantify any differences between group demographic data. Intraclass correlation coefficients (ICC) were used to compare measurements taken between visits one and two, one and three, and one and four. Reliability for ICC values greater than 0.75 was considered good while those below were considered poor to moderate.(110) Additionally, disease state (diabetes) was entered as a between groups variable in the repeated measures ANOVA. Findings based on disease state can be found in Appendix B.

3.3.2 Results

Demographics: Sixteen people participated in the study, eight with diabetes and eight without diabetes. Ages ranged from 19- to 78-years-old with a mean age of 48.5 ± 19.23 years. Specific demographic statistics can be seen in Table 3.1. Mean body mass index (BMI) for the whole group was 31.5 ± 7.61 . The Centers for Disease Control and Prevention classify a BMI score of 30 or more as obese, whereas scores of between 25 and 29.9 are classified as overweight. Increased disease risk is associated with both overweight(111) and obesity(112) classifications of BMI.

Mean self-reported activity levels were 1.3 ± 1.3 which fell between minimally active and sedentary. Sedentary (0) was defined as performing activity on less than or equal to one day per week while minimally active (1) involved doing activity on less than or equal to two days per week. Moderately active (2) was reported if a subject performed physical activity on less than or equal to three days per week. Finally, a subject reported

being active (3) if they participated in activity more than three days per week. The range of activity levels across all participants included all four categories.

Gender groups were not equivalent within this study. It was not expected to impact the results. Protective sensation was intact across all subjects without diabetes. Half of the subjects with diabetes had a loss of protective sensation which placed them at a higher risk for plantar ulceration.(30)

Table 3.1 Subject demographic profile.

Subjects	Age (years ± SD)	Gender	BMI (± SD)	USG (± SD)	Activity Level (± SD)	Protective Sensation (Intact)
N=16	48.5±19.2	11F/5M	31.5±7.61	1.021±0.008	1.31±1.3	12
Ranges:	19-78		22.1-47.3	1.01-1.029	0-3	

3.3.2.1 ***TID v1:***

As the sites were tested, there was a mild stress softening that was apparent with the first three measurements taken during repeated testing. Mean stiffnesses across trials were 506.064, 502.855, 499.819, 502.796, and 503.592 from trial one to trial five, respectively. No statistical differences were present between trials. There were also no main effect differences for stiffness across time as was measured by visits ($p=0.294$ Greenhouse-Geisser).

When TID measurements were compared using repeated measures ANOVA, there were main effect differences between the locations ($p=0.000$) (Figure 3.6). Using partial eta squared, more than 47% of the variability was related to the location. With further evaluation using pairwise comparisons with Least Significant Difference adjustment, stiffness variations could be readily seen. The great toe was less stiff than the

first metatarsal head ($p=0.000$), the lateral midfoot ($p=0.000$), and the heel ($p=0.000$).

The first metatarsal head tended to be less stiff than the lateral midfoot ($p=0.056$) and the heel ($p=0.051$). The third metatarsal head was less stiff than the lateral midfoot ($p=0.006$) and the heel ($p=0.013$). All relationships can be seen in Table 3.2.

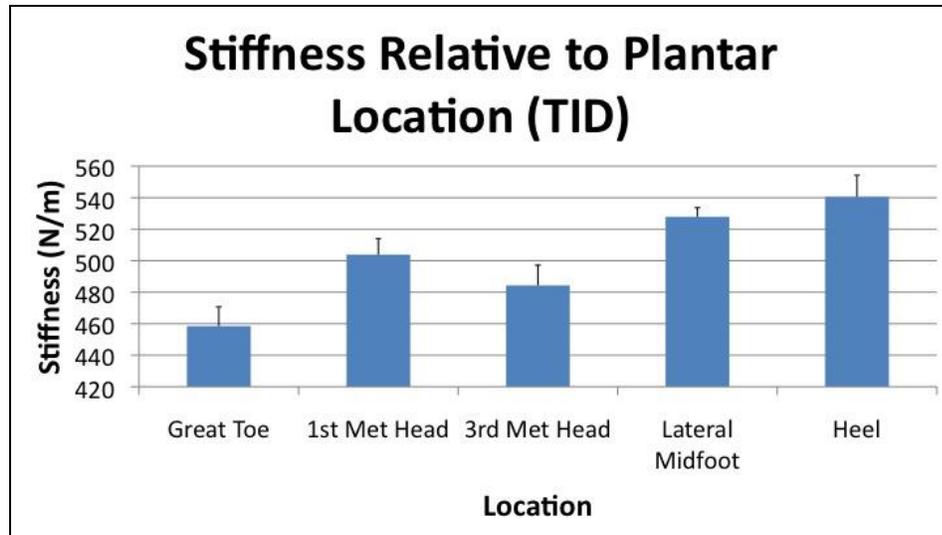


Figure 3.6 Stiffness relative to plantar location

Table 3.2 P-values for pairwise comparisons of the stiffness by location. # denotes that the location in the left column is less stiff than the location in the top row.

LOCATION	First met head	Third met head	Lateral midfoot	Heel
Great toe	$p = 0.000$ #	$p = 0.192$ #	$p = 0.000$ #	$p = 0.000$ #
First met head		$p = 0.284$	$p = 0.056$ #	$p = 0.051$ #
Third met head			$p = 0.006$ #	$p = 0.013$ #
Lateral midfoot				$p = 0.372$ #

The environmental condition also approached main effect differences ($p=0.086$).

Using partial eta squared, it was noted that 24.5% of variation was secondary to the environmental condition. The skin measured in the environmental chamber tended to be less stiff than the skin measured in the typical room temperature environment (Figure 3.7). Large variation across and within subjects was present with TID v1 measurements as well (Figures 3.8).

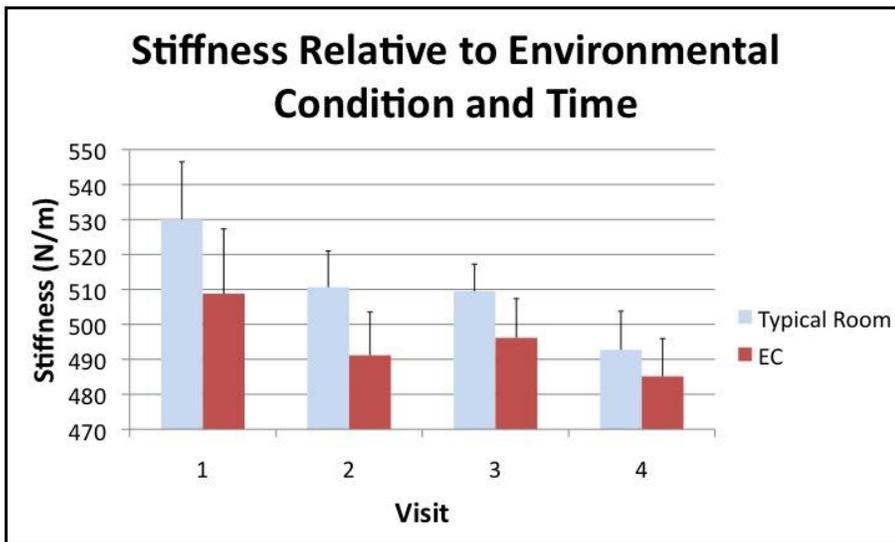
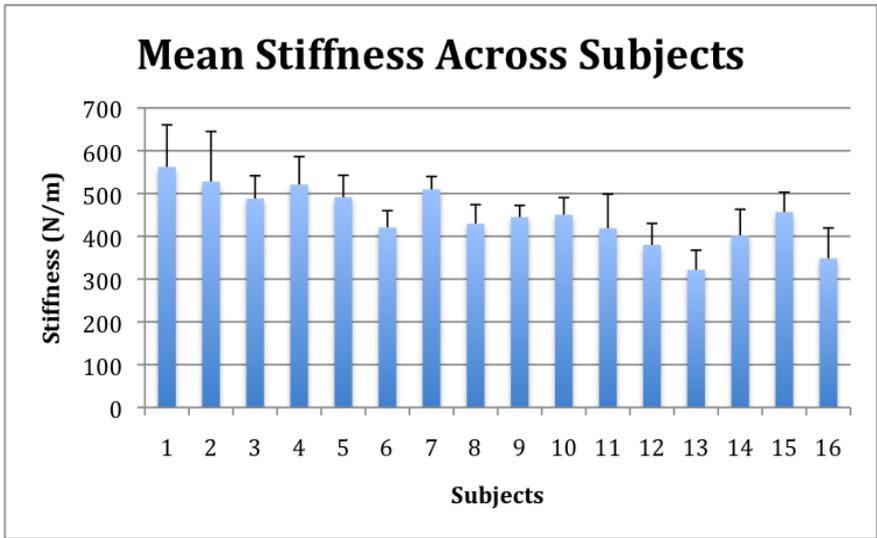
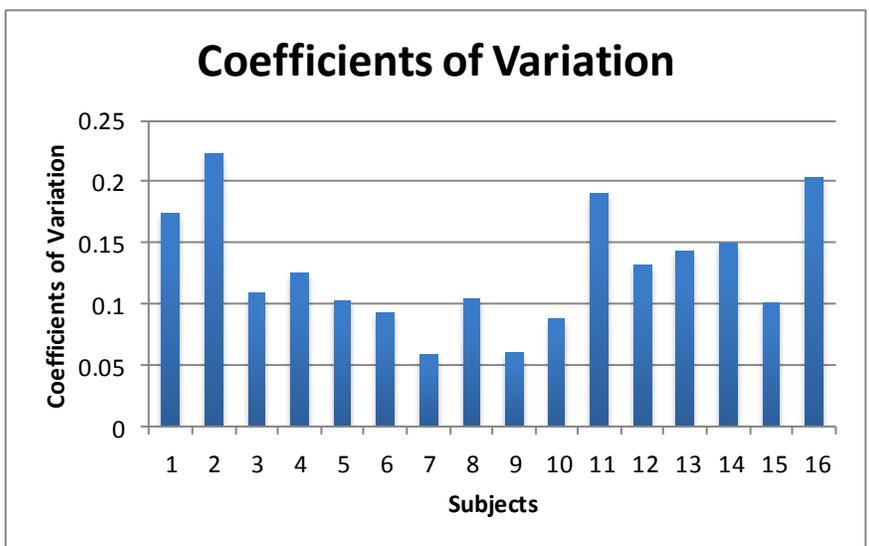


Figure 3.7 Stiffness as measured with TID v1 relative to environmental condition and time.



a. Mean stiffness variation across subjects at great toe.



b. Coefficients of variation across subjects at the great toe.
 Figure 3.8 Sample variations occurring with great toe stiffness.

Using ICC, it was noted that the TID v1 measurements showed various levels of reliability over time. While five locations were tested, emphasis was placed on the great toe and first metatarsal head. Examining average measure ICC values in both the great toe and the first metatarsal head revealed variation across time. For the shortest time

period (visit one compared with visit two), the ICC for the great toe was 0.83. For the same comparison at the first metatarsal head, the value was 0.71. When visits one and three were compared at the great toe, the value was 0.74. The first metatarsal head ICC values comparing visits one and three was 0.74. Finally, when visits one and four were compared at the great toe and first metatarsal heads, the ICC values showed poor reliability at both the great toe and the first metatarsal head. See Appendix D for other ICC values at the other testing locations.

3.3.2.2 Myotonometer:

Compliance was represented by tissue displacement at specified load. Using repeated measures ANOVA, there were main effect differences for compliance by location ($p=0.000$) (Figure 3.9). Using pairwise comparison with Least Significant Difference adjustment, the third metatarsal head was more compliant than all other locations (great toe ($p=0.000$), first metatarsal head ($p=0.000$), lateral midfoot ($p=0.023$), and heel ($p=0.000$)). Similarly the lateral midfoot was more compliant than all other locations except the third metatarsal head (great toe ($p=0.001$), first metatarsal head ($p=0.000$), third metatarsal head ($p=0.023$), and heel ($p=0.000$)). There were no differences between compliance at the great toe, the first metatarsal head, and the heel (p -value ≥ 0.2). Mean displacement for the whole group was 4.443 mm.

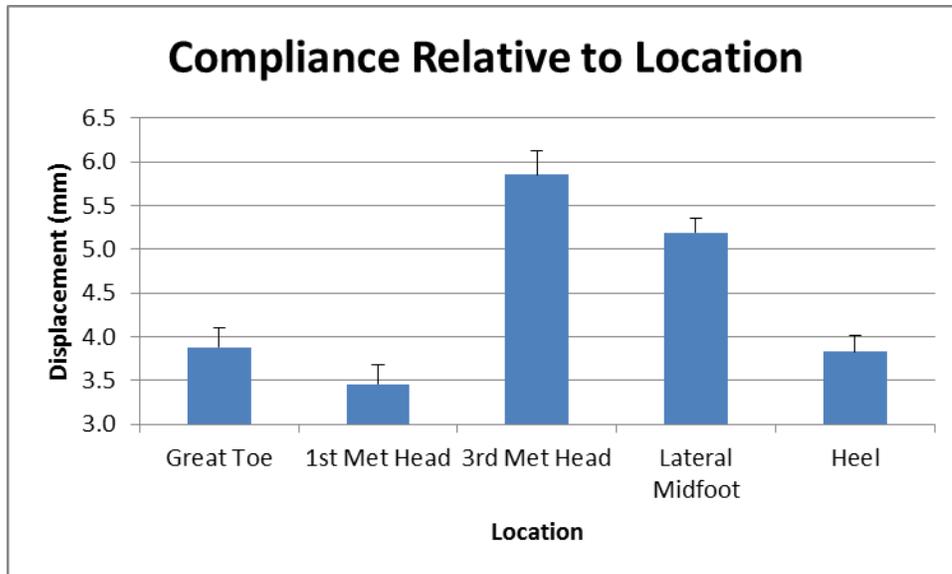


Figure 3.9 Compliance relative to location

There were no main effect differences ($p=0.214$) for compliance over time as measured by visits. Compliance related to the environmental condition also showed no main effect differences ($p=0.366$).

Using ICC, it was noted that the myotonometer measurements showed consistency over time. While five locations were tested, emphasis was placed on the great toe and first metatarsal head. Examining average measure ICC values in both the great toe and the first metatarsal head exhibited variation across time. Within a day (visit one compared with visit two), the ICC for the great toe was 0.89. For the same comparison with the first metatarsal head, the value was also 0.89. When visits one and three were compared, the value at the great toe was 0.80. The first metatarsal head ICC values comparing visits one and three was 0.87. Finally, when visits one and four were compared at the great toe and first metatarsal heads, the ICC values were 0.82 at the great

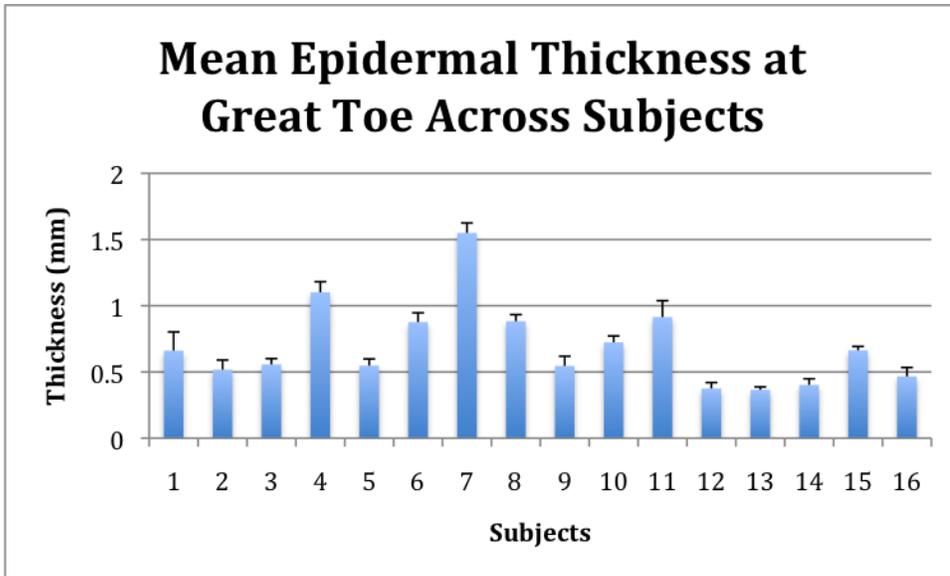
toe and were 0.79 at the first metatarsal head. See Appendix D for other ICC values at the other testing locations.

3.3.2.3 Ultrasound:

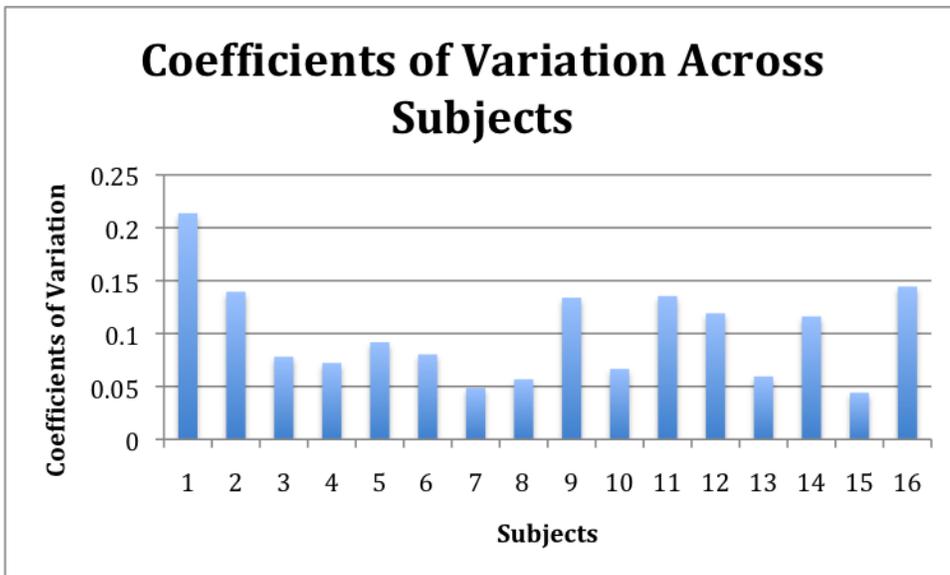
Epidermal and dermal thickness across the five different sites produced interesting results. Once again, the main effect of anatomical location indicated differences in thickness of the epidermis ($p=0.000$) and dermis ($p=0.044$). No main effect differences were noted for any of the plantar skin thicknesses across time (epidermal: $p=0.755$, dermal: $p=0.335$, total: $p=0.596$). No main effects were noted for any plantar skin thickness across environmental conditions (epidermal: $p=0.626$, dermal: $p=0.696$, total: $p=0.846$).

Skin thickness varied across subjects. Epidermal thickness ranged from a low of 0.35 mm at the great toe to a high of 1.61 mm at the heel. Even within a single location such as the great toe, the range across subjects was 0.35 mm to 1.57 within the environmental chamber. Similarly, the coefficients of variance also range widely. Figure 3.10 is a representative sample of the variation that occurs on the plantar foot.

The main effect difference in epidermal thickness across anatomical locations was evaluated with pairwise comparisons with Least Significant Difference adjustment (Figure 3.11). Differences could be found such that the third metatarsal head and heel were thicker than the great toe ($p=0.005$; $p=0.001$, respectively), the first metatarsal head ($p=0.001$; $p=0.000$, respectively), and the lateral midfoot ($p=0.008$; $p=0.000$, respectively). The third metatarsal head was not different in thickness compared with the heel ($p=0.099$).



a. Mean epidermal thickness variation across subjects at great toe



b. Coefficients of variation across subjects at the great toe

Figure 3.10 Sample variations occurring at the great toe (epidermal).

