THE INFLUENCE OF SEASONAL VARIATION ON IN-SHOE TEMPERATURE AND RELATIVE HUMIDITY DURING MODERATE EXERCISE IN A MALTESE POPULATION: IMPLICATIONS FOR DIABETIC FOOT ULCERATION

by

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Abstract

Temperature and humidity (microclimate) are key factors implicated in the development of pressure ulceration, however, microclimate in-shoe has been relatively understudied in research related to diabetic foot ulceration (DFU). Additionally, the influence of ambient climate on these parameters in-shoe has also been overlooked. Such information is needed since footwear guidelines to prevent DFU commonly emerge from countries with cooler climates and it is not known whether their application in warmer Mediterranean climates is beneficial.

Preliminary validation studies demonstrated suitability of the thermistors (ICC $r = 1$; Bland and Altman limits of agreement of -0.42^0C and 95% CI -1.96, 1.14) and relative humidity sensors (ICC $r = 1$; Bland and Altman limits of agreement of -0.6^0C and 95% CI -1.8, 0.6) for use in in-shoe measurement during ambulation when compared with the gold reference instruments. A reliable repeated measure of in vivo application during shod gait with a thermistor and RH sensor attached between first and second toe and beneath the navicular, was demonstrated. To assess influence of season on in-shoe microclimate, 14 healthy participants walked for 38 minutes on a treadmill in winter and in summer, establishing normative data which was then compared with data from diabetic participants (n=5) using the same protocol.

Results demonstrated that seasonal variation has a significant influence ($p < 0.01$) on in-shoe temperature, while no difference was exhibited on in-shoe RH kinetics ($p > 0.05$). It has been demonstrated that after 20 minutes of walking in Summer, in-shoe skin parameters exceeded 30^0C and 70% RH in both healthy and DM participants, levels previously stipulated as indicative of unfavourable parameters to skin resilience in other areas of the body.

Therefore, this study provides new Mediterranean-relevant evidence related to in-shoe temperature and RH kinetics during activity, suggestive of negative implications to tissue viability, and also highlighting the need for more climate-specific guidelines related to the use of closed footwear, prescribed to prevent diabetic foot ulceration. It is hoped that this novel information will increase awareness on high in-shoe temperature and RH levels, as potential and influential factors within the pathway of diabetic foot ulceration, in Malta and countries with similar climates.

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This thesis is dedicated to my family and friends who have supported me and helped me succeed. There is not enough space in this thesis to express my appreciation.

To my wife Anabelle and daughter Katrina

This work is for you. I will always take care of you and will be always present.

Love you

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Abbreviations

A

AA	After Adjustment

B

BA	Before Adjustment
BMI	Body Mass Index

C

CI	Confidence Interval

D

DFU	Diabetic Foot Ulceration
DM	Diabetes Mellitus

G

GEE	Generalized Estimated Equations

I

ICC	Intra-class Correlation Coefficient

M

MCCAA	Malta Competition and Consumer Affairs Authority - Standards and Metrology Institute Malta
MTPJ	Metatarsophalangeal Joint

P

p	Statistical significance
PAD	Peripheral Arterial Disease
PRT	Platinum Resistance Thermometer

R

RCT	Randomized Control Trial
RH	Relative Humidity
RH_{FF}	Relative Humidity – Toe Area
RH_{Nav}	Relative Humidity – Arch Area
RH_{Ref}	Reference Platinum Resistance Thermometer
RPE	Rate of Perceived Exertion
RTD	Resistance Temperature Detectors

T

T_{FF}	Temperature – Toe Area
T_{Nav}	Temperature – Arch Area
T_{Ref}	Reference Standard Precision Hydrometer

Chapter 1

Introduction

1.1 Background to the Study

Diabetes Mellitus (DM) is a serious, chronic metabolic disease, which is increasing in prevalence worldwide at an alarming rate (Mehta, Karki et al. 2006, Nather, Bee et al. 2008). In 2009, 347 million individuals were suffering from Diabetes Mellitus, and it is predicted that there will be 500 million cases by 2030, which equates to a prevalence of 5.4% of the global population (Shaw, Sicree et al. 2010). In Malta, there is a distinctly high prevalence, of approximately 10%, which ranks the island as a country with one of the highest frequencies in Europe (Federation, Atlas 2013). Globally, DM is the fifth leading cause of mortality with 32 million deaths per annum (Roglic, Unwin et al. 2005, Federation, Atlas 2013).