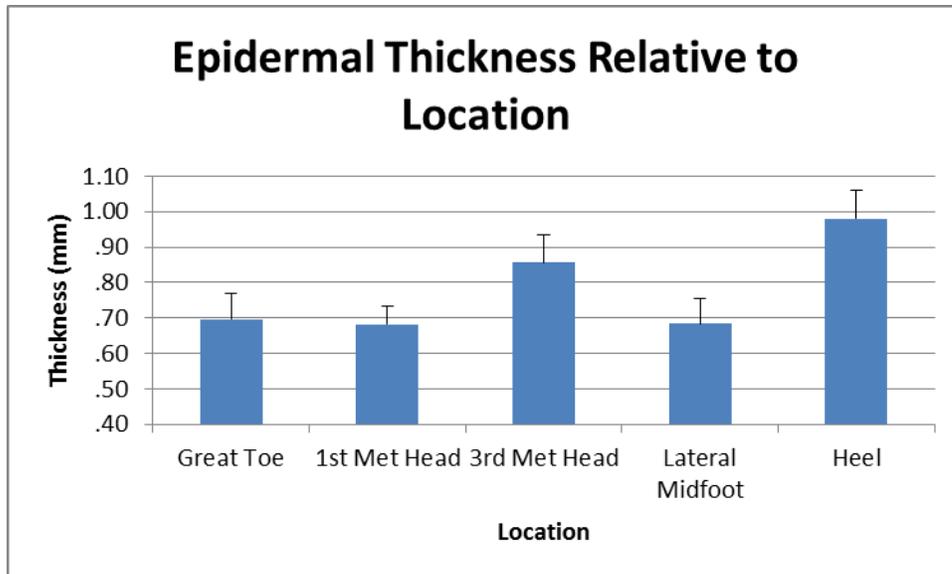
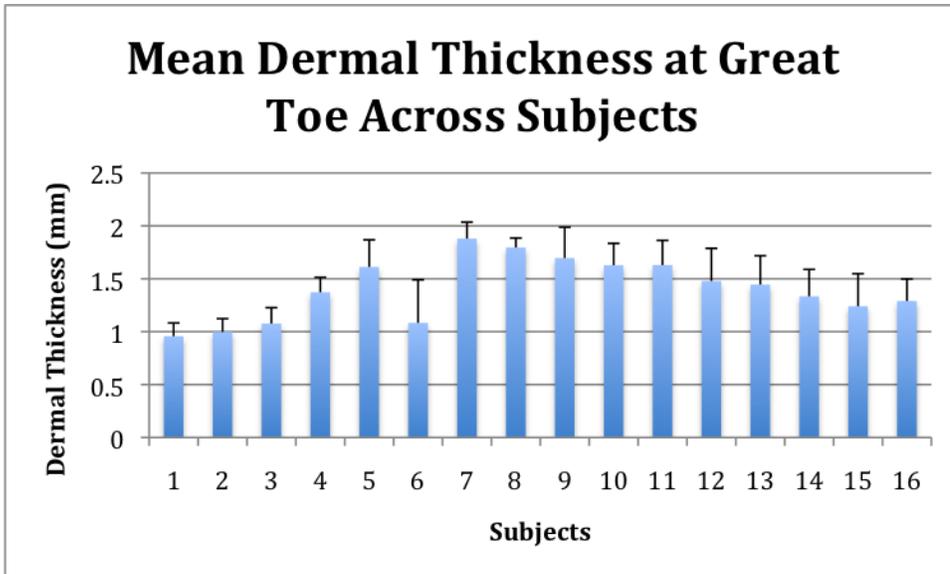


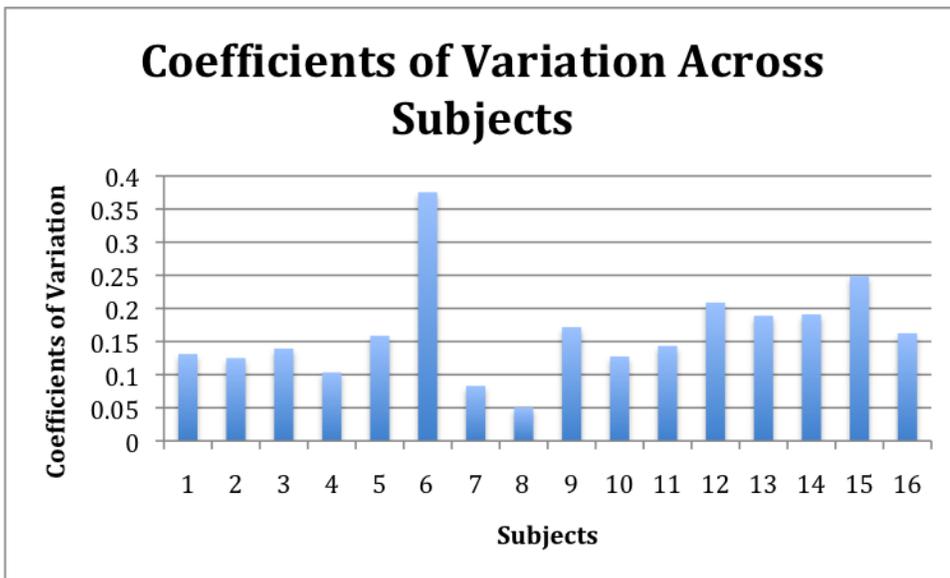
Figure 3.11 Epidermal thickness relative to location

Variations across the dermal thickness measurements revealed similar variations as those seen with epidermal thickness. For instance, the mean dermal thickness range across locations extended from a low of 0.83 mm at the third metatarsal head to a high of 2.10 mm at the first metatarsal head. Again, even within a single location such as the great toe, the range across subjects was 0.95 mm to 2.01 mm within the environmental chamber. The coefficients of variance also varied widely. Figure 3.12 is a representative sample of the variation that occurred in the dermis of the plantar foot.

When anatomical difference for dermal thickness was compared using pairwise comparison, specific differences could be seen (Figure 3.13). The great toe had a thicker dermis than the third metatarsal head ($p=0.006$) and tended to be thicker than the first metatarsal head as well ($p=0.080$). The first metatarsal head also had dermal measurements that were thinner when compared to the lateral midfoot ($p=0.041$).



a. a. Mean dermal thickness variation across subjects



b. Coefficients of variation across subjects at the great toe
 Figure 3.12 Sample variations occurring at the great toe (dermal).

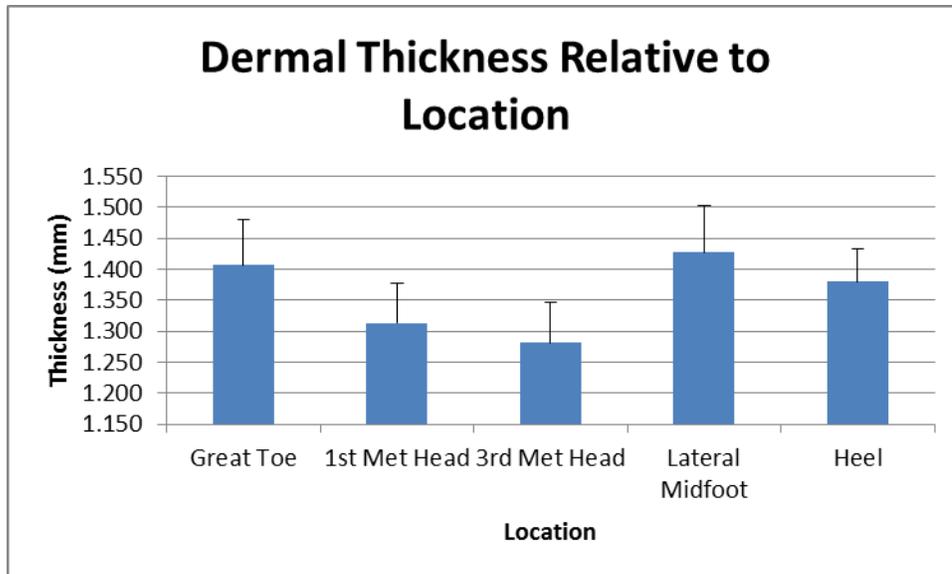


Figure 3.13 Dermal thickness relative to location

Using ICC, it was noted that the US measurements showed a wide range of consistency over time, particularly in the case when dermal thickness was measured. While five locations were tested, emphasis was placed on the great toe and first metatarsal head. Examining average measure ICC values in both the great toe and the first metatarsal head exhibited variation across time for both epidermal and dermal thickness measurement comparisons. For epidermal measurement, ICC values at the great toe between visits one and two, between visits one and three, and between visits one and four varied little. The range was 0.96 to 0.98. For the first metatarsal head measurements of epidermal thickness, ICC values varied between 0.93 and 0.95 between visits one and two and visits one and three. Decreased but good reliability was apparent between visits one and four with ICC values of 0.79.

Dermal thickness ICC values for the US varied widely across the great toe with ranges from 0.48 to 0.87. The first metatarsal head location had even larger variations.

The dermal ICC values tended to show poor to moderate reliability. All ICC values can be referred to in Appendix D.

3.3.3 Discussion:

The aim of this study was to characterize the stiffness and thickness of plantar skin across environmental condition and time. The null hypothesis being tested was that skin would not vary across environmental condition and time. Shorter and longer time frames were tested. The results of this study were that skin did not, in fact, differ across environmental condition and time when assessed for stiffness (TID), compliance (myotonometer), and thickness (US) using repeated measures ANOVA. Despite this failure to reject the null hypothesis, consideration must be made for all of the study's findings to better allow the interpretation of these data.

When stiffness, compliance, and thickness of the plantar skin were assessed, there were wide variances across the group. And, with each measure, stiffness, compliance, and thickness of the plantar skin varied across locations. Variation in stiffness across location was consistent with the literature.(45, 50)

The stiffness across location differed between the TID and the myotonometer (Figures 3.6, 3.10). Stiffness as measured by the TID v1 can be characterized as tensile stiffness. This measurement was taken as a tangential or traction force was applied to the skin rather than by the more common normal (orthogonal) loading that was applied by the myotonometer, an indenter. Furthermore, the nature of this tangential force application resulted in stiffness measurements that were more reflective of the skin anatomy and its skin properties than the subcutaneous structures. In distinction, compliance, the stiffness correlate measured using a normal force, was heavily influenced

by the total anatomy, particularly the subcutaneous structures, including fat pads, ligaments, tendons, and bone. Difference in the measurement technique may, in itself, result in the variation.

The TID with its traction force focused on the measurement of the tensile properties of skin. Collagen and elastin, located in the dermis, are largely responsible for the tensile properties of skin. The dermis varies in thickness from one to four millimeters. Divided into papillary and reticular layers, the content of each differs. Collagen in the more superficial papillary layer tends to be thinner in diameter compared to that found in the reticular layer. The elastin also shows an increase in size from superficial dermis to deeper dermis.(42) Structural differences are likely to affect the mechanical properties of the tissue.

The other component of skin that cannot be ignored is its anisotropy. The direction of traction must matter. All measurements were taken using the same TID orientation. Skin has well-known anisotropic characteristics(113) so the tensile stiffness in other orientations may be different than the ones measured. Assessing the magnitude of the anisotropic nature of the plantar skin should be considered for a future study.

Compliance, as measured by the myotonometer, also varied by location. It is important to recognize that the anatomy surrounding each testing location likely affected the compliance outcome. With an orthogonal force, the layers of each tissue deep to the skin were impacted as increased force was applied. For example, some sites had tendinous structures or fat pads beneath the skin while other sites had very superficial bone. The bone would limit displacement whereas softer, more elastic structures would

allow larger displacements to occur. Thus, resultant displacement measures would be reflective of not only the skin but also the underlying tissue.

Epidermal and dermal thickness varied across anatomical locations. When thickness measurements were reviewed, there was a main effect for difference across anatomical locations for both the epidermis and the dermis. This was consistent with the literature.(107)

The stability over time of the properties of the plantar foot including stiffness, compliance, and thickness were important to assess to inform future interventional study design. Stiffness measurements taken with the TID did not demonstrate difference across visits. Compliance measures showed no main effect differences over time (visits). Similarly, thickness measurements (epidermal, dermal, and total) did not show any main effect differences across visits.

The ICC values were utilized to determine the reliability of the measurements across the visits. This reliability was assessed between visits occurring on the same day (visits one and two), within the same week (visits one and three), and within the month (visits one and four). When the values were reviewed for measurements taken at the great toe and first metatarsal head, one could see that the TID had moderate to good reliability within the first three visits. It did not have acceptable reliability for the comparison between the first and four visits. The myotonometer, on the other hand, did have good reliability across the entire time span for all locations except at the lateral midfoot where reliability was moderate between the first and fourth visits. US measurements similarly had good reliability when measuring epidermal thickness across all four visits for the great toe and first metatarsal head. The dermal thickness measurements showed poor

reliability. This poor reliability was likely related to the difficulty of determining the lower border of the dermis within the custom LabView program.

There was a large variability between subjects, but, there was also a certain stability that was apparent within an individual over time. But, taking both the repeated measures ANOVA findings and the ICC values together, one must consider how future testing should be supported. These results support the conclusion that the testing of skin stiffness (TID) is sufficiently stable within an individual within a week. Utilizing the myotonometer and the US for epidermal thickness, one could reliably test at the great toe and the first metatarsal head within a month with sufficient stability. This allows for repeated measurements to be taken over these timeframes.

Most materials behave differently in different environments. Material has a tendency to become less stiff and less viscous with rising temperatures.(114) In a shoe, the foot is certainly in an environment that is not only warmer, but also more humid. When the properties of the plantar skin were compared in different environments (typical versus warm and humid), several findings were apparent. First, with the TID v1 stiffness measurements, a main effect difference was approached ($p=0.086$) such that the skin measured in the EC tended to be softer than the skin in a typical room environment (Figure 3.7). With the environmental condition being responsible for approximately 24% of variance (partial eta squared=0.245) when using the TID, there was an observed power of 0.406. This study was under-powered to show differences between environmental conditions. Conversely, tissue compliance, as measured by the myotonometer, did not indicate main effect differences across different environments ($p=0.366$). Similarly

plantar skin thickness was not different when the environmental condition alone was considered (epidermal: $p=0.626$, dermal: $p=0.696$, total: $p=0.846$).

Taken together, these findings indicate that the environment may affect the plantar skin. Because this study was under-powered relative to the TID measurement, it is critical to recognize that it may be important to perform testing in the same environment for future work, particularly when using the TID. With this possibility, for future studies, one should consider the value of testing in an environment that is consistent with that of a typical environment for a foot. Or, at a minimum, one should consider controlling the environmental conditions in which testing is completed.

In this study, examining people with and without diabetes as comparison groups was not an objective. However, interesting findings could be seen by making a comparison between these groups. This comparison may provide insight and power for future studies and can be reviewed in Appendix B.

Additionally, urine measures were taken to assess hydration level and BMI was calculated as well. While these were not a part of this study's specific aim, correlation between these measures (USG, Uosm, and BMI) and tissue properties can be useful to inform future studies. These correlational statistics can be found in Appendix E.

3.3.4 Summary

This study points to a number of findings that help to characterize the skin of people with and without diabetes. The skin varies across anatomical locations regardless of assessment technique used (TID, myotonometer, or US). These findings were consistent with variable anatomy that is present in areas tested.

One objective of the study was to assess the variation in skin properties that occur

within a day and over a few weeks. This objective was meant to inform research studies that may require measurements to be taken over time. If natural variation occurs in skin due to variations in activities of daily living, then interventional effects may be obfuscated. Similarly, one risks assigning effect to an intervention whereas differences could be explained by natural variation.

The data indicate two important results: 1) a relatively large variation in skin properties exist across people, and 2) no statistical differences in skin properties (stiffness, compliance, or thickness) existed over time. The first result is well-documented so the results of this study are confirmatory. The finding that group differences of within-subject variables did not differ over multiple testing sessions can be applied to future research studies. However, the finding of a lack of differences in group results does not mean that individual differences did not exist. This study purposely did not control for many factors that might affect skin, such as food intake or levels of activity. Sessions 1 and 2 were taken on the same day and were intended to assess skin after different levels of activity, weight-bearing, and dietary activity within one day. Researchers that seek to limit variation may consider tighter controls on subject behavior but this can be problematic.

A second objective of the study was to assess how the environmental condition affects the skin. While no statistical differences existed across conditions, one must realize that an under-powered study could obscure differences that may actually be present. In the case of the TID and the variability associated with environmental condition, additional control of the environmental condition should be considered for future studies looking at skin properties, particularly the most superficial.

This study had several associated limitations. The skin of people is highly variable. While the variability within human anatomy is unavoidable, some variation can be minimized. The study was designed to measure skin characteristics across days, and while statistical differences did not exist, variation in measurements increased. Subjects were not instructed to eat or drink in any particular manner prior to any visit or to limit the amount of activity that they performed. Each of these could be a potential contributor to the large amount of variability that was present. In fact, weak correlation to skin thickness was noted relative to USG and Uosm values (Appendix E). For future studies, these may be additional factors that could be controlled and investigated further.

4 Identification of the Impact of a 10-minute Bout of Walking on the Foot

4.1 Specific Aim #3: Identify the impact of a 10-minute bout of walking on skin tissue.

Multiple studies have reported that people with diabetes have different skin stiffness compared to those without diabetes.(45-47, 50) While some investigators reported increased stiffness in all groups with diabetes,(50) others found that stiffness or tissue hardness, compared to controls, varied relative to the location(45) or level of neuropathy.(45, 46, 51) The studies evaluating stiffness relative to neuropathic changes have typically found that more severe neuropathy is related to more profound skin stiffness.(45, 46, 51) This change in stiffness is often related to structural changes that happen with poor glycemic control. Namely, the formation of advanced glycosylation end products (AGEs) has been associated with changes in collagen formation with an increase in cross-linking.(3, 4)

People with diabetes and neuropathy have long been known to be at risk for the development of plantar ulceration.(2) In fact, until 2007, people with peripheral neuropathy were encouraged to participate in non-weight-bearing (NWB) exercise to minimize risk to the plantar skin.(16) A few studies have found that people who were more active and walked more were actually less likely to ulcerate on the plantar surface of their feet.(17, 18, 20) These findings support the use of walking as a mode of exercise, rather than restricting exercise to that which is NWB. Similarly, one study found that those who developed ulcers had lower activity levels and higher variability in activity prior to ulceration.(19) Also, another study reported re-ulceration following a sudden increase in activity level.(21) Given these findings, one must consider the immediate

effect that walking has on the skin properties of the plantar foot. Specific Aim #3 was intended to address this question.

The aim of this study was to investigate how a 10-minute bout of walking acutely changes the stiffness and viscosity of skin relative to disease state. Specifically, this study was meant to test the central hypothesis that skin will demonstrate a decrease in stiffness and increased compliance as a result of a 10-minute bout of walking. Answers to the following four questions were sought:

1. Does stiffness, compliance, or viscosity of the plantar skin differ in people with and without diabetes?

2. Do plantar tissue properties, such as stiffness, compliance, and viscosity, change following walking?

3. If changes in the skin or tissue of those with or without diabetes occur following walking, then how long do those changes persist?

4. Does the plantar skin of people with diabetes behave differently compared to the plantar skin of people without diabetes in response to a 10-minute bout of walking?

4.1.1 Methods

Subjects: The subjects were people with and without diabetes. The diabetes group included people who were between 18 and 85 years old, had a diagnosis of diabetes, and were able to ambulate on a treadmill for 10 minutes. The non-diabetes control group included people without diabetes who were age- and gender-matched to the people in the diabetes group. These people also had to be able to ambulate on a treadmill for 10 minutes. Exclusion criteria for both groups included the presence of any other neurological diagnosis that affected a person's ability to walk or to feel the plantar

surface of his or her feet. Additionally, subjects were excluded if they had a skin disorder that affected the plantar foot. G*Power was used *a priori* for power analysis from a small pilot of TID v2 data. It was determined that 20 subjects per group were needed for the study to be adequately powered (0.8). Results were not based on any myotonometer data.

Equipment: The myotonometer (Neurogenic Technologies, Inc.) is a device that measures compliance in response to the application of an orthogonal force applied to the skin (displacement/force). Displacements were measured at the following forces: 0.25 kg, 0.43 kg, 0.61 kg, 0.79 kg, 0.96 kg, 1.14 kg, 1.32 kg, and 1.5 kg. These forces were selected by the manufacturer to allow for an adequate range of force-displacement measurements.

Tissue Interrogation Device (TID v2): This non-commercial, research tool measures the stiffness and viscosity of a tissue or material. The TID v2 measures the tensile stiffness and viscosity of tissue using a small tweezers-like probe that applies gentle traction force to the skin at frequencies of five Hz. Each trial with this device lasts five seconds each for an unloaded condition as well as a loaded condition. A 1.5 N normal force was applied to give adequate friction to load the skin in traction. A constant force spring aligned with the plastic housing reduced variability of the normal force applied.

A positioning boot (Figure 4.1) was designed to hold the foot in neutral plantarflexion/dorsiflexion using double upright braces. To maintain this position, the foot and ankle were held in place, within the boot, using hook and loop fastener strapping. Additionally, a steel plate was affixed to the sole of the boot for attachment of the TID v2 mount.



Figure 4.1 Positioning boot side and bottom views

A magnetic-based arm (Figure 4.2) was attached to the positioning boot. The upper portion of the arm had a platform to which the TID v2 was attached. With this design, a screw moved the platform and thus allowed precise control of the orthogonal pressure that was applied by the TID v2 to the plantar foot.

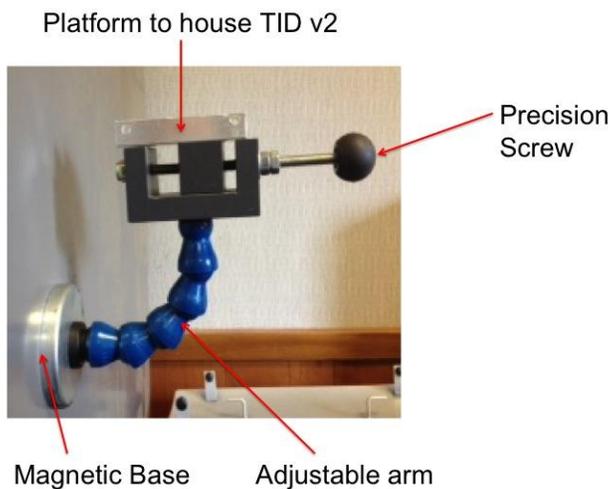


Figure 4.2. Magnetic-based arm

Temperature and relative humidity sensors (MSR Electronics GmbH, Seuzach, Switzerland) (Figure 4.3) were applied to the foot and shoe of each subject. Modular Signal Recorder (MSR) software was used to download the data collected. Accuracy for the temperature sensors within the range of temperatures used was $\pm 0.1^{\circ}$ C. Accuracy for relative humidity sensors was $\pm 4\%$ relative humidity at this range of temperatures.

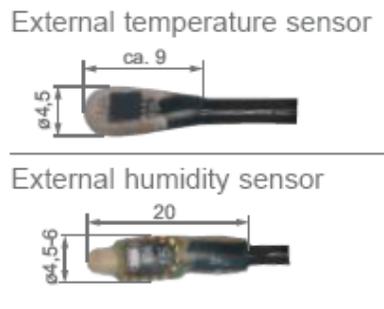


Figure 4.3 Temperature and relative humidity sensors

The StepWatch Activity Monitor (Orthocare Innovations, Oklahoma City, OK) was used to count the strides during the treadmill walking. StepWatch is a United States Food and Drug Administration (FDA) cleared class II device intended for long-term assessment of community walking. The device was 75 x 50 x 20 mm and weighed approximately 38 grams.(108)

A heart monitor (Polar Electro Inc., Lake Success, NY) with a chest strap and watch band was worn during the treadmill walking to provide continuous heart rate monitoring.

Protocol: Following initial screening and informed consent, subjects were tested. Prior to testing, the subjects were offered an opportunity to walk on the treadmill for practice. If this option was chosen, the subjects needed to complete the practice session at least one

day before the experiment began. The subjects did not have to complete this practice to complete the trial. Also, prior to the experiment, each subject was encouraged to drink an additional one to two glasses of water or fluid to ensure full hydration at the beginning of the experiment. Finally, each subject was asked to avoid physical activity (intentional exercise) prior to the morning session of testing.

All testing was completed in the morning. On the day of the experiment, each subject was asked to urinate into a specimen container so that his or her hydration level could be determined using urine color and urine specific gravity (USG). A USG of 1.020 or less was required prior to the initiation of the experiment. Sensation was also tested on the plantar feet using a 5.07 Semmes Weinstein monofilament to test protective sensation and a biothesiometer to test vibration sense. These are standard clinical measurement tools for this purpose. Additionally, a finger stick was performed on each subject using a lancet. The blood taken was used to test his or her hemoglobin A1C using an A1CNow+ (Bayer) home unit. This unit was National Glycohemoglobin standardization Program certified, Clinical Laboratory Improvement Amendments (CLIA) waived, and approved by the FDA. Subjects completed a short questionnaire including a basic history and level of physical activity (Appendix F). Height and weight were also collected.

Following this initial demographic and data collection, each subject went into the environmental chamber (EC) set to 32 degrees Celsius and 66% relative humidity. This environment simulated the temperature and relative humidity of a foot within a shoe as was determined by testing related to Specific Aim #2. There the subject acclimated to the environmental condition in the EC, while in a reclined position on a plinth for approximately 10 minutes. During the acclimation period, the positioning boot was

placed on the subject's right foot over a tubigrip stocking placed to protect the leg and provide a consistent environment across subjects. The first metatarsal head and great toe were left exposed and the outline of the myotonometer was marked. Within the marking, the skin to be tested under the plunger of the myotonometer was identified and marked for future testing. Following the acclimation period, baseline measurements at the 1st metatarsal head and the great toe were collected using the myotonometer and the TID v2. The order of the measurements was randomized, both by location and by measurement device (TID v2 and myotonometer).

The procedure for the myotonometer testing was performed as follows. At each marked site, the probe was held perpendicular to the skin with light pressure. Then, pressure was applied with the central plunger component of the probe, progressively to 1.5 kg over the course of one to two seconds. This was repeated eight times at each site. The myotonometer software calculated the load and amount of tissue displacement. The changes in force and displacement were recorded at 0.25 kg, 0.43 kg, 0.61 kg, 0.79 kg, 0.96 kg, 1.14 kg, 1.32 kg, and 1.5 kg. Displacement under load was used as a means to describe the compliance of the tissue that was deformed by the plunger.

The TID v2 was used to test skin stiffness and viscosity by applying a tangential force across the skin. To test, the device was lowered with the magnetic-based arm using the precision screw to apply 1.5 N of orthogonal force. This force provided enough friction so that the piezoelectric benders/delrin boots did not slip when the traction force was applied. In the case when the subject's skin did not have the requisite friction, a thin layer of hypoallergenic body glue (It Stays, Sigvaris Inc., Peachtree City, Georgia) was rolled onto the skin to prevent slippage. The glue was not used if the subject had an

allergy to adhesives. Each site was tested three times, which was consistent with the validation findings from Specific Aim #1. The skin area was wiped clean of any body glue following measurement.

Immediately after baseline measurements were taken, the subjects began the interventional component of the study. This portion of the study took place outside of the EC in the typical room temperature environment. First, the small probe sensors were applied to measure the temperature and relative humidity of the foot as well as the same in the individual's shoe. Temperature and RH sensors were placed at the medial longitudinal arch of the foot and, similarly, temperature and RH sensors were placed at the medial wall of the shoe (Figure 3.2). Sensors were adjusted so that they did not impose increased pressure on the area or affect the subject's gait pattern. All subjects were given the same type of socks to wear over the sensors so that the immediate foot environments were the same across subjects. Each subject wore his or her typical walking shoes for the intervention component of the study. Pumps, heels, open toe sandals, or boots were not permitted. To monitor physical activity for the study, participants wore a StepWatch activity monitor just above the right ankle. The StepWatch was calibrated according to manufacturer's instructions using short bouts of gait. Accuracy was calculated to be at least 95% accurate during calibration. Neither the foot environmental sensors (temperature or RH) nor the activity monitor interfered with the subject's walking. Once placed, the sensors collected data throughout the rest of the experiment. A heart rate monitor was also applied.

The intervention consisted of a 10-minute bout of treadmill walking at 2.1 miles per hour (mph). The walking speed chosen was slower than typical walking speed for

those without diabetes and approximately the median walking speed for people with type 2 diabetes.(115) During treadmill walking, each subject was asked to rate how hard he or she was working using the Borg Rating of Perceived Exertion (6 to 20).(116) If the subject reported a rating above 15 (perception of working hard), his or her heart rate became excessively elevated, or if the subject requested a slower speed, the treadmill was slowed to 1.5 mph. Each control group subject matched the speed of his or her matched counterpart with diabetes unless he or she met the criteria to be slowed down. In that case, the control subject's speed was slowed, regardless of the speed of the person with diabetes. Each subject was allowed to hold onto the treadmill for balance at all times and most subjects did. Immediately following the intervention, the subject returned to the EC and the boot was re-applied. The tubigrip stocking and boot stayed in place throughout the remainder of the study session. Temperature and relative humidity sensors remained in place on the medial foot as well. Testing was completed to the plantar surface of the foot in the same manner as was done at baseline. After the immediate testing (0post) was completed, the subject was able to sit outside the EC until the next testing. Then, to determine how long any effects persisted, plantar measurements were taken in the EC again at 30 minutes (30post), 60 minutes (60post), and 90 minutes (90post) following the treadmill walking. The total experimental session took approximately 4 hours.

Using SPSS Statistical software, statistical analyses utilized descriptive statistics as well as repeated measures ANOVA where the disease state (diabetes) was used as the between groups variable. The great toe and first metatarsal head sites were treated separately because the locations were not expected to behave in the same manner. Effect size using partial eta squared and observed power were included with repeated measures

ANOVA. T-tests were used to compare demographic group means. Also, G*Power version 3.1.5 was used to estimate sample sizes necessary for future studies based on the findings from this study.

4.1.2 Results

Demographics: Thirty-two subjects completed the study, 16 with diabetes and 16 age- and gender-matched controls. None of the subjects took the opportunity to practice treadmill walking prior to participating in the study. Only one subject without diabetes walked at a slower speed than his diabetes group counterpart. The group with diabetes had a mean age of 63.13 ± 12.87 years and the matched group had a mean age of 62.81 ± 12.28 years. USG findings showed that subjects were similarly hydrated at 1.014 ± 0.005 for the diabetes group and 1.011 ± 0.005 for the matched controls ($p=0.086$). BMI, A1C, and vibration testing differed between groups (p -values range from 0.000 to 0.001) Protective sensation also differed between groups ($p = 0.036$ equal variance not assumed) (Table 4.1).

Table 4.1 Subject Demographic Profile

**Groups showing difference with $p \leq 0.001$; *Groups showing difference with $p < 0.05$

Group	Age	Gender	USG	BMI **	A1C **	Biothesiometer Great toe (Volts)**	Biothesiometer 1 st met (Volts)**	Prot. Sens. (intact) *
DM	63.1 ± 12.9	8M/8F	1.01 4 ± 0.01	32.75 ± 12.87	8.12 ± 1.17	31.77 ± 15.48	25.18 ± 12.70	10
No DM	62.8 ± 12.3	8M/8F	1.01 1 ± 0.01	24.84 ± 3.12	5.68 ± 0.35	12.67 ± 5.43	11.65 ± 6.75	15

4.1.2.1 TID v2 Output:

The TID v2 device broke midway through the entire project and it could not be repaired. Because of this, data were analyzed from 18 subjects (diabetes group = 10, non-diabetes group = 8). Analyses were performed separately for the great toe and first metatarsal head because results were expected to differ.

Great Toe: Looking at the time points when measurements were taken (pre, 0post, 30post, 60post, 90post), repeated measures ANOVA revealed that there was a difference in stiffness (mean ± standard deviation) between the group with diabetes (mean stiffness = 663.705 ± 4.796 N/m) and the group without diabetes (mean stiffness = 647.753 ± 5.328 N/m) ($p = 0.040$, partial eta squared = 0.237, observed power = 0.554). There was also a difference when comparing stiffness testing before and after TM walking (Figure 4.4) shown by the within subject main effect of time ($p = 0.000$, partial eta squared =

0.467, observed power = 0.999 with Greenhouse-Geisser correction). The graphed response to TM walking exhibited a similar response in both groups. Using pairwise comparison with Least Significant Differences adjustment, the mean stiffness immediately following treadmill walking (0post) did not significantly differ from pre-walking stiffness ($p = 0.191$, partial eta squared = 0.104, observed power = 0.250). Stiffness subsequent to 0post increased compared to that time point (30post, 60post, 90post with $p = .002, 0.000, 0.000$, respectively). The only time points stiffer than pre TM walking were 60post ($p=0.000$) and 90post ($p=0.001$).

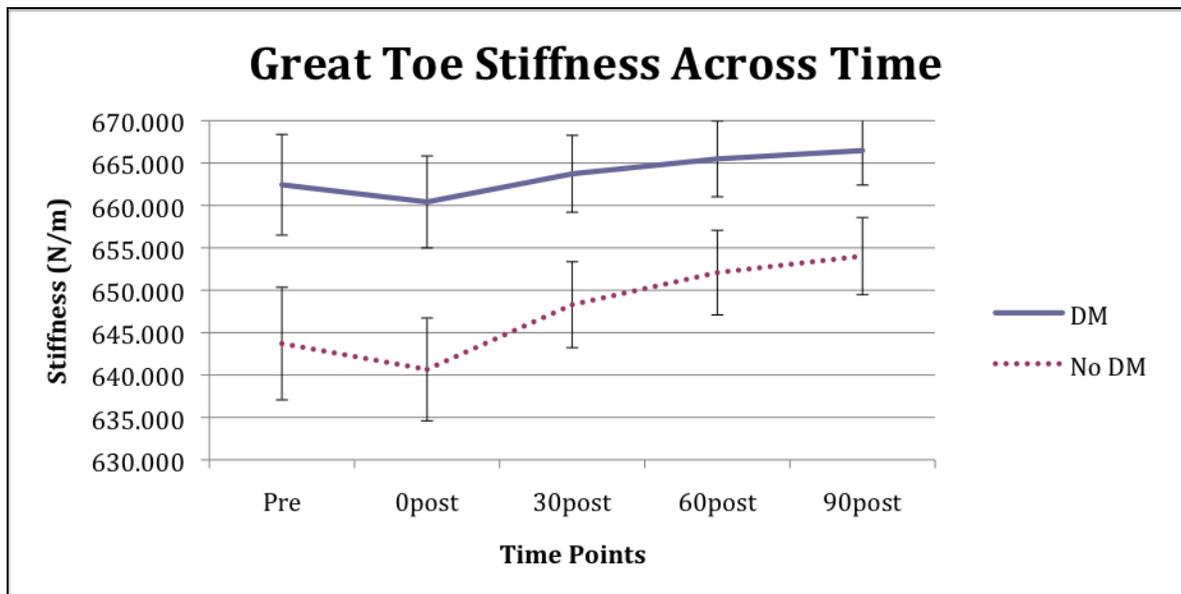


Figure 4.4. Great toe stiffness (N/m) across time as measured by the TID v2. Error bars represent standard error of the mean.

Skin viscosity at the great toe approached but did not reach significance between groups with the diabetes group grand mean \pm standard error of the mean equal to 6.054 ± 0.032 versus the non-diabetes group equal to 5.957 ± 0.035 ($p=0.060$, partial eta squared

= 0.204, observed power = 0.477) (Figure 4.5). There were no differences with respect to timing of viscosity ($p=0.643$). There were not within subject interactions.

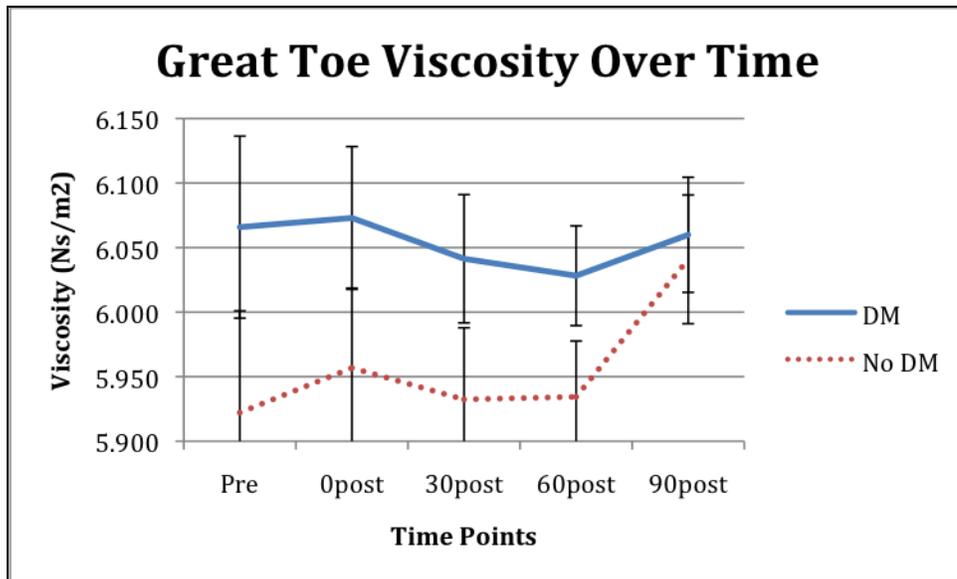


Figure 4.5 Great toe viscosity (Ns/m²) across time as measured by the TID v2. Error bars represent standard error of the mean.

1st Metatarsal Head: Again, comparing time points when testing was completed, repeated measures ANOVA demonstrated that there were no differences in stiffness between the groups with and without diabetes ($p = 0.258$, partial eta squared = 0.079, observed power = 0.197). The mean stiffness (mean stiffness \pm standard error) for the diabetes group was 667.323 N/m \pm 2.994 while the group without diabetes had a mean stiffness of 662.056 N/m \pm 3.347. At baseline, stiffness between the two groups exhibited a substantial difference with the diabetes group = 668.133 \pm 3.129 and non-diabetes group = 658.799 \pm 3.499.

Following treadmill walking, main effect differences in stiffness relative to time approached significance ($p = 0.080$, partial eta squared = 0.171, observed power = 0.437).

While the overall response was similar, there was a large decrease in the mean stiffness immediately following activity for the group with diabetes (Figure 4.6). This change in stiffness resulted in stiffness (mean stiffness \pm standard error) at 0post being very close between the groups (diabetes group 657.306 ± 6.968 and non-diabetes group 658.266 ± 7.790), but with a concomitant increase in variability. Subsequent measurements had smaller amounts of variability. The response following TM walking was graphically similar to the response at the Great Toe. If only the Pre and 0post time points were compared using repeated measures, then there is a difference between the time points ($p=0.041$, partial eta squared = 0.236, observed power = 0.551).

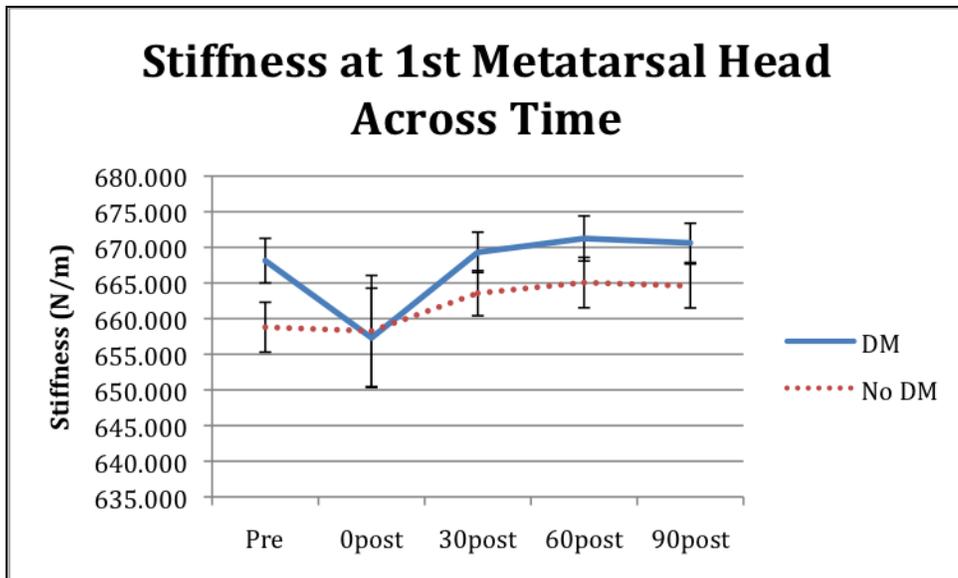


Figure 4.6 First metatarsal stiffness (N/m) across time as measured by the TID v2. Error bars represent standard error of the mean. There were no differences with respect to time.

Using repeated measures ANOVA and pairwise comparisons with Least Significant Difference adjustment, first metatarsal viscosity was shown to have no difference between groups with the diabetes group grand mean viscosity \pm standard error

of the mean equal to 6.015 ± 0.051 versus the non-diabetes group equal to 6.098 ± 0.054 ($p=0.280$, partial eta-squared= 0.077, observed power = 0.183). There were no differences with respect to timing of the measurement ($p=0.137$, partial eta-squared= 0.131, observed power = 0.360 using Greenhouse-Geisser correction) (Figure 4.7).

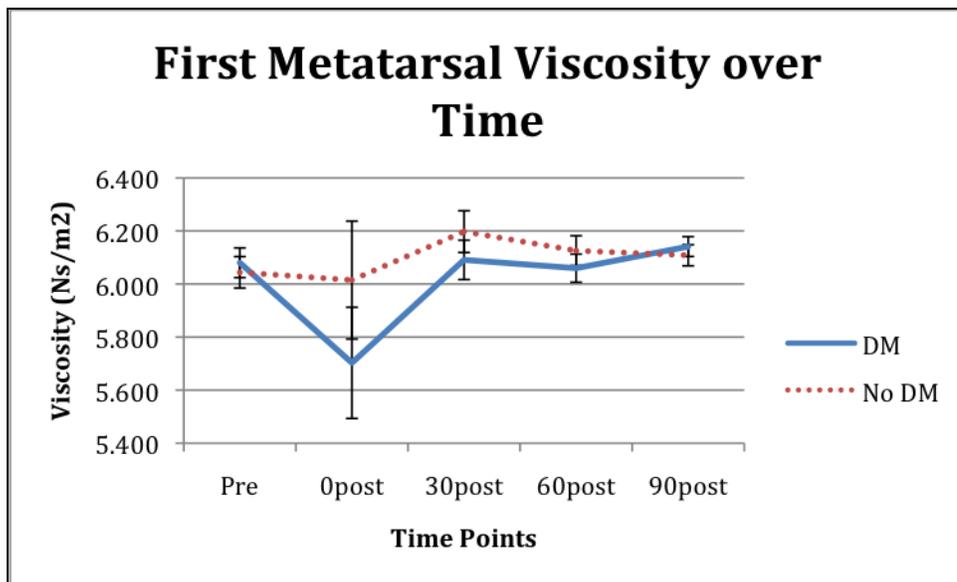


Figure 4.7 First metatarsal viscosity (Ns/m^2) across time as measured by the TID v2. Error bars represent standard error of the mean. There were no significant differences between groups ($p=0.280$) or time ($p=0.137$).