Patients with diabetes are at risk of developing foot ulceration, which can lead to serious complications (including amputation) with significant impact on the healthcare system (Kerr, Rayman et al. 2014). Diabetic foot ulceration remains one of the most common complications of diabetes, having an annual incidence rate of 2% per year in European countries (Prompers, Huijberts et al. 2008b), with studies suggesting that diabetic patients have a 25% lifetime risk of developing a foot (Singh, Armstrong et al. 2005). Diabetic foot ulcers take time to heal and during this time patients require specialist continuous care. The ulcer may become complicated by infection and can lead to gangrene which would require long hospital stays and, in the most serious cases, amputation (Boulton, Vileikyte et al. 2005, Frykberg, Lavery et al. 1998, Edmonds, Bates et al. 2000). Furthermore, foot ulceration may have a significant impact on the quality of life of the sufferer due to reduced mobility, impairment of the ability to perform simple everyday tasks, absence from work and reduction in participation in leisure and social activities (Brod 1998, Nabuurs-Franssen, Huijberts et al. 2005, Sekhar, Thomas et al. 2015).

The development of diabetic foot ulceration (DFU) is thought to be a multi-factorial (Dinh & Veves, 2005), with various contributing factors acting in combination with each

other to initiate a process which results in ulcer formation. The primary contributing factors in the development of DFU are foot deformity, trauma, peripheral neuropathy and peripheral arterial disease. A universally (Boulton, 2013) accepted fundamental factor within the pathway to foot ulceration in diabetic patients is abnormal mechanical load applied to soft tissues in the form of pressure or shear, often due to ill-fitting footwear (Birke, Novick et al. 1991), generally over a bony prominence, resulting from a foot deformity (Pecoraro et al., 1990; Reiber et al., 1999). This abnormal load is not detected by diabetic patients because of neuropathy – a secondary effect of diabetes – which causes loss of sensitivity in the periphery, such as the foot (Vinik et al., 2003; Duby et al., 2004). Furthermore, the healing of any tissue breakdown and ulceration is impaired by poor tissue perfusion, due to peripheral arterial disease – another secondary effect of diabetes (Foster, Edmonds 2001).

The identification of the key contributing factors that exacerbate ulceration in this abnormal loading situation, has led to some significant research studies over the last 30 years, providing valuable insight into the mechanism of diabetic foot ulceration, with the ultimate aim of intervening to reduce ulceration rates (Birke, Novick et al. 1991, Pecoraro, Reiber et al. 1990, Boulton 2013). However, research investigating ulceration in other parts of the body (such as 'bed pressure sores') has also included measurement of temperature and relative humidity at the interface between the skin and supporting surface. This has been referred to as 'microclimate', and suggested as a key factor, having an important role in the pathway to tissue breakdown (Clark, 2010). However, the possible influence of these parameters (temperature and humidity) appears to have been overlooked in diabetic foot ulceration research and the identification of this gap in the literature highlights the need to investigate in-shoe temperature and humidity kinetics during activity and whether they might contribute to ulceration, particularly because diabetic patients are often prescribed closed therapeutic footwear as a preventive measure for diabetic foot ulceration. Findings about the in-shoe microclimate may influence advice to patients.

The use of 'appropriate' therapeutic footwear is recommended by various bodies including the International Working Group on the Diabetic Foot (Bus, Armstrong et al. 2016), with the aim of reducing ulceration risk, despite limited clinical evidence supporting the efficacy of such footwear (Bus, Valk et al. 2008, Maciejewski, Reiber et al. 2004). The term 'appropriate footwear' is often understood to mean, both by the practitioner and the patient, as a closed shoe, made of soft leather (Tulley 2008). However, there is a paucity of information regarding the use of such footwear in a typical Mediterranean climate, where there are high ambient temperatures and humidity levels, with the majority of relevant publications emerging from studies in cooler temperate climates.

1.2 The Maltese Context Relevant to People with Diabetes.

The origin of the work of this thesis emerged from personal clinical experiences of patient need, and awareness of a gap in the literature around the contribution of in-shoe temperature and humidity associated with closed therapeutic footwear, to diabetic foot ulceration. Diabetes mellitus (DM) is a major health care concern in Malta because of the elevated prevalence of diabetes in this population. The DECODE study group (2003) established that age-specific prevalence of diabetes was higher in Malta than in other populations and epidemiological studies indicated that currently 10% of the Maltese population suffer from DM, compared to 2-3% prevalence in neighboring European countries (WHO, 2012). Additionally, Malta leads the EU in having one of the highest overweight and obesity rates (22% of the population is obese while a further 36% are overweight), according to the Department of Health Information and Research report (2008). It also ranks third highest for obesity in Europe (after Andorra and Turkey), confirming previous studies that Malta tips the weighing scales beyond the healthy levels, which further predisposes this population to a higher risk of developing diabetes mellitus.