4.1.2.2 Myotonometer Output:

The myotonometer malfunctioned during one testing session; hence, data are available for 16 subjects in the diabetes group and for 15 subjects in the group without diabetes. Stiffness was represented by tissue compliance at specified loads.

Great Toe: When compliance was analyzed relative to the forces applied, increased force resulted in an increased compliance ($p = 0.000$ using the Greenhouse-Geisser correction).

When timing relative to the treadmill walking intervention was considered, compliance

was different relative to time points ($p = 0.036$, partial eta squared = 0.103, observed power 0.648 using the Greenhouse-Geisser correction) (Figure 4.8). Significantly increased compliance was found between pre-TM walking and immediately after walking (0post) ($p = 0.003$) as well as at 30 minutes post walking ($p = 0.005$). Therefore, post-activity compliance was higher than baseline levels for up to 30 minutes before compliance decreased and compliance values returned toward baseline. While groups with and without diabetes did not show between group differences ($p = 0.888$), a graphic comparison of the two groups provides interesting insight. At each of the different forces, the graphical representation showed that the increase in compliance peaked at 0post in the diabetes group and at 30post in the non-diabetes group (Figure 4.9). A peak in compliance is consistent with the decrease in stiffness from the TID data. These differing peaks were not significantly different.

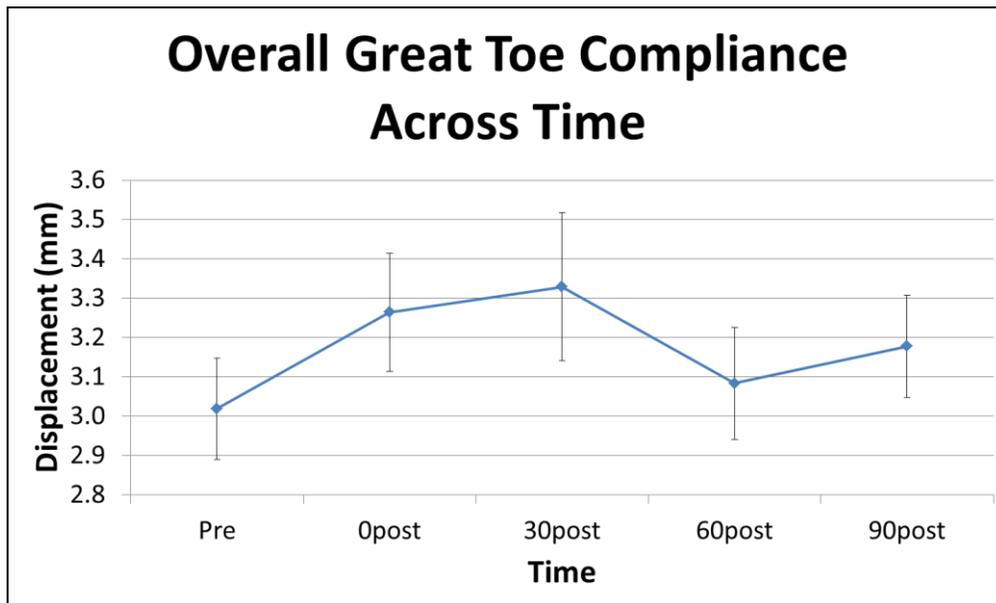
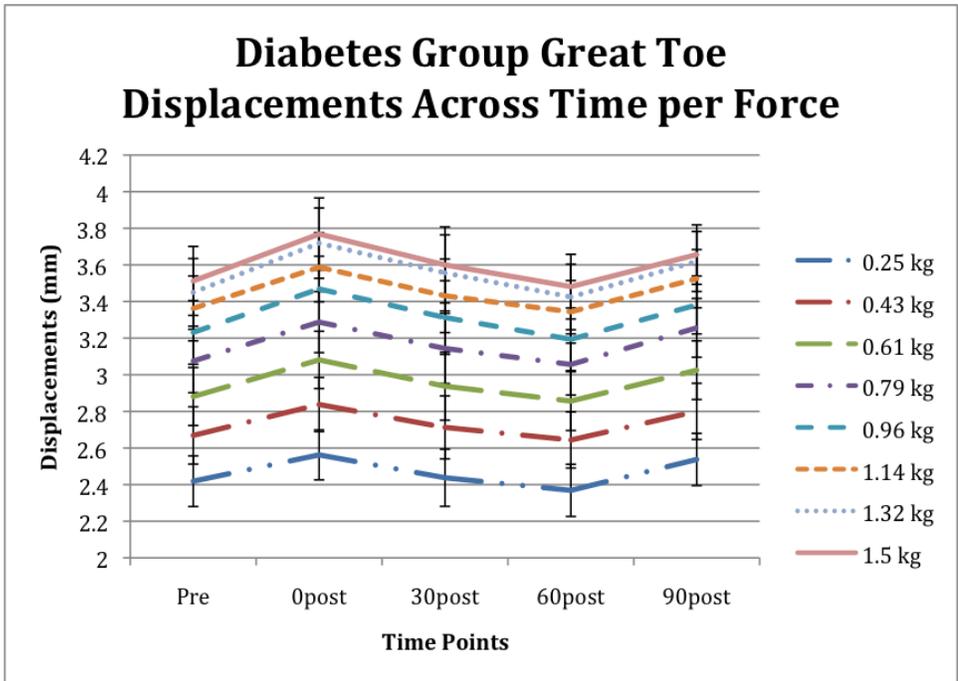
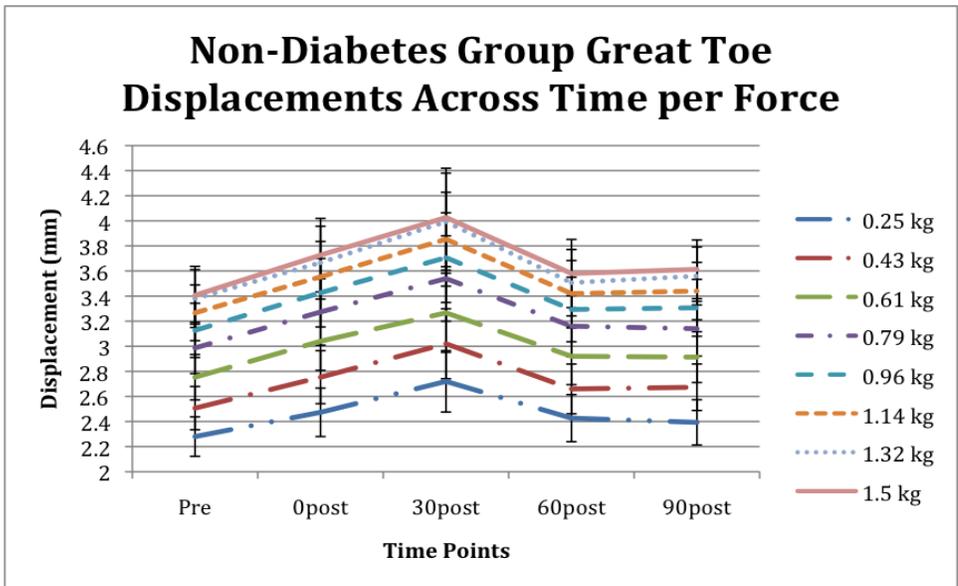


Figure 4.8 Using the myotonometer, compliance as described with grand mean raw displacement (mm) across time. Error bars represent standard error of the mean.



A. Diabetes group



B. Non-diabetes group

Figure 4.9 Great toe raw displacement. A. Diabetes group and B. Non-diabetes group compliance as measured by the myotonometer. Error bars represent standard error of the mean.

1st Metatarsal Head: When compliance as measured by raw displacement was used as the dependent variable in a repeated measures ANOVA, there were main effect differences ($p = 0.000$ using Greenhouse-Geisser correction) as well as across different time points ($p = 0.047$, partial eta squared = 0.079, observed power = 0.695). There was no difference between groups ($p = 0.443$, partial eta squared = 0.020, observed power = 0.117). Across time, compliance increased compared with pre-gait measurements (Figure 4.10). Pairwise comparisons with Least Significant Difference adjustment showed differences between the pre-TM walking values and measurements taken at 30 minutes after treadmill walking ($p = 0.039$) as well as at 90 minutes after treadmill walking ($p = 0.019$), with a trend at sixty minutes ($p = 0.052$). When graphical comparison was made, both groups showed an overall trend of a steady rise in compliance across each of the time points for testing (Figure 4.11).

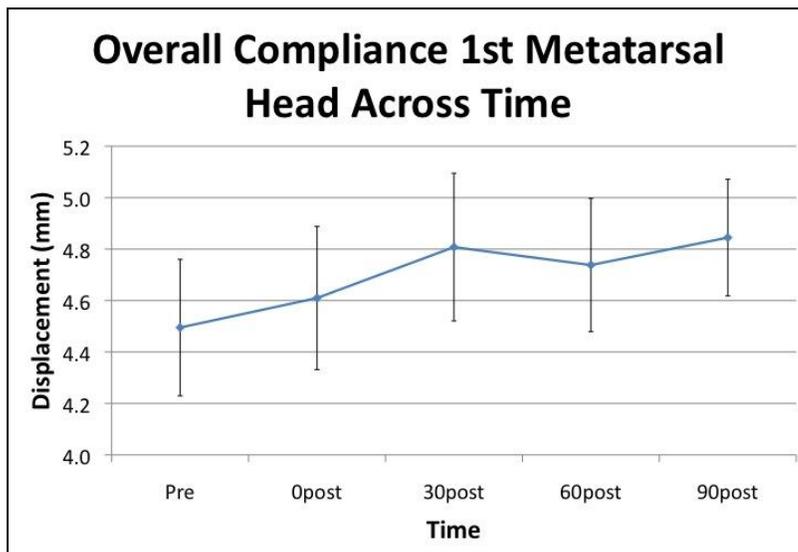
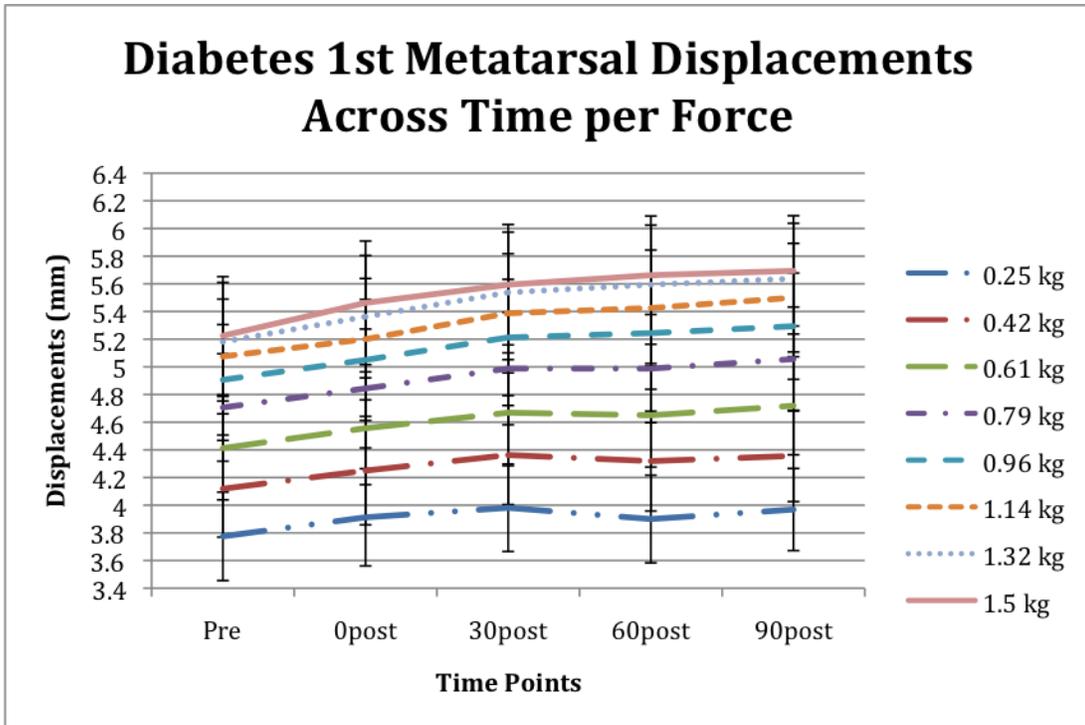
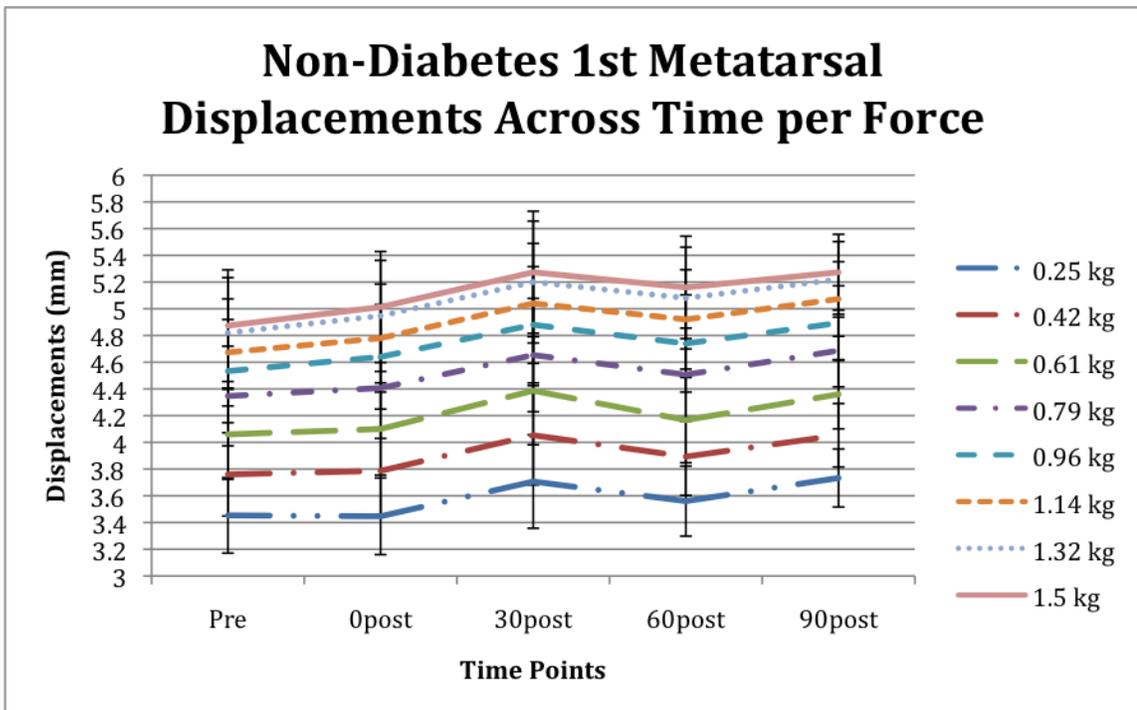


Figure 4.10 Using the myotonometer, compliance as measured with grand mean raw displacement (mm) across time for both groups. Error bars represent standard error of the mean.



A. Diabetes group



B. Non-Diabetes group

Figure 4.11 First metatarsal head raw displacement. A. Diabetes group and B. Non-diabetes group compliance as measured by the myotonometer. Error bars represent standard error of the mean.

4.1.3 Discussion

The aim of this study was to investigate how a 10-minute bout of walking acutely changes the stiffness and viscosity of skin relative to disease state. Specifically, this study was meant to test the central hypothesis that skin will demonstrate a decrease in stiffness and increased compliance as a result of a 10-minute bout of walking. The null hypothesis was that skin will not demonstrate a change in stiffness or compliance as a result of a 10-minute bout of walking. The discussion that follows will address the four specific questions presented:

1. Does stiffness, compliance, or viscosity of the plantar skin differ in people with and without diabetes?

2. Do plantar tissue properties, such as stiffness, compliance, and viscosity, change following walking?

3. If changes in the skin or tissue of those with or without diabetes occur following walking, then how long do those changes persist?

4. Does the plantar skin of people with diabetes behave differently compared to the plantar skin of people without diabetes in response to a 10-minute bout of walking?

When the body's response to walking was assessed, there was evidence that the group with diabetes had stiffer skin, either pre-intervention or overall. Using the TID v2, the overall stiffness measured at the great toe was greater in people with diabetes than without ($p=0.0040$). Stiffness was not greater at the first metatarsal head in those with diabetes; however, the initial stiffness measurement with the TID v2 tended to be stiffer in the group with diabetes. Together, these findings for stiffness tested with traction forces (TID) were consistent with measurements of stiffness taken with indenter systems in the literature.(45, 46, 51) It should be noted that there was a wide variation among the

group with diabetes as to the severity of peripheral neuropathy. Over half of the subjects had protective sensation intact while others had more severe neuropathy as was demonstrated by the absence of protective sensation and difficulty feeling the biothesiometer. These findings were consistent with the literature that people with severe neuropathy are more likely to have more profound skin stiffness.(45, 46, 51) It is likely that larger differences in stiffness were not seen between the groups because the group with diabetes lacked many people with severe peripheral neuropathy.

The increase in stiffness (TID) among those with diabetes may be related to the change in collagen cross-linkage associated with diabetes and advanced glycation end products (AGEs).(117) Such a structural change in the skin tissue is supported in the study by Reihnsner et al.(117) where the investigators looked at non-enzymatic glycation effects on healthy skin as a model for changes that occur with diabetes.

Compliance as defined with raw displacements measured with the myotonometer and viscosity measured with the TID v2 were not different between groups. Viscosity approached significant differences at the great toe between groups ($p=0.060$) with the diabetes group demonstrating higher levels of viscosity. Because viscosity measures resistance to flow, it is not surprising that the results were similar to that of stiffness. At the first metatarsal, the viscosity behavior of the diabetes group was graphically similar to that of stiffness at the same location (Figures 4.6 and 4.7).

The answers to questions 2 and 3, and thus the results of hypothesis testing, can be identified with main effect changes in regard to the timing of measurements taken relative to when treadmill walking occurred. Significant changes were apparent in select measurements at both the great toe and the first metatarsal head. Walking affected

stiffness as assessed by the TID v2 where stiffness increased subsequent to the 0post testing time. In this case, the null hypothesis was rejected but the direction in which it was directed was different than that which was anticipated. Compliance, as measured by the myotonometer, was also affected by treadmill walking so that compliance increased. Again, the null hypothesis was rejected and the central hypothesis was supported. Viscosity was not affected by the intervention. In this case, the null hypothesis was not rejected.

Timing of changes to the stiffness, as measured by the TID v2, relative to treadmill walking demonstrated similar trends (Figures 4.4 and 4.6). Generally, it was noted that an initial decrease in stiffness occurred immediately after walking (0post only) followed by a subsequent rise in stiffness. Differences were only evident when later measurements were compared to the pre-intervention values in the case of the great toe. But, the measurements immediately after walking had lower mean values compared with pre-gait measurement. These values were accompanied by a large variability in tissue responses. This was particularly marked with the response to treadmill walking in the first metatarsal head (Figure 4.6).

Interestingly, the myotonometer captured changes in compliance where displacements increased over time with statistical differences beginning immediately at the great toe and at 30post at the first metatarsal head. The compliance changes had longer lasting effects at the first metatarsal head. These effects continued through 90post. The changes at the great toe, on the other hand, returned to baseline at 60post.

The difference in skin property responses must be viewed with respect to how the data was gathered. The devices were not measuring the same thing. The TID v2 data

reflected more superficial measurements when compared to the myotonometer data, which assessed displacements up to 6 mm. One must consider that the myotonometer compliance changes were not merely coming from an increase in compression of the underlying tissue. But rather, the measurement also included the tissue surrounding the plunger of the indenter. As forces increased, not only was compression increasing but also the surrounding tensile forces of that skin and tissue. The amount of force utilized resulted in more or less tissue and skin being included in the overall stiffness.

Another component that may have affected differences in measurements across time was related to superficial skin temperature. Because of a larger distance from the heat source and exposure to the air, the skin likely more rapidly cooled compared with deeper tissues. This temperature behavior could certainly result in a quicker stiffening of superficial tissue compared to deep. It was consistent with the literature that increasing temperature decreases stiffness.(114) Thus, if temperature plays a role, it was expected that the TID v2 measurements would return to baseline more quickly than measurements taken with the myotonometer.

Variability in findings among subjects could also be related to the variability of human gait and anatomy. In the case of the first metatarsal head, gait patterns and speed relative to each subject's typical speed could have largely influenced the amount of extension that occurred at the first metatarsal phalangeal joint. Despite the same gait speed, different subjects had different gait patterns to accomplish the task. The result was great toe extension that was affected by multiple components including cadence, muscle recruitment, and flexibility. The range of motion at this joint was variable among subjects. This repetitive motion and tissue stretching in gait with a subsequent decrease in

stiffness or increased compliance as was seen in this study is consistent with the literature for the effects, in general, of repeated movement and stretching.(118) With the great toe alone, gait pattern could have influenced the amount of force applied to the plantar skin and tissue during gait. Unfortunately, the subjects' gait patterns were not directly assessed and these direct measurements were not taken.

The anatomy is another source of variance among individuals that must be considered. Although general anatomy is similar, different body types have different amounts and composition of tissue. Some people have thicker fat pads while others do not. The possibilities of human structural and tissue variation are immense.

The last specific question addresses the behaviors of plantar skin in individuals with and without diabetes in response to acute physical activity (q.4). While there were sometimes differences between the groups relative to stiffness, largely the groups had similar patterns of response to activity. When graphs are viewed together, one can see that with stiffness as measured by the TID v2, all groups had non-significant decreases in stiffness followed by rises in stiffness. The magnitudes of change were different but the general patterns were similar. Likewise, viscosity patterns mimicked one another. When myotonometer output patterns were reviewed, first metatarsal compliance and stiffness patterns were mostly consistent across groups. However, the great toe responses showed some variability with stiffness responses and consistent variability with a longer time to peak compliance with the non-diabetes group. Overall, both groups had similar responses.

Finally, some of the TID measurements approached significance, but were underpowered, largely because of a faulty device. Given the effect size presented with

partial eta squared and an alpha level of 0.01 to adjust for repeated measures, G*Power was used to calculate necessary sample size to adequately power future work. Table 4.2 lists, along with the effects size f from the current study (TID v2), sample sizes required to adequately power future work. It should be noted that partial eta squared values were from differences between Pre and 0post. In the case of the first metatarsal head and the TID measurements, the current study found significant differences that occurred at a later time than 0post.

Table 4.2 Sample sizes required for adequate power for future work. Great toe (GT); First metatarsal head (MH1)

Test	Effect size f	Sample size for 0.7 power	Sample size for 0.8 power	Sample size for 0.9 power
GT TID	0.3407	18	20	24
MH1	0.5558	24	28	34
GT myotonometer	0.5820	22	26	32
MH1 myotonometer	0.3202	62	76	96

4.1.4 Summary

Together, this study points to the fact that physical activity causes acute changes in the skin and tissue properties of the plantar foot in individuals with type 2 diabetes as well as those without diabetes. Because of these changes, health care providers need to know how these changes may impact a person's health. Considerations are needed so that a person with diabetes is encouraged to ambulate to best capitalize on the benefits of walking for diabetes control as well as to mitigate one's risk for skin breakdown.

Unfortunately, a complete picture of the mechanism of these changes and the

ramifications of the alteration of the mechanical properties of skin is not known. Thus, physical activity must be further investigated to clarify the possible ramifications of decreased stiffness to the plantar tissue. Care must be taken to insure that plantar ulceration risk is minimized and diabetes control is maximized.

This study has several associated limitations. First, because of the heat of the chamber, it was necessary to allow the subject to move out of the chamber when tissue measurements were not being made. The possible consequence of such a location change could be that the skin more rapidly cooled than the deeper tissue. This may have affected the length of time that changes in stiffness were evident. When the temperature was monitored to assess any changes in tissue temperature and relative humidity, the location of the sensors minimized significant drops in skin temperature because the area was contained within the tubigrip within the positioning boot. Unfortunately, the first metatarsal head and great toe were not similarly maintained within the sock/ tubigrip environment. Also, the TID v2 device results could not be compared to previous TID v1 studies because the devices were actually different.

Another possible limitation involved subject matching. While subjects were matched on age and gender, subjects were not BMI matched. This failure to match on weight and height could offer an additional mechanism for changes that may have been seen. Height influences cadence of gait while weight influences load on tissue so both of these factors contributed to variation. With the large variability among subjects, difference in subject BMI was unlikely to affect group results.

5 Conclusions

5.1 *Summary Findings*

The goal of this project was to determine how skin responds to a short bout of walking. The hypothesis that was to be tested was that skin would demonstrate a decreased stiffness and an increased compliance as a result of a 10-minute bout of walking. The skin properties of interest included skin stiffness and viscosity as measured in response to a traction force and skin compliance as measured in response to an orthogonal force. To complete this goal, multiple steps were required as three specific aims were addressed. Specific Aim #1 was designed to validate the instrumentation necessary to characterize skin tissue. Specific Aim #2 was intended to characterize the skin tissue of the foot across environmental conditions and time. And, with the insight gained from Specific Aims #1 and #2, Specific Aim #3 was proposed to identify the impact of a 10-minute bout of walking on skin tissue. The outcomes of the process follow.

Specific Aim #1 required the testing of a number of pieces of equipment to ensure that they were reliable for the examination of plantar skin properties. Following testing in both a typical room environment and a warm, humid environment (EC), reliability of the TID v1 and TID v2 was demonstrated. TID v1 was reliable only for stiffness testing with either handheld or mechanical lowering. The TID v2, on the other hand, was reliable for both stiffness and viscosity measurements. Unlike the earlier version, reliability for TID v2 was better using the mechanical lowering mechanism. Both devices were found to test consistently, regardless of the temperature and relative humidity of the environment in which they were tested. Similarly, the StepWatch was validated in a population who used

an assistive device and the myotonometer was found to be reliable across typical, and warm, humid environments, except at the initial pressure point (0.25 kg). Importantly, these devices were found reliable for measuring plantar skin testing. All of these findings were incorporated into the design and analysis of subsequent study components.

Next, plantar skin was characterized across time and within different environmental settings for people with and without diabetes (Specific Aim #2). Multiple studies have evaluated various properties of skin in a single visit,(45, 47, 48, 50) but few have looked at properties across days.(107) To design future interventional studies, the element of time was especially critical if multiple visits were required to test more than one intervention. Additionally, it was important to consider the environment of testing because of the various environments to which feet are exposed. With these factors, a clearer understanding of the behavior of skin was obtained by using a four-visit protocol over the course of a month.

Skin stiffness (TID), compliance (myotonometer), and epidermal and dermal thickness (US) did not change statistically across environmental conditions or over time. It was noted that there was a wide amount of variability between subjects when skin was tested. This is consistent with the literature.(45, 119) When ICCs were reviewed at the great toe and the first metatarsal head, the TID was most reliable across the first week while the myotonometer and US (epidermal measurement only) were acceptable across a month's time. Also, since skin may tend to respond based on the environment, particularly when measured using the TID, one must consider the environment in which testing is performed.

Together, this four-visit study provides insight into future testing on the skin of people with and without diabetes. First, if future testing needs to compare multiple conditions, the stability of skin within an individual found in the four-visit study supports the ability to test on different days as long as the testing is within a week for the TID and within a month for any myotonometer or epidermal thickness testing. Given that some variance occurred within different environments, it is suggested that the environment in which testing is performed should be considered. Finally, sample size must take into consideration the variability of skin between individuals.

Specific Aim #3 examined the impact of a 10-minute bout of walking on skin. From this single-visit study, it was determined that acute changes were present in the stiffness and compliance of the skin following walking. The measure taken using perpendicular forces did, in fact, reveal an increased compliance (decreased stiffness) that was still significant at 90 minutes after walking in the case of the first metatarsal head and at 60 minutes in the case of the great toe. For this case, the null hypothesis was rejected. However, when skin was tested using traction forces with the TID v2, stiffness did not change initially following walking, but then increased over time. While stiffness (TID) changed, it did not change in the way that was anticipated. Viscosity did not differ and the null hypothesis was not rejected. Together, walking certainly affected the plantar skin. Possible physiological contributors to this change may include stretching of soft tissue, increased skin temperature secondary to exercise or friction, tissue loading, or physiological changes associated with exercise including an elevation in heart rate and blood pressure. (Figure 5.1) Unfortunately, the ramifications of change in stiffness or

compliance relative to risk for injury are not specifically known. But, recall the general risks for ulceration as described in Chapter 1 and summarized in Figure 1.1.

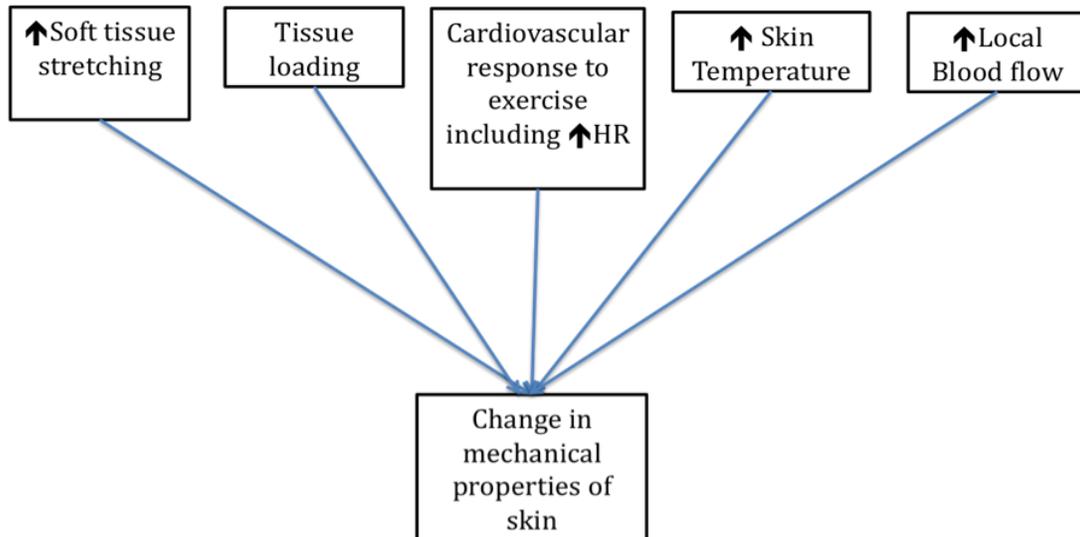


Figure 5.1 Potential physiological contributors to change in the mechanical properties of skin

Because the body's response to skin changes are not fully known, possible effects of the stiffness and compliance changes that come with walking must be considered. Previous studies have found that those with a history of foot ulceration actually were less active as was measured with an accelerometer over a week(20) or with a validated questionnaire every 17 weeks over two years.(17) Similarly, when using an activity monitor for at least 25 weeks or until ulceration, subjects who ulcerated had lower levels of activity.(19) In fact, those who were up on their feet more actually ulcerated less.(17, 19) When a group of subjects was encouraged to increase their walking activity, no increased rate of breakdown was seen compared to a control group without additional activity intervention.(18) However, other studies have reported that more variable

rates(19) or higher quantity walking(21) was associated with ulceration or re-ulceration. Given these findings of previous studies relative to skin breakdown, it is clear that further work is needed to understand the changes to the skin properties related to the associated change in injury risk. While the increased flexibility of tissue with walking may allow the foot to better accommodate to its surrounding surface, it may also put one at risk for injury if flexibility becomes too great. Such excessive flexibility is likely to result in a bottoming out of the tissue. Further evidence is required to enable more specific prescription of exercise in response to the findings of these studies. The goal of exercise in a population with diabetes continues to aim for improved diabetes control while minimizing risk for injury and complications.

5.2 *Future Directions*

There are a number of questions that need further study based on the findings from these projects such that two general areas can be clarified. First, it is critical to better understand the physiological mechanisms causing changes in the mechanical properties of skin. Second, it is also imperative to address how any changes in these properties (stiffness and compliance) could actually affect one's risk for injury. Together, advances in these areas could enable clinicians to not only understand more fully the effects of exercise, but also to apply that information to design walking and exercise programs for their clients and patients.

Potential physiological causes or contributors to the mechanical changes (stiffness and compliance) that were seen may include soft tissue stretching secondary to joint movement, loading of tissue, the cardiovascular response to exercise including an elevation of heart rate and increased blood flow, and/or increased skin temperature

(Figure 5.1). For an improved understanding related to the source of possible changes, these likely contributors should be experimentally differentiated. To distinguish among these or other possibilities, there is a need to first compare the effects of different modes of exercise on the stiffness of skin. These modes should be selected so that component contributions can be isolated and causality more closely determined. This may be accomplished by comparing skin stiffness and compliance following a variety of interventions including different activity bouts that highlight certain expected contributors of change. In figure 5.2, one can see a sampling of potential interventions and modes of activity along with their expected mechanisms of skin changes. Using such interventions may help, at a minimum, to rule out what components of the exercise behavior are unrelated to skin stiffness and compliance changes. Beyond these modes of exercise, intensity of exercise can be modulated to determine whether exercise intensity has an effect on stiffness and compliance or not. Additionally, duration of exercise can also be modulated to determine whether or how duration affects skin property changes and the extent to which any of these changes may last.

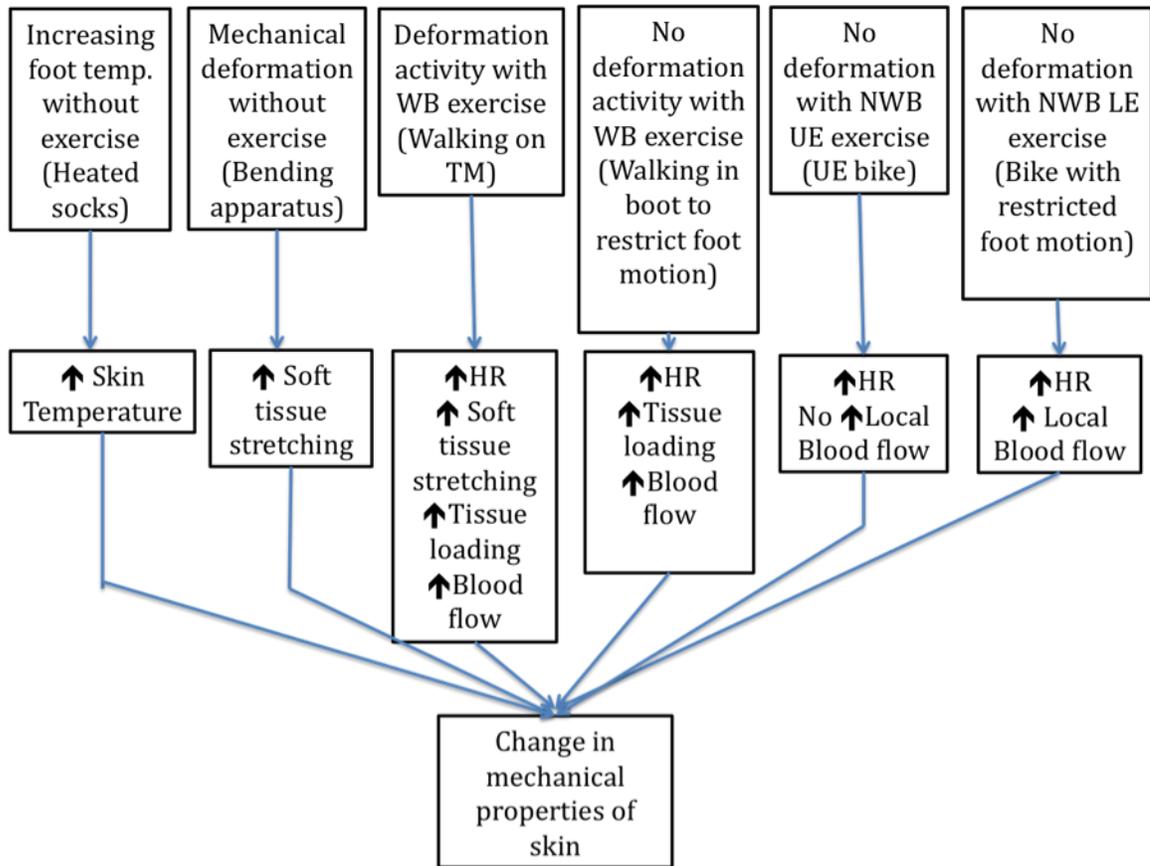


Figure 5.2 Clinical interventions to help identify contributors of changes in skin stiffness

The interventions suggested in Figure 5.2, along with modifications to intensity and duration of activity, can be utilized in studies of a similar design to that which was described in Chapter 4. Importantly, baseline measures should be compared to measurements taken subsequent to the intervention strategy. In the case of myotonometer testing, in particular, it will be important to extend the length of time for measurements, beyond 90 minutes, so that a return to baseline is captured. This will allow a more complete understanding of the behavioral changes that are happening with an activity or intervention. Additionally, in the case of examining exercise intensity, caution must be used to insure a safe exercise protocol among the group that may be at a higher risk for

exercise-induced injury. Again, subsequent measurements would be taken following all exercise bouts.

Another important implication for stiffness changes in people with diabetes is how stiffness changes longitudinally. With simple devices such as the TID v2 or indentors, stiffness and compliance measures could be easily added in the clinic as part of a yearly diabetic foot exam. These mechanical changes could then be tracked so that foot orthotic devices could be better prescribed and utilized. In fact, with the tracking of stiffness and compliance, one may be able to determine the effect that orthotic devices have on the feet. Additionally, efforts should be made to assess the chronic effects that exercise might have on the mechanical properties of tissue.

The second consideration for future work is the need to address how stiffness and compliance changes can actually affect one's risk for injury. The reason for this objective is to better understand to what extent a decrease in stiffness or increase in compliance is desirable. This work would need to occur at a skin tissue level where a porcine or cadaveric skin model could be utilized so that following stiffness and compliance manipulation, the forces required for tissue failure could be measured. With such an experimental design, a better understanding of how a range of stiffness or compliance alter tissue response.

In conclusion, this study investigated the effect that physical activity had on the stiffness, viscosity, and compliance of the plantar skin in people with and without diabetes. The findings of this study, along with the preceding background studies, have implicated the need for future work as well as clinical consideration for exercise prescription that may promote better glycemic control through exercise, improved health

through fewer complications, and an improved quality of life in people living with diabetes.

Activity monitor accuracy in persons using canes

Deborah Michael Wendland, PT, DPT, CPed;^{1*} Stephen H. Sprigle, PhD, PT¹⁻²

¹School of Applied Physiology and ²School of Industrial Design, Georgia Institute of Technology, Atlanta, GA

Abstract—The StepWatch activity monitor has not been validated on multiple indoor and outdoor surfaces in a population using ambulation aids. The aims of this technical report are to report on strategies to configure the StepWatch activity monitor on subjects using a cane and to report the accuracy of both leg-mounted and cane-mounted StepWatch devices on people ambulating over different surfaces while using a cane. Sixteen subjects aged 67 to 85 yr (mean 75.6) who regularly use a cane for ambulation participated. StepWatch calibration was performed by adjusting sensitivity and cadence. Following calibration optimization, accuracy was tested on both the leg-mounted and cane-mounted devices on different surfaces, including linoleum, sidewalk, grass, ramp, and stairs. The leg-mounted device had an accuracy of 93.4% across all surfaces, while the cane-mounted device had an aggregate accuracy of 84.7% across all surfaces. Accuracy of the StepWatch on the stairs was significantly less accurate ($p < 0.001$) when comparing surfaces using repeated measures analysis of variance. When monitoring community mobility, placement of a StepWatch on a person and his/her ambulation aid can accurately document both activity and device use.

Key words: accelerometry, accuracy, ambulation aids, assistive device, canes, elderly, gait, mobility limitation, physical activity monitoring, walking.

INTRODUCTION

Physical activity is important for health and has been associated with a decrease in all-cause mortality [1–3]. Additionally, physical activity has been connected to health benefits including improved cardiorespiratory fitness, metabolic health, weight maintenance, functional health, cancer risk reduction, and improved mental health [4].

Furthermore, according to a report from the Urban Institute of the U.S. Department of Health and Human Services, there has been an increased prevalence of assistive device use to facilitate increased independence with activities of daily living such as mobility and instrumental activities of daily living [5]. Simple equipment such as walkers, canes, and crutches showed the largest growth in usage [5]. While research offers some indication that assistive devices may replace human help in some cases, the effect of this is unknown [5].

Given the increase in assistive device use and the value of physical activity, it is important to recognize that activity varies across populations [6–7]. With such variation, it is also essential to acknowledge that facilitation of physical activity must reflect these differences. One useful means to promote and measure activity is with an activity monitor that, by measuring activity, can help to grade activity changes [8] as well as motivate people to be active [9].

The StepWatch (Orthocare Innovations; Mountlake Terrace, Washington) is one example of a commonly used accelerometer-based monitor that reflects physical activity by reporting strides during ambulation. It has been utilized and/or validated in a large number of populations, including the elderly [10–12] and those with

Abbreviations: ANOVA = analysis of variance, SCI = spinal cord injury.

*Address all correspondence to Deborah Michael Wendland, PT, DPT, CPed; 3001 Mercer University Dr, Davis Building, 100A, Atlanta, GA 30341-4155; 678-547-6775; fax: 678-547-6202. Email: wendland_dm@mercer.edu
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spinal cord injury (SCI) [13–14], amputation [15], stroke [16–18], muscular dystrophy [19], and diabetes mellitus [20–22]. Some people who use mobility assistive devices have been studied in the context of other, larger studies using StepWatch [13–14,16–17,23–24].