The high prevalence of DM together with increased predisposing factors to this disease in Malta, have led the health authorities and the Government to put prevention of complications associated with diabetes high in the priority list, within the current healthcare system (Directorate for Health Information and Research 2014). The current Maltese health care system has been in place since the 1970s when the government had introduced primary care clinics in addition to secondary care, both of which are funded by central taxation and are free at point of delivery for all Maltese citizens. Since then both government and health authorities have sought to improve standards of health care and at the same time have encouraged health professionals enhance their evidenced-based practices by improved academic study to underpin their work. Since Malta joined the European Union (EU) in 2004, improvement of standards has been mandatory in all areas and health authorities are striving to achieve an improved health service. Together with other European countries, Malta follows international recommendations associated with DM care and prevention of foot ulceration, with the free provision of therapeutic footwear to all individuals living with DM.

Therapeutic footwear is typically a closed shoe with special features such as a 'high toe box' prescribed with the intention of protecting the insensate foot from trauma which may lead to skin breakdown (Cavanagh 2004). Despite this, diabetic foot ulceration and amputation are still occurring at high rates in Malta. This was explored through a scoping study (Appendix II) conducted in the local general hospital in Malta. Retrospective analysis of medical records from patients who were referred to the hospital due to diabetic foot ulcerations revealed 872 referrals over 24 months, with the most common location being the hallux, accounting for 26% of ulcerations, with 38% of foot ulcers resulting in amputation. The results from this scoping study indicated that effective foot ulcer prevention is not being achieved despite free optimal healthcare and footwear to all patients. As suggested in the literature (Maciejewski, Reiber et al. 2004), the current efficacy of diabetic therapeutic footwear is therefore brought into question. Additionally, through personal clinical experience, humid hosiery was often observed when patients

attended the clinic wearing closed footwear (especially in summer), hence suggesting that the clinical recommendations for this footwear, emerging from cooler climates, might not be transferrable to countries having a distinct Mediterranean climate, like Malta.

Malta is an island located in the Mediterranean Sea, south of Sicily (Italy), having a distinct subtropical Mediterranean climate with very mild winters and warm to hot summers (see Figure 1.1). Rain occurs mainly in winter, with summer being generally dry. The summer climate generally starts from around mid-April to mid-November with temperatures rising to an average of 31^0C during July and August. Figure 1.2 shows the variability of the relative atmospheric humidity over the Maltese Islands (Galdies 2011). The annual average relative humidity is 73%, which varies from a minimum of 61 per cent in July to a maximum of 87 per cent in January. In such summer climates, clinicians in Malta still follow recommended guidelines and advise the use of closed therapeutic footwear. To date, there is currently limited research investigating the influence of ambient climate on in-shoe temperature and humidity when wearing closed footwear, neither in healthy individuals nor in individuals with DM.

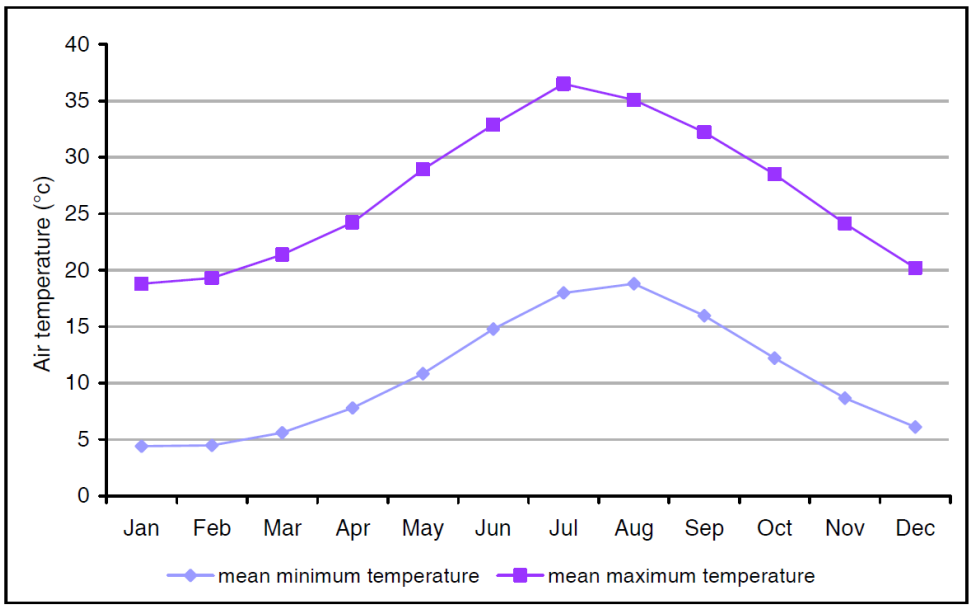


Figure 1.1: Malta's mean minimum and maximum air temperature based on the 30-year climate (Galdies 2011)