Bowden and Behrman used StepWatch to monitor ambulation in 11 persons with incomplete SCI, 9 of whom used an ambulation device [13]. For those using ambulation aids, the overall stride accuracy of the 10-meter walk was 97 percent, with individual stride accuracy ranging between 85 and 100 percent [13].

In a study of activity in persons after stroke, Haeuber et al. studied 17 people, 15 of whom used an ambulation device [17]. StepWatch accuracy was measured using 1 min bouts of walking at self-selected and faster speeds. For all subjects, the StepWatch reported a 94 percent accuracy using the default configuration settings of cadence = 80 and sensitivity = 12 [17]. Both of these studies conclude that the StepWatch is accurate enough to monitor the activity of persons using ambulation aids indoors.

In a different study looking at gait in people after a stroke, Mudge et al. described the subjects as being able to “walk independently” with their customary orthotic devices, but no mention was given to any ambulatory aid [23]. While this study utilized different surfaces, there was no clear use of ambulation aids. In a population that uses ambulation aids such as canes, documenting gait accuracy of the monitoring device on different surfaces consistent with everyday mobility would be beneficial because gait patterns often change while ambulating over outdoor surfaces, including ramps and uneven terrain [25–27]. These surface changes and subsequent gait pattern modifications might affect StepWatch accuracy as well. Moreover, documenting the accuracy of a StepWatch mounted to a cane can be beneficial to studies investigating the use of ambulation aids during everyday mobility.

The aims of this technical report were to report on (1) strategies to individualize StepWatch configuration parameters for leg and cane-mounted monitors, (2) the accuracy of the StepWatch as persons ambulate over different surfaces and grades while using a cane, and (3) StepWatch accuracy in measuring cane-ground contact as a practical means of documenting cane usage.

METHODS

Participants

People who used canes during ambulation were recruited from Senior Centers and Senior Housing in metropolitan Atlanta, Georgia. To be included in the study, subjects had to be over 18 years old, use an assistive device for gait, have the ability to ambulate at least 10 m without rest, and be able to give informed consent.

Instrumentation

StepWatch is a U.S. Food and Drug Administration-cleared class II device designed for long-term assessment of community walking function. It is 75 × 50 × 20 mm and weighs 38 g. StepWatch output consists of stride counts over a user-defined epoch. Two parameters, cadence and sensitivity, can be adjusted for individuals. Cadence limits the rate at which the StepWatch detects steps. As cadence settings are raised, the device will increase the time delay before the next stride can be detected. Therefore, higher cadences should be chosen for slow walkers and for people with long legs [28]. Sensitivity, on the other hand, is a measure of the amount of movement required by the StepWatch before it detects a step. As the sensitivity settings are raised, the device requires more movement to record a step [28].

Protocol

Subjects were fitted with a StepWatch device. The device was placed on each subject's right ankle just proximal to his or her lateral malleolus using a Velcro band. A second StepWatch device was placed on the distal end of his or her regular cane using double-sided tape and porous tape to further secure the device. No adjustments were made to either the subjects' canes or their gait patterns.

The protocol included two stages (**Figure 1**). In the first stage, the StepWatch parameters were evaluated and optimized using short bouts of mobility. Leg-monitor accuracy was defined by comparing the number of strides recorded by StepWatch with the number of strides observed by a researcher. Strides were manually counted each time the right leg had forward progression. Cane-mounted accuracy was defined by comparing the number of “strides” recorded by StepWatch to the number of times that the cane made contact with the ground as observed by a researcher. Separate researchers used

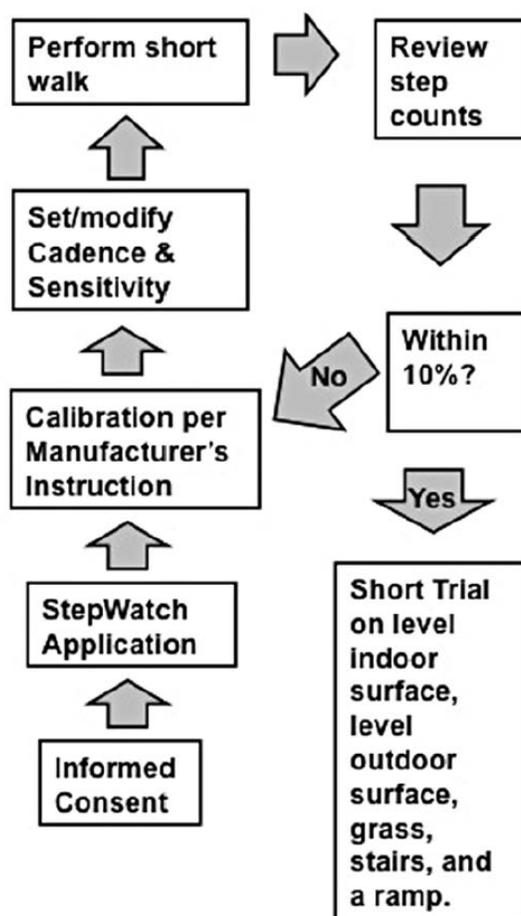


Figure 1.
Two-stage study protocol.

handheld counters (GOGO, Atafa; Cambridge, Massachusetts) to document strides and cane-ground contacts for comparison with the recorded data from the leg- and cane-mounted StepWatch devices, respectively. In the second stage, bouts of ambulation were taken over different surfaces and grades. Leg strides and cane-ground contacts were manually counted by separate researchers to calculate accuracy.

For stage 1, both the leg-mounted and cane-mounted StepWatch devices were initially configured according to the manufacturer's specification. The cadence setting

was defined using the individual's height, and the suggested sensitivity setting relative to each device's calibrated threshold was initially used [28]. These numbers were then input into the advanced programming setting along with a 6 s epoch time.

Calibration trial ambulation was performed in a room such that each subject ambulated in one direction, around a table in a circle, at a given speed. The subject then turned around and ambulated in the opposite direction at a slower speed. Both self-selected and slower speeds were used to calculate accuracy. The total distance covered with each calibration trial was typically between 30 and 50 strides. Acceptable accuracy for both devices was defined when the recorded strides were within 10 percent of the manually counted strides and cane-ground contacts.

Parameters for the StepWatch were modified in a manner similar to Bowden and Behrman [13]. If the StepWatch overcounted strides, sensitivity was raised an increment. When the monitor undercounted during the trial, sensitivity was lowered an increment. Normally, to minimize subject fatigue, if the device's recorded strides were 10 or more counts different than the counted strides or cane-ground contacts, sensitivity was increased or decreased by two increments rather than one. This process was repeated until StepWatch values were within 10 percent of the manual count. **Figure 1** illustrates the calibration procedure.

Once calibrated, the StepWatch devices were remounted to each participant's right leg and cane. Each subject then proceeded with the validation protocol. The validation protocol required that participants ambulate over an indoor level surface (linoleum), an outdoor level surface (sidewalk), an outdoor uneven surface (grass), up and down a ramp, and up and down stairs. Two 10 m distances were completed for each surface. At least 30 s of rest was given between bouts. Leg strides and cane-ground contacts were manually counted by separate researchers using the same handheld tally counters. The indoor and outdoor level surfaces were always completed first to allow for an assessment of the participant's safety during ambulation. Following those trials, the remaining three tasks were performed in a randomly assigned order to minimize the effect of fatigue on the gait pattern.

Upon completion of the protocol, the StepWatch monitors were removed and the data were downloaded per the manufacturer's specification. StepWatch software was used to process data and report the number of strides taken during each bout of activity. Data were analyzed using

PASW Statistics software (IBM: Armonk, New York). Descriptive data analysis was performed comparing the manual counts with the recorded StepWatch data for both stages of this study. A repeated measures analysis of variance (ANOVA) with post hoc pairwise comparison tests was used to compare accuracy across surfaces and device location. Huynh-Feldt was used to correct for sphericity. A Bonferroni correction was used to correct for multiple comparisons. Finally, a *t*-test was used to compare the accuracy between the leg- and cane-mounted StepWatch devices. Alpha level was set at $\alpha < 0.05$.

RESULTS

Sixteen subjects ranging in age from 67 to 85 yr (mean 75.6) met qualification criteria and agreed to participate. On level surfaces, every subject ambulated with a 2-point gait pattern using his or her straight cane. In all but one case, cadence settings were effectively set for both leg- and cane-mounted monitoring devices according to the person's height without any adjustment. The subject with an ineffective height-based cadence setting exhibited an antalgic gait pattern, which changed from trial to trial. With this individual, we were unable to calibrate either StepWatch device. That subject did not complete the study.

Sensitivity setting adjustments were required to calibrate the leg-mounted StepWatch monitors in only seven of the subjects completing the study (Table). Five of these subjects required lowering the sensitivity setting, while two required that the sensitivity setting be raised. Six of the seven subjects requiring sensitivity modification needed an adjustment of one unit from the default value. In the remaining case, the subject required a two unit adjustment from the default setting.

Calibration of the cane-mounted StepWatch devices required sensitivity setting adjustment in all but one of the 15 subjects completing the study (Table). The sensitivity setting had to be lowered for the cane-mounted monitors in 12 of these cases. The remaining two subjects needing modification required the sensitivity setting be raised. Of the 14 requiring adjustment to the sensitivity setting, 8 required a lowering of the sensitivity by three or four units and 1 required a decrease in the sensitivity setting by two units.

During stage 1 of the protocol, the calibrated leg-mounted StepWatch monitors had 98 percent accuracy,

Table.

Sensitivity calibration for leg- and cane-mounted StepWatch devices.

Subject	Leg-Mounted Sensitivity Modification	Cane-Mounted Sensitivity Modification
1	↓ 1 unit	↓ 3 units
2	No adjustment	↓ 3 units
3	No adjustment	↓ 4 units
4	↑ 1 unit	↓ 3 units
5	↑ 1 unit	↓ 1 unit
6	No adjustment	↓ 4 units
7	No adjustment	↓ 3 units
8	Unable to calibrate	Unable to calibrate
9	↓ 1 unit	↓ 4 units
10	No adjustment	↑ 1 unit
11	No adjustment	↓ 3 units
12	↓ 2 units	↑ 1 unit
13	↓ 1 unit	No adjustment
14	No adjustment	↓ 2 units
15	No adjustment	↓ 1 unit
16	↓ 1 unit	↓ 1 unit

while the calibrated cane-mounted monitors had 97 percent accuracy.

For stage 2 of the protocol, aggregated manual and recorded counts over the entire trial, both indoor and outdoor, resulted in an accuracy measure for the leg-mounted device of 93.4 percent. The cane-mounted device recorded an aggregate accuracy of 84.7 percent. These values were significantly different when compared using a *t*-test ($p < 0.001$).

Specific surfaces were found to have different levels of accuracy for the leg-mounted and the cane-mounted monitors (Figure 2). When the surfaces were compared using the repeated measures ANOVA, there was a main effect ($p < 0.001$). With post hoc pairwise comparisons, the accuracy on the stair surface was significantly lower than all the other surfaces ($p < 0.01$). There was no interaction between surface and device. If stairs were excluded in the aggregation, leg-mounted accuracy was improved to 95.8 percent and cane-mounted accuracy was improved to 89.1 percent.

On the whole, the cane-mounted device had lower accuracies than the leg-mounted monitor. The lowest accuracies for both leg- and cane-mounted StepWatch monitors were recorded during ambulation on stairs ($85.9\% \pm 10.4\%$ and $64.0\% \pm 25.7\%$, respectively). The surfaces with the highest accuracy for the cane-mounted StepWatch were the

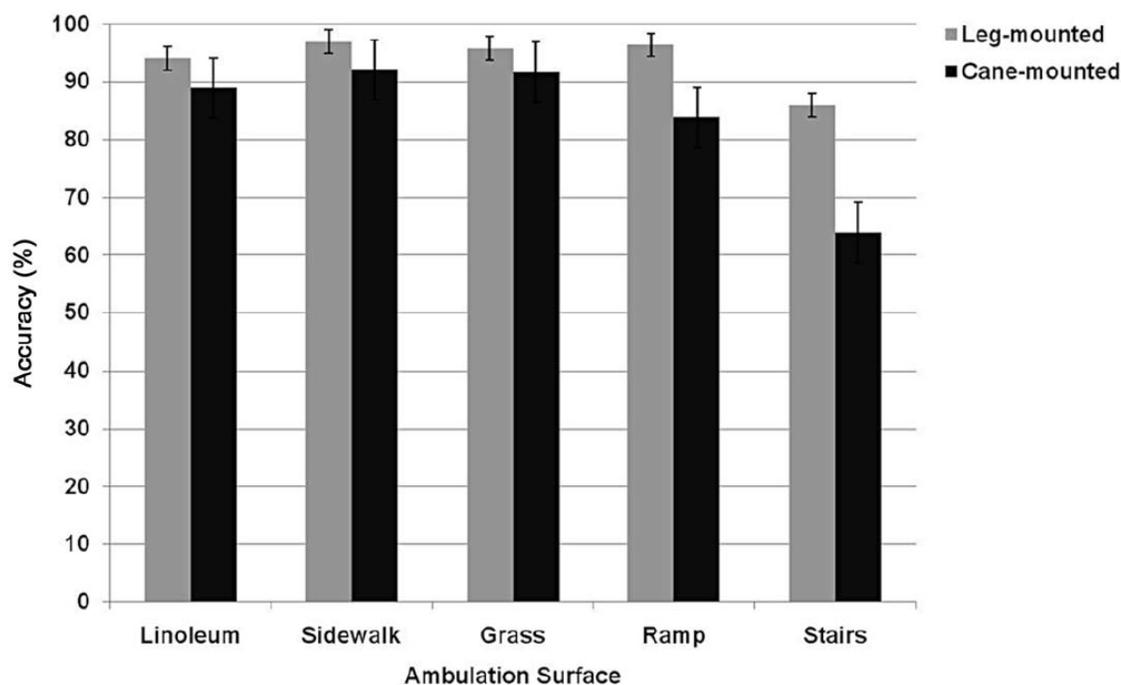


Figure 2.

Accuracy of StepWatch during validation trial compared with manually counted leg strides and cane-ground contacts. Stair values were significantly different from other surfaces when compared using pairwise comparison post hoc for repeated measures analysis of variance ($p < 0.01$, $\alpha < 0.05$ with Bonferroni correction). Bars indicate standard error of mean.

sidewalk ($92.1\% \pm 7.9\%$) and the grass ($91.7\% \pm 4.7\%$). Accuracy of the StepWatch on the leg for the same surfaces was 97.0 ± 2.8 percent (sidewalk), and 95.8 ± 3.7 percent (grass) (Figure 2). Aggregated accuracy was affected by individual surface accuracy as well as strides recorded between trials.

DISCUSSION

We found that short ambulation bouts can be used to optimize cadence and sensitivity settings for both leg- and cane-mounted StepWatch devices. Ease of calibration was affected by the limited stamina of this elderly cohort, so settings should be optimized using as little iteration as possible. For leg-mounted units, a judicious approach is to begin with the settings as recommended by the manufac-

turer. For cane-mounted devices, we recommend starting the calibration process slightly differently. The cadence setting may be based on the person's height, but the starting point for sensitivity calibration may be adjusted to two units below the default setting to increase calibration efficiency. Alternatively, "jumps" of two sensitivity units could be made when the trial ambulation bouts report a mismatch between recorded and observed scores. Tight turns, leg movement, cane tapping, and cane carrying affected StepWatch accuracy on the cane-mounted device in particular. In addition, we found that an antalgic gait can be prohibitive to accurately calibrate leg- and cane-mounted StepWatch devices.

For cane users, we found that the leg-mounted StepWatch accurately recorded the number of strides for ambulation over various surfaces and grades with a combined accuracy of 93.4 percent. The surface accuracy

ranged from 86 percent on stairs to 97 percent on the sidewalk. The StepWatch device was more accurate on nonstair surfaces for the leg-mounted device. Given that people spend little time walking on stairs, the leg-mounted device accuracy will allow clinicians to use the StepWatch in this population with confidence that everyday mobility will be appropriately reflected in longer-term trials.

The cane-mounted StepWatch had an aggregate accuracy across all surfaces of 85 percent. The specific surface accuracy ranged from 64 percent on stairs to 92 percent on the sidewalk. Accuracy was improved when stairs were excluded (89.1%). This is likely related to the variability with which a cane is utilized. During ambulation on level surfaces, participants consistently utilized a 2-point gait pattern, which the StepWatch monitored accurately. While traversing stairs, participants used a nonreciprocal step-to pattern while gripping a railing, which altered the need for supplementary balance assistance from the cane. Based on visual feedback, the decreased accuracy resulted from the tendency of the subjects to use the cane differently, including carrying the cane or cane tapping.

In certain populations, monitoring cane usage can be helpful to the immediate health of the individual. For example, people with diabetes with plantar ulceration should ambulate in a way that the ulcer is off-loaded and often includes the use of an assistive device. By closely monitoring the use of an assistive device in conjunction with leg-monitoring, further injury from an excessive increase of physical stress (steps) [29] can be avoided [8]. Additionally, spikes in physical activity levels have been associated with an increased risk of skin breakdown [30]. Potentially, using an activity monitor for feedback may facilitate consistency in physical activity levels, which could reduce the risk to the integumentary system.

Feedback indicated that the StepWatch can be used on a cane with little effect on the user's daily life. Since the person never has to interact with the StepWatch, compliance is not an issue and its measurements therefore reflect the utilization of the cane.

Limitations of this study are primarily due to the cohort studied and the resulting variability of how they utilized a cane. Cane behaviors like tapping or carrying may be measured with an activity monitor even though they may not constitute a true step. Additionally, tight turns will affect the distance that canes are moved, thus affecting whether strides are actually recorded or not. In

addition, gait patterns that change secondary to pain, for instance, may be prohibitively difficult to calibrate. This will limit the generalizability of the findings to that component of this population.

Also, accuracy for both the leg- and cane-mounted StepWatch devices may also be biased lower secondary to the surface protocol used for stage 2 of this study. Because participants used a step-to pattern on stairs, the number of strides required to complete the 10 m level surface walks was approximately half the number required to complete the stair task. With the disproportionate strides taken on the stairs, the increased weighting of stair surface accuracy depressed overall accuracy. Because the prevalence of traversing stairs in real life is minimal compared with negotiating level surfaces, we believe the cane-mounted StepWatch accurately reflects cane usage.

For future work, an analogous StepWatch validation could be completed on other assistive devices, such as standard walkers and crutches, which are used by various populations with mobility disabilities. With further validation, the StepWatch could be used to monitor ambulation of persons using an array of assistive devices in order to study the everyday activities of persons with disabilities.

CONCLUSIONS

Calibration of StepWatch monitors can be performed according to manufacturer's instructions for both leg- and cane-mounted devices in a population that uses ambulation aids. When monitoring community mobility, placement of a StepWatch on a person and his or her ambulation aid can accurately document both activity and device use.

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Author Contributions:

Study concept and design: D. M. Wendland, S. H. Sprigle.

Acquisition of data: D. M. Wendland.

Analysis and interpretation of data: D. M. Wendland, S. H. Sprigle.

Drafting and critical revision of manuscript: D. M. Wendland, S. H. Sprigle.

Statistical Analysis: D. M. Wendland, S. H. Sprigle.

Study supervision: S. H. Sprigle.

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Participant Follow-Up: The authors have no plans to notify the study subjects of the publication of this article because of a lack of contact information.

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Appendix B: Characterization of people with and without diabetes

Because it is of interest to study groups with and without diabetes, comparisons between the people with and without diabetes may be helpful. With that in mind, comparisons were made between the people in this study who had and did not have diabetes. First, if people with and without diabetes were considered as comparison groups, comparisons of means using independent t-tests showed similarities between groups for body mass index (BMI), USG, and activity levels (Table B-1).

Table B-1 Subject demographic profile. *Statistical difference where $p < 0.05$; **Statistical difference where $p = 0.000$ between groups.

Group	Age**	Gender	BMI	USG	Activity Level	Protective Sensation (Intact)*
Diabetes	63.25±10.86 years	6F/2M	29.4±8.03	1.0221± 0.008	1.5±1.2	4
No Diabetes	33.75±13.31 years	5F/3M	33.6±7.05	1.0189± 0.007	1.1±1.4	8

Further comparisons were made across the testing measurements including measurements made using the TID, the myotonometer, and US. Such comparisons follow. When the disease states were compared with TID measurements, there was a main effect difference ($p = 0.002$). This difference revealed that the group without diabetes had stiffer skin than the group with diabetes (Figure B.1). Like the group as a whole, both the people with diabetes and those without had similar stiffness responses relative to the environmental condition to which they were exposed (Figure B.2).

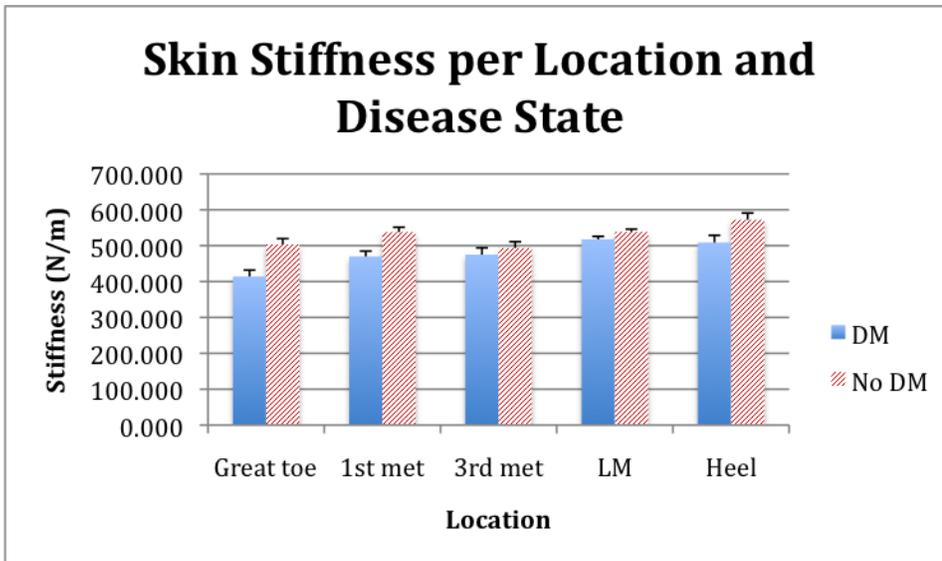


Figure B.1 Skin stiffness per location and disease state

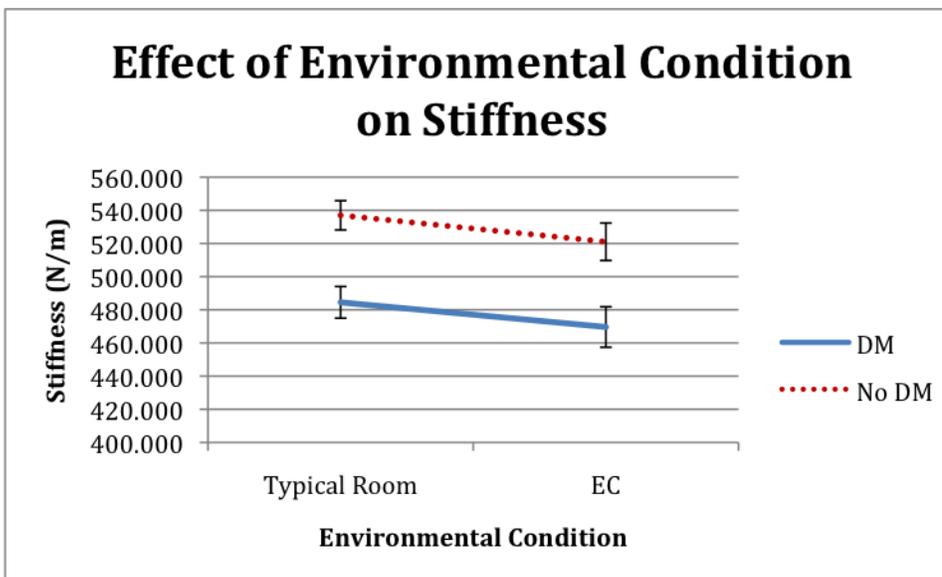


Figure B.2 Effect of environmental condition on stiffness

When myotonometer data was considered, the group with diabetes had consistently lower compliance relative to the amount of force applied ($p = 0.000$ Greenhouse-Geisser) (Figures B.3). Compliance according to location and relative to disease is shown in Figure B.4.

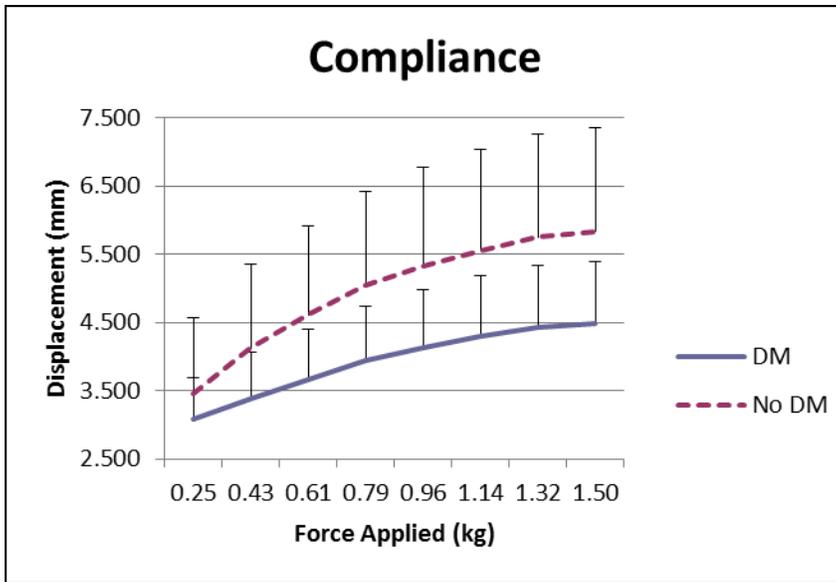


Figure B.3 Compliance relative to force. Error bars represent standard deviations.

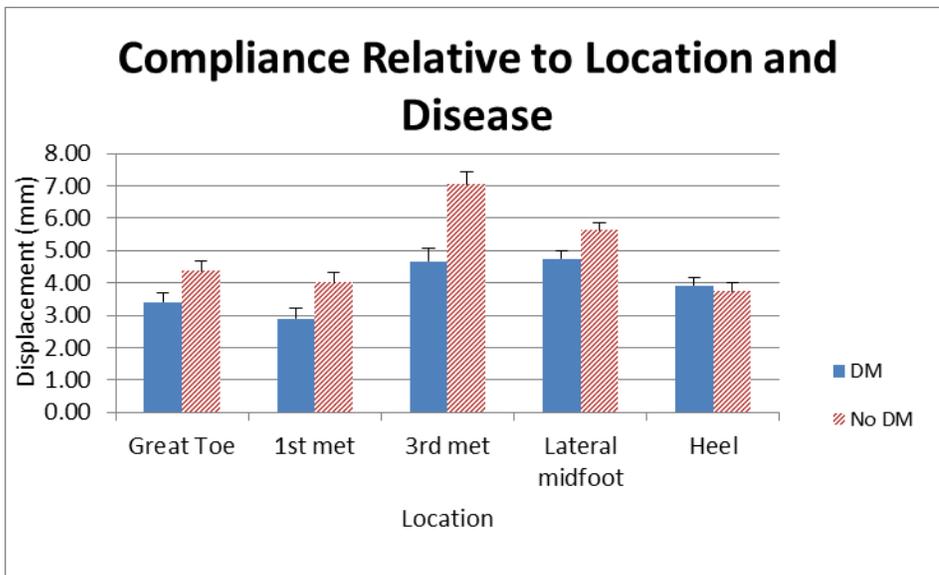


Figure B.4 Compliance relative to disease and location

Thicknesses, on the other hand, did not reach statistical significance across disease state in the epidermis. People with diabetes had a mean epidermal thickness (\pm Standard deviation) of 0.690 ± 0.111 mm and those without diabetes had a mean epidermal thickness of 0.869 ± 0.162 mm ($p=0.171$). However, using partial eta squared,

13% of variation in epidermal thickness was attributed to the disease state. Similarly, there was no difference between the dermal thickness in people with diabetes (1.444 ± 0.099 mm) or those without diabetes (1.280 ± 0.097 mm) ($p=0.173$). Again, using partial eta squared, 12.9% of variation in dermal thickness was attributed to the disease state (Figures 3.12 and 3.13).

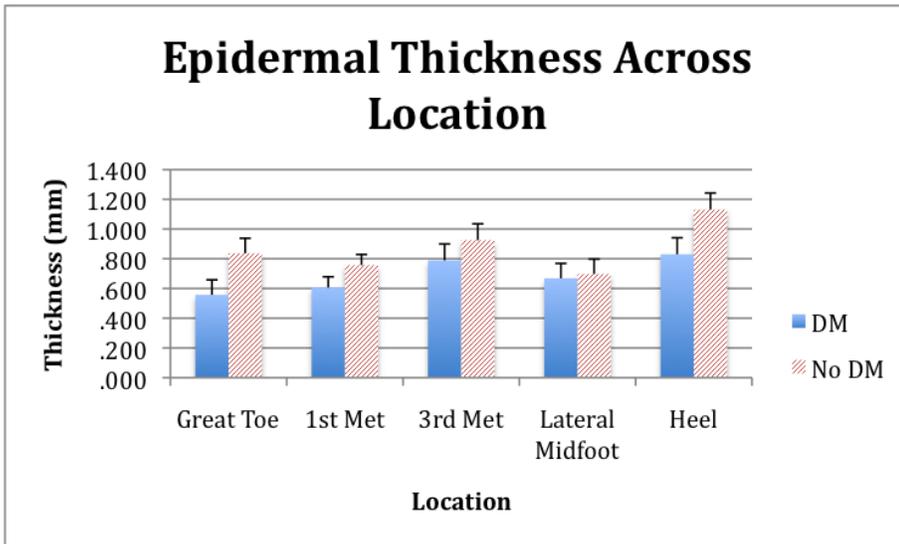


Figure B.5 Epidermal thickness across location and disease state

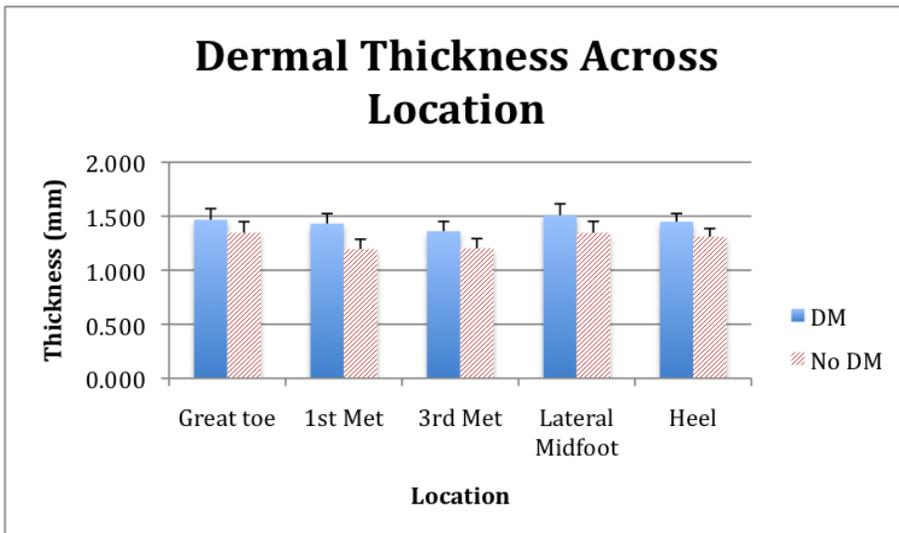


Figure B.6 Dermal thickness across location and disease state

Discussion

When the different groups were compared, this study found that with the application of traction forces, stiffer skin was present in people without diabetes. This result was not what one would expect compared to the literature. Stiffness is reportedly increased in people with diabetes in much of the literature.(46, 47, 49-51) But, this literature represents stiffness that was determined with indentation or suction equipment. The TID v1 does not measure stiffness in the same way.

The TID stiffness outcome, which differs from the literature, was likely related to the difference in measurement technique. In this case, the more superficial TID v1 measurement would more likely be affected by the skin anatomy. With the tendency for the epidermis to be thinner in the group with diabetes, the TID v1 could be capturing more of the dermal properties in the group with diabetes than in the group without diabetes. Given that the dermis is where the elastic properties of skin are located, the resultant measurement, taken closer to the dermis, would more likely include the increased compliance associated with the dermis.

Or, despite the different mechanism of measurement, the direction by which measurements were taken may have also played a role in the differences. Measurements were taken with the probe stretching skin in a medial-lateral direction. Because the direction that the foot bends during the progression of the gait cycle is actually in an anterior-posterior direction, expected changes to the skin structure in response to diabetes may also occur along this direction. By not measuring in this direction, it was possible that the resulting stiffness may not be reflective of the changes typical of people with diabetes. One must consider that for subsequent studies, it may be more helpful and

informative to test stiffness that is in response to traction forces in the same direction that this tissue typically moves. Thus, measurement where the traction is applied in an anterior-posterior direction may be preferable. Additionally, the variation across groups and within subjects must be noted.

Tissue compliance as represented by displacement under orthogonal load decreased in the group with diabetes compared to the group without diabetes. Mean displacements for the group with diabetes was 3.924 mm compared to the group without diabetes with a mean displacement of 4.962 mm. The decrease in compliance was consistent with increased stiffness found in people with diabetes using indenter systems.(45, 46, 51) Compliance relative to the disease process revealed that large differences occurred at the third metatarsal head, great toe and the first metatarsal head (Figure A.4). Interestingly, these locations are also some of the most common sites for skin breakdown. Little difference was noted at the heel between the two groups which was consistent with the findings of Klaesner et. al.(45)

There was not a significant difference in either the epidermis or the dermis but the effect sizes were large. Given the partial eta squared values of 0.13 for epidermis and 0.129 for dermis, the need for further study using a greater sample size was indicated. Interestingly, epidermal thickness was greater in persons without diabetes, whereas dermal thickness was greater in persons with diabetes. Graphs of thickness across anatomical sites also suggested that additional investigation onto interaction is worthwhile. The tendency for those in the diabetes group was to have a thinner epidermis and a thicker dermis compared to the matched controls. This thinner epidermis in the group with diabetes was consistent with the literature, particularly in a population with

neuropathy.(50) The mechanism of epidermal thinning secondary to neuropathic changes was also consistent with several rat studies that looked at the epidermal thickness following denervation. With denervation, the animals demonstrated a rapid thinning of the epidermis.(120, 121) Additionally, changes associated with age cannot be ruled out. The thickening of the dermis in people with diabetes was also consistent with the literature.(4) When total thickness of skin was considered, some studies have found that people with diabetes have increased thickness of the skin(4, 48, 50, 106) while others have found no difference in the thickness when people with diabetes were compared to controls.(46) So, by showing no difference in total thickness, the results were likely related to the extent of diabetes progression among those in the diabetes group. Age variation could also be a factor.

Interestingly, when epidermal results were viewed graphically (Figure B.5), the largest differences in thickness were noted at the great toe and the heel. These sites take large amounts of force during the gait cycle. Another possible contributor to a difference in thickness could be related to the way that people with diabetes walk compared to those without diabetes. People with diabetes have a tendency for decreased push-off during their gait cycle compared to people without diabetes.(34) Subsequent decreased gait pressures and friction could result in smaller epidermal thicknesses in response to the minimization of forces. Finally, while the dermis tends to be thicker in persons with diabetes, these thicknesses were 12.8% greater compared to that in persons without the disease (Figure B.5).

While skin in people is highly variable, some differences were found between people with and without diabetes. First, stiffness as was measured with traction forces

was lower in people with diabetes compared to those without diabetes. This finding, although unexpected, could have been influenced by the thickness of the epidermis or the directionality of the application of traction forces. On the other hand, tissue compliance was lower in people with diabetes. Finally, the thickness of the epidermis tends to be less in people with diabetes and the dermal thickness tends to be increased. In this study, total thickness was not different between the two groups.

Additionally, the small number of subjects in this study had a wide range of ages. A limitation of the study is that the two group mean ages are disparate. Escoffier, et al. report that there are few changes to the skin between the ages of 15 and 65.(53) Also, Reihnsner and Menzel found that there were not significant differences relative to age regarding “orthotropic biomechanical behavior” of skin.(113) The ages included in their study ranged from 30 to 80 years old.(113) With such acknowledgements, one should consider that skin differences within an adult population may be more related to the presence or absence of diabetes and less related to age differences, but certainly age could still be a factor.

Appendix C: Demographic survey for characterization study

Subject ID: _____

Date: _____

Date of Birth: _____

Gender: Male Female

Medical History:

HIGHEST LEVEL OF EDUCATION COMPLETED: (Select single best option)

- Some or No high school
- High School Diploma or GED
- Associates degree
- Bachelor's degree
- Graduate degree
- Other (please specify): _____

CURRENT OCCUPATION: (Select single best option)

- Paid employment
- Non-paid work, such as volunteer/charity
- Student
- Keeping House / Home Maker
- Retired
- Unemployed (health reasons)
- Unemployed (other reasons)
- Other (please specify): _____

RACE OR ETHNICITY: (You may select more than one option)

- Asian American
- American Indian / Alaskan Native
- Black / African American
- Native Hawaiian / Other Pacific Islander
- White
- Hispanic or Latino
- Other (please specify): _____

LIVING SITUATION:

- Lives alone
- Lives with spouse
- Lives with other family
- Lives with friend
- Caregiver support
- Other (please specify): _____

Subject ID: _____

Date: _____

FUNCTIONAL STATUS:

General activity level: (Select single best option)

- Sedentary (exercises \leq 1 days/week)
- Minimally Active (exercises \leq 2 days/week)
- Moderately Active (exercises \leq 3 days/week)
- Active (exercises $>$ 3 days/week)

Time spent in standing: (Select single best option)

- $<$ 25% of awake hours
- 25 to $<$ 50% of awake hours
- 50 to $<$ 75% of awake hours
- 75 to $<$ 100% of awake hours
- 100% of awake hours

Ambulation: (Select single best option)

- Ambulates $>$ 500 feet
- Ambulates $>$ 150 feet and $<$ 500 feet
- Ambulates 50 feet to 150 feet
- Ambulates $<$ 50 feet
- Ambulates with contact assist only

Activity within the last 2 hours prior to visit:

Additional Information: _____

Appendix D: ICC for characterization study

Table D.1 Average measures ICC values for TID

Location	Visits 1-2	Visits 1-3	Visits 1-4
Great Toe	0.83	0.74	0.31
1 st Met Head	0.71	0.74	0
3 rd Met Head	0.52	0.62	0
Lateral midfoot	0.27	0.05	0
Heel	0.77	0.62	0.29

Table D.2 Average measures ICC values for myotonometer

Location	Visits 1-2	Visits 1-3	Visits 1-4
Great Toe	0.89	0.80	0.82
1 st Met Head	0.89	0.87	0.79
3 rd Met Head	0.94	0.92	0.92
Lateral Midfoot	0.92	0.78	0.74
Heel	0.76	0.85	0.77

Table D.3 Average measures ICC values for US measures of the epidermis

Location	Visits 1-2	Visits 1-3	Visits 1-4
GreatToe	0.98	0.94	0.97
1 st Met Head	0.95	0.93	0.79
3 rd Met Head	0.95	0.84	0.73
Lateral Midfoot	0.90	0.75	0.76
Heel	0.94	0.95	0.96

Table D.4 Average measures ICC values for US measures of the dermis

Location	Visits 1-2	Visits 1-3	Visits 1-4
GreatToe	0.87	0.48	0.70
1 st Met Head	0.63	0.53	0.18
3 rd Met Head	0.68	0.68	0.61
Lateral Midfoot	0.69	0.40	0.36
Heel	0.69	0.63	0.63

Appendix E: Correlations of biological and skin measures

Correlation statistics between USG, Uosm, and BMI and skin measures (stiffness, compliance, and thickness) as measured in characterization study (Specific Aim #2)
 Correlations between biological measures and stiffness (TID):

Table E.1 Correlation between USG and stiffness (TID)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	-0.12	0.177	0.199	0.251
Sig (2-tailed)	0.924	0.163	0.114	0.046
N	64	64	64	64

Table E.2 Correlation between Uosm and stiffness (TID)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	-0.032	0.200	0.182	0.212
Sig (2-tailed)	0.802	0.113	0.150	0.092
N	64	64	64	64

Table E.3 Correlation between BMI and stiffness (TID)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	-0.052	-0.097	0.017	0.208
Sig (2-tailed)	0.681	0.445	0.895	0.100
N	64	64	64	64

Correlations between biological measures and compliance (myotonometer):

Table E.4 Correlation between USG and compliance (myotonometer)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	0.071	0.054	0.195	0.268
Sig (2-tailed)	0.575	0.669	0.123	0.032
N	64	64	64	64

Table E.5 Correlation between Uosm and compliance (myotonometer)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	0.036	0.067	0.159	0.257
Sig (2-tailed)	0.779	0.598	0.209	0.041
N	64	64	64	64

Table E.6 Correlation between BMI and compliance (myotonometer)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	0.149	0.010	0.271	0.192
Sig (2-tailed)	0.239	0.936	0.030	0.128
N	64	64	64	64

Correlations between biological measures and thickness (US--epidermis):

Table E.7 Correlation between USG and thickness (US--epidermis)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	0.273	0.278	0.162	0.134
Sig (2-tailed)	0.029	0.026	0.200	0.292
N	64	64	64	64

Table E.8 Correlation between Uosm and thickness (US--epidermis)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	0.281	0.284	0.184	0.177
Sig (2-tailed)	0.025	0.023	0.146	0.163
N	64	64	64	64

Table E.9 Correlation between BMI and thickness (US--epidermis)

	Great Toe (typical room)	Great Toe (EC)	First Met Head (typical room)	First Met Head (EC)
Pearson Correlation	-0.129	-0.166	-0.100	0.284
Sig (2-tailed)	0.310	0.191	0.430	0.023
N	64	64	64	64

Sample correlation scatterplots:

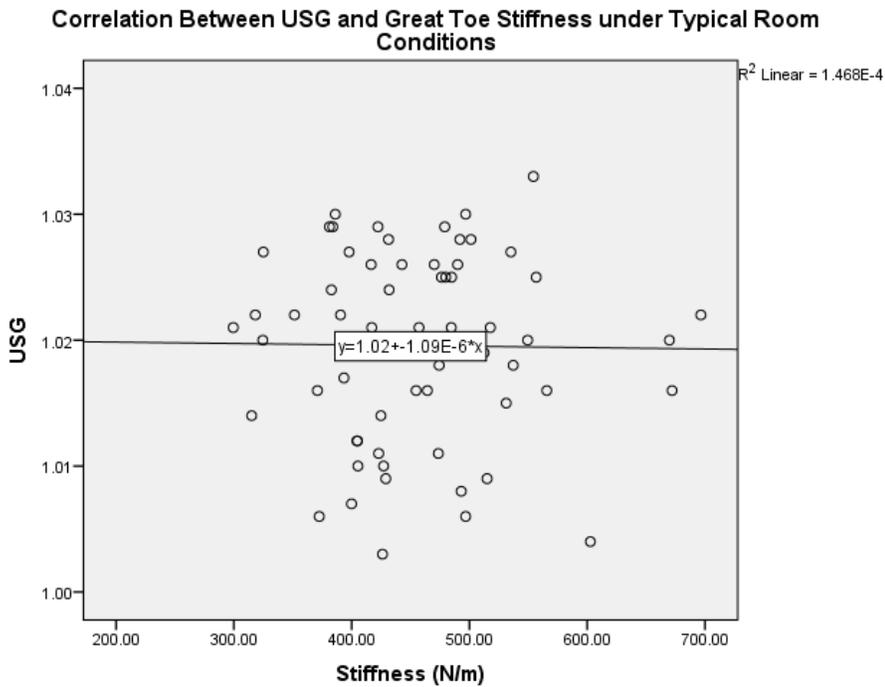


Figure E.1 Correlation between USG and great toe stiffness (TID)

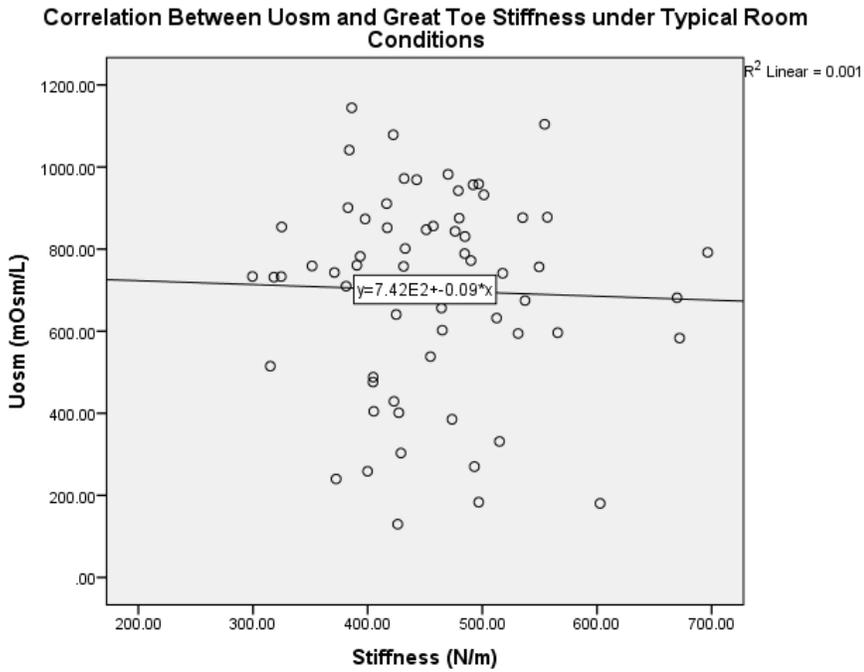


Figure E.2 Correlation between Uosm and great toe stiffness (TID)

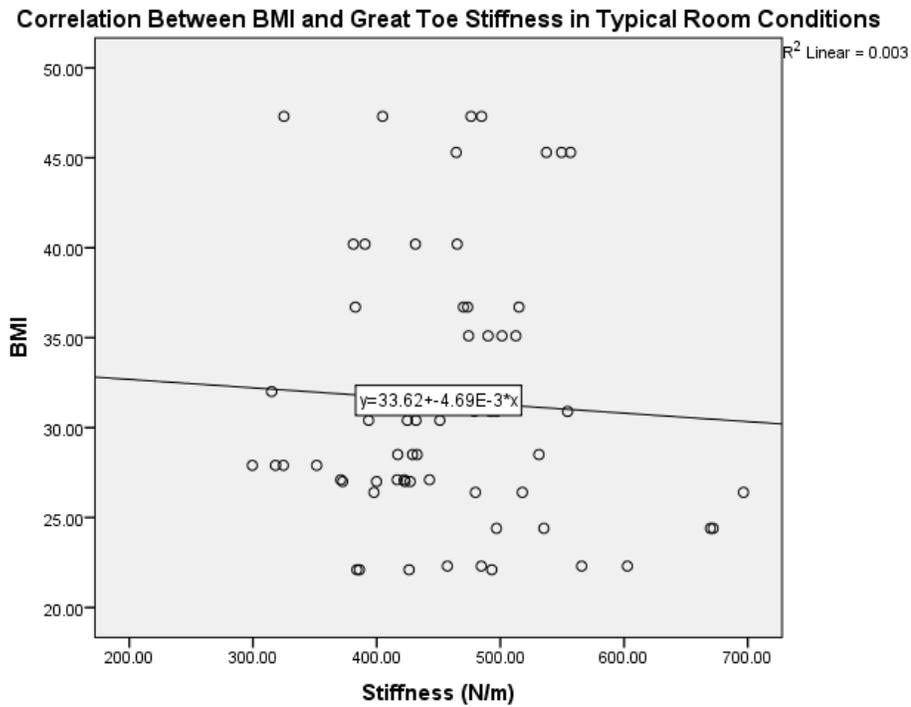


Figure E.3 Correlation between BMI and great toe stiffness (TID)

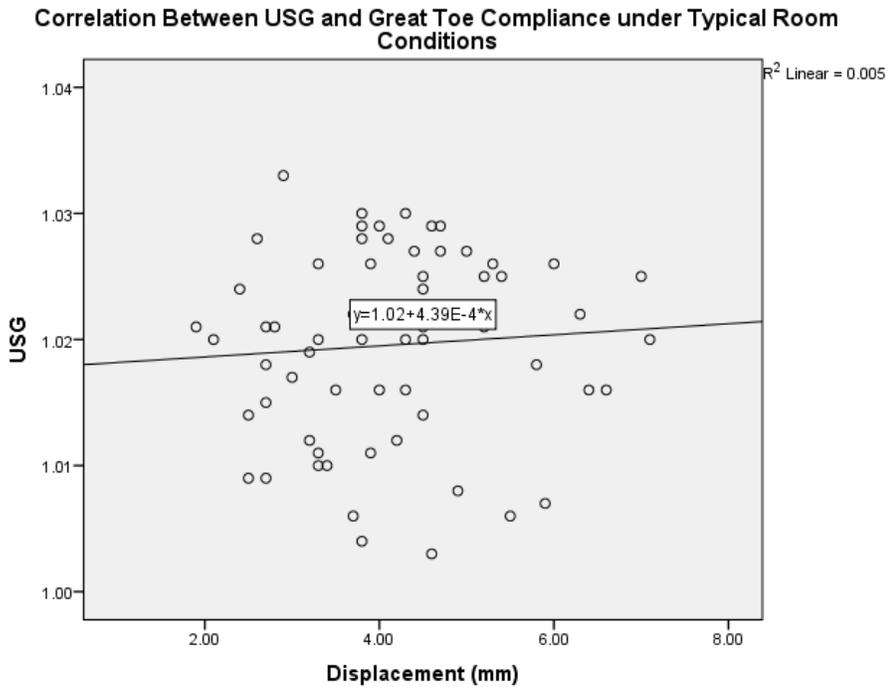


Figure E.4 Correlation between USG and great toe compliance (myotonometer)

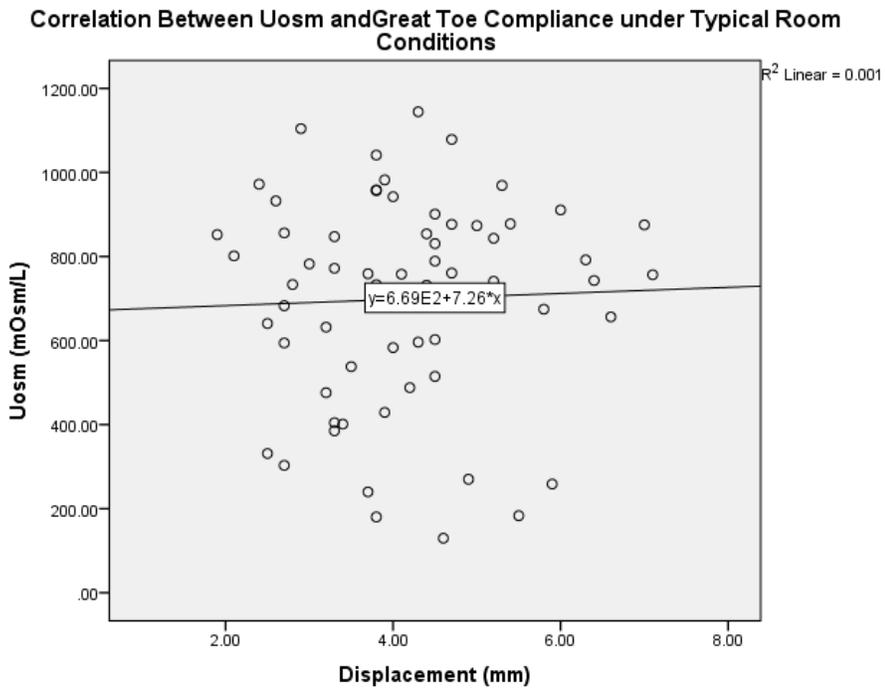


Figure E.5 Correlation between Uosm and great toe compliance (myotonometer)

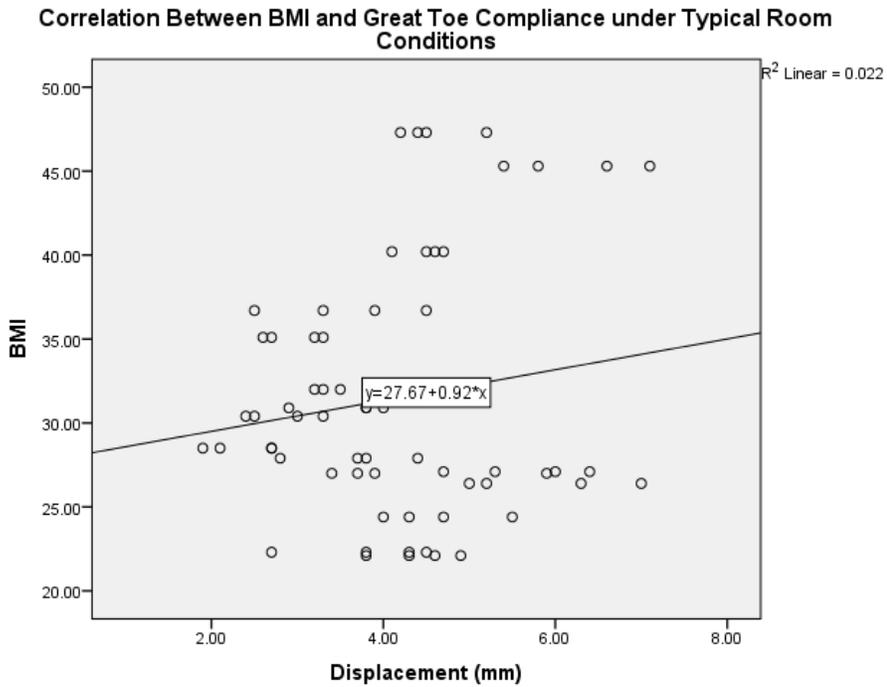


Figure E.6 Correlation between BMI and great toe compliance (myotonometer)

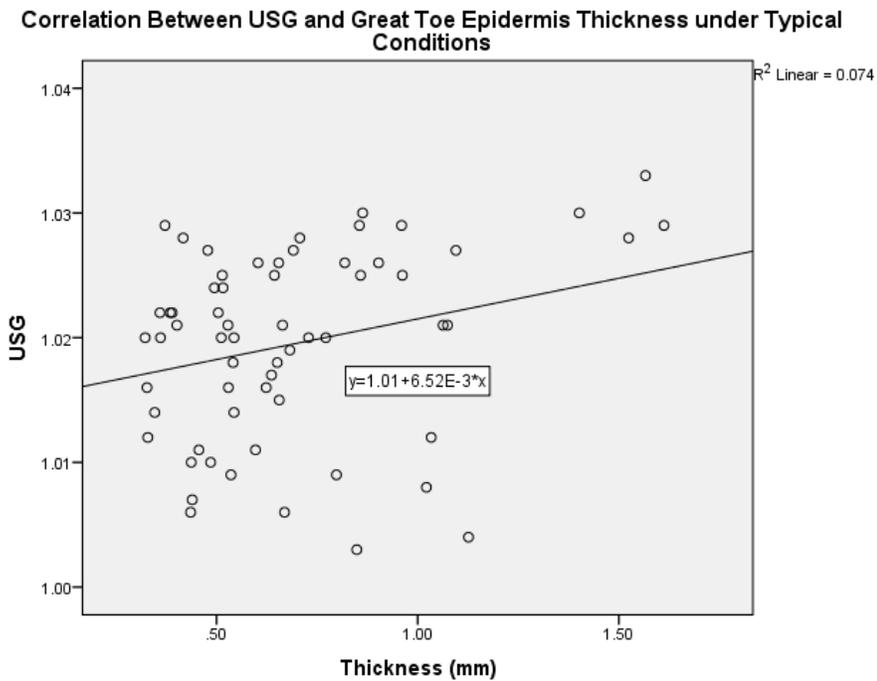


Figure E.7 Correlation between USG and great toe thickness (epidermis)

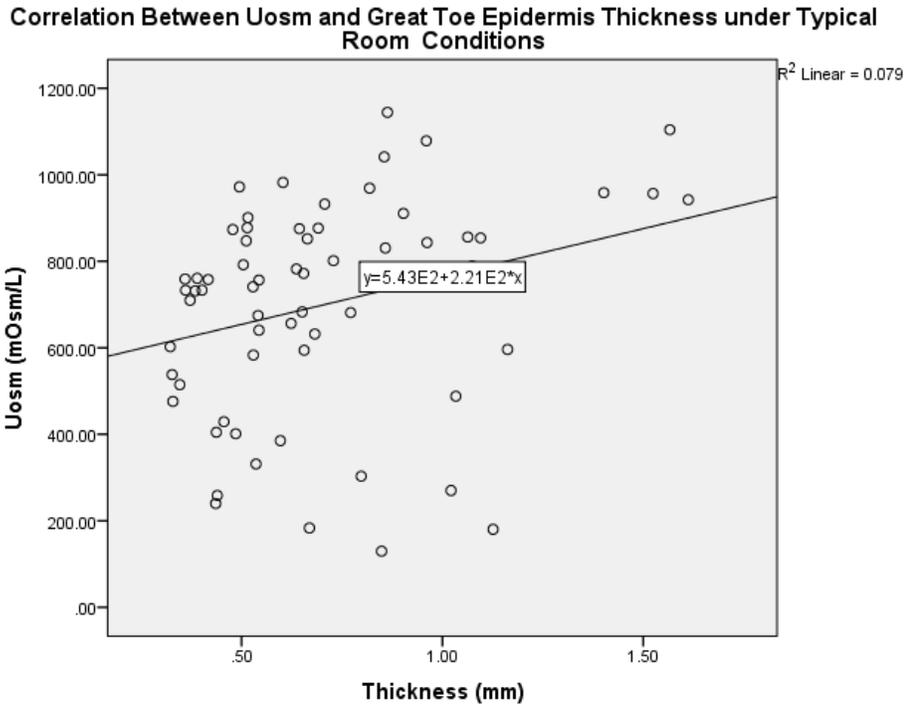


Figure E.8 Correlation between Uosm and great toe thickness (epidermis)

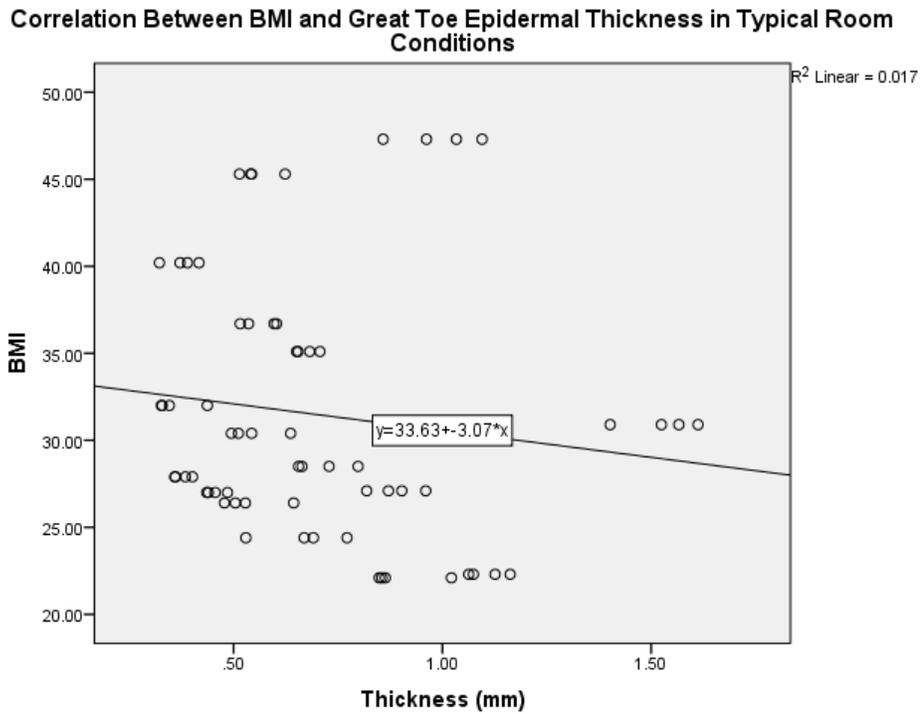


Figure E.9 Correlation between BMI and great toe thickness (epidermis)

Appendix F: Demographic survey for walking study

Subject ID: _____

Date: _____

Year of Birth: _____ **Current Age:** _____

Gender: Male Female

Medical History:

Allergies: Please include any allergy or sensitivity to glue, gel, or latex.

HIGHEST LEVEL OF EDUCATION COMPLETED: (Select single best option)

- Some or No high school
- High School Diploma or GED
- Associates degree
- Bachelor's degree
- Graduate degree
- Other (please specify): _____

CURRENT OCCUPATION: (Select single best option)

- Paid employment
- Non-paid work, such as volunteer/charity
- Student
- Keeping House / Home Maker
- Retired
- Unemployed (health reasons)
- Unemployed (other reasons)
- Other (please specify): _____

RACE OR ETHNICITY: (You may select more than one option)

- Asian American
- American Indian / Alaskan Native
- Black / African American
- Native Hawaiian / Other Pacific Islander
- White
- Hispanic or Latino
- Other (please specify): _____

FOOT CARE: Have you used in the last 6 months?

- Pumice stone
- Sandpaper/ File
- Pedicure
- Callus care by medical professional
- Callus care by another

Other (please specify): _____

Subject ID: _____

Date: _____

FUNCTIONAL STATUS:

General activity level: (Select single best option)

- Sedentary (exercises \leq 1 days/week)
- Minimally Active (exercises \leq 2 days/week)
- Moderately Active (exercises \leq 3 days/week)
- Active (exercises $>$ 3 days/week)

Time spent in standing: (Select single best option)

- $<$ 25% of awake hours
- 25 to $<$ 50% of awake hours
- 50 to $<$ 75% of awake hours
- 75 to $<$ 100% of awake hours
- 100% of awake hours

Ambulation: (Select single best option)

- Ambulates $>$ 500 feet
- Ambulates $>$ 150 feet and $<$ 500 feet
- Ambulates 50 feet to 150 feet
- Ambulates $<$ 50 feet
- Ambulates with contact assist only

Activity within the last 2 hours prior to visit:

Additional Information: _____

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