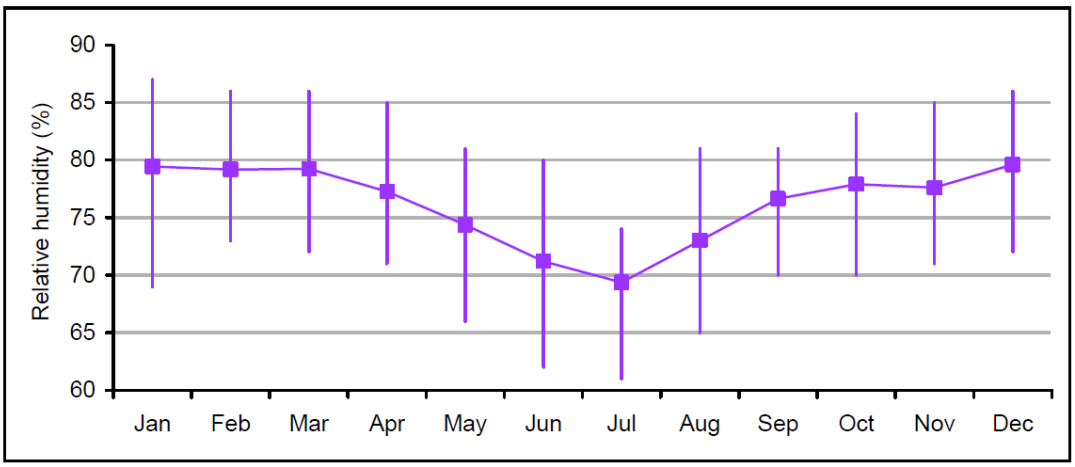


Figure 1.2: Malta's monthly means and variability of the relative humidity, based on the 30-year climate (Galdies 2011)

Therefore, this study aims to investigate in-shoe microclimate, namely temperature and humidity during ambulation and the possible influence of ambient climate on these parameters. It is hoped that this research will help fill the knowledge gap which is currently missing in research related to in-shoe temperature and humidity and perhaps provide additional information to better understand whether these parameters may have a role in the development of diabetic foot ulceration.

1.3 Research Question

The experimental studies presented in this thesis were planned after carrying out a review of the current literature (Chapter 2), which identified a knowledge gap associated with in-shoe temperature and relative humidity (RH) and the influence of seasonal variation on these parameters. Therefore, the following research question was developed:

Does seasonal variation have an influence on in-shoe temperature and relative humidity during moderate exercise in a Maltese population?

1.4 Aims and Objectives

The purpose of this study is to explore in-shoe temperature and RH kinetics during ambulation, which may in turn help in better understanding the tissue response to various in-shoe mechanical forces, which have been implicated to be detrimental factors in the development of diabetic foot ulceration (DFU) in high risk patients, as discussed in Chapter 2. This study therefore **aims**:

- To establish in vitro and then in vivo with healthy volunteers the validity and reliability of RH and temperature sensors to be used in a novel application to measure in-shoe variables at the interface between the shoe and the skin (Chapter 5).
- To establish normative data of in-shoe temperature and in-shoe RH levels during 38 minutes of moderate exercise in summer and winter (Chapter 6)
- To investigate the influence of seasonal variation on the in-shoe temperature and RH levels during ambulation in a healthy (Chapter 6) and a diabetic participant group (Chapter 7).

Objectives:

- To measure in-shoe temperature and relative humidity (RH) in a healthy and diabetic participant group during ambulation in summer using previously validated instrumentation.
- To measure in-shoe temperature and relative humidity (RH) in a healthy and diabetic participant group during ambulation in winter using previously validated instrumentation.
- To assess and compare seasonal variation on in-shoe temperature and RH kinetics during ambulation between a healthy and diabetic participant group.

The flow-chart below illustrates the organizational pathway employed within this thesis. It illustrates the methodological process of the studies undertaken in preparation for the main study and how conclusions were derived in order to answer the research question posed in this thesis.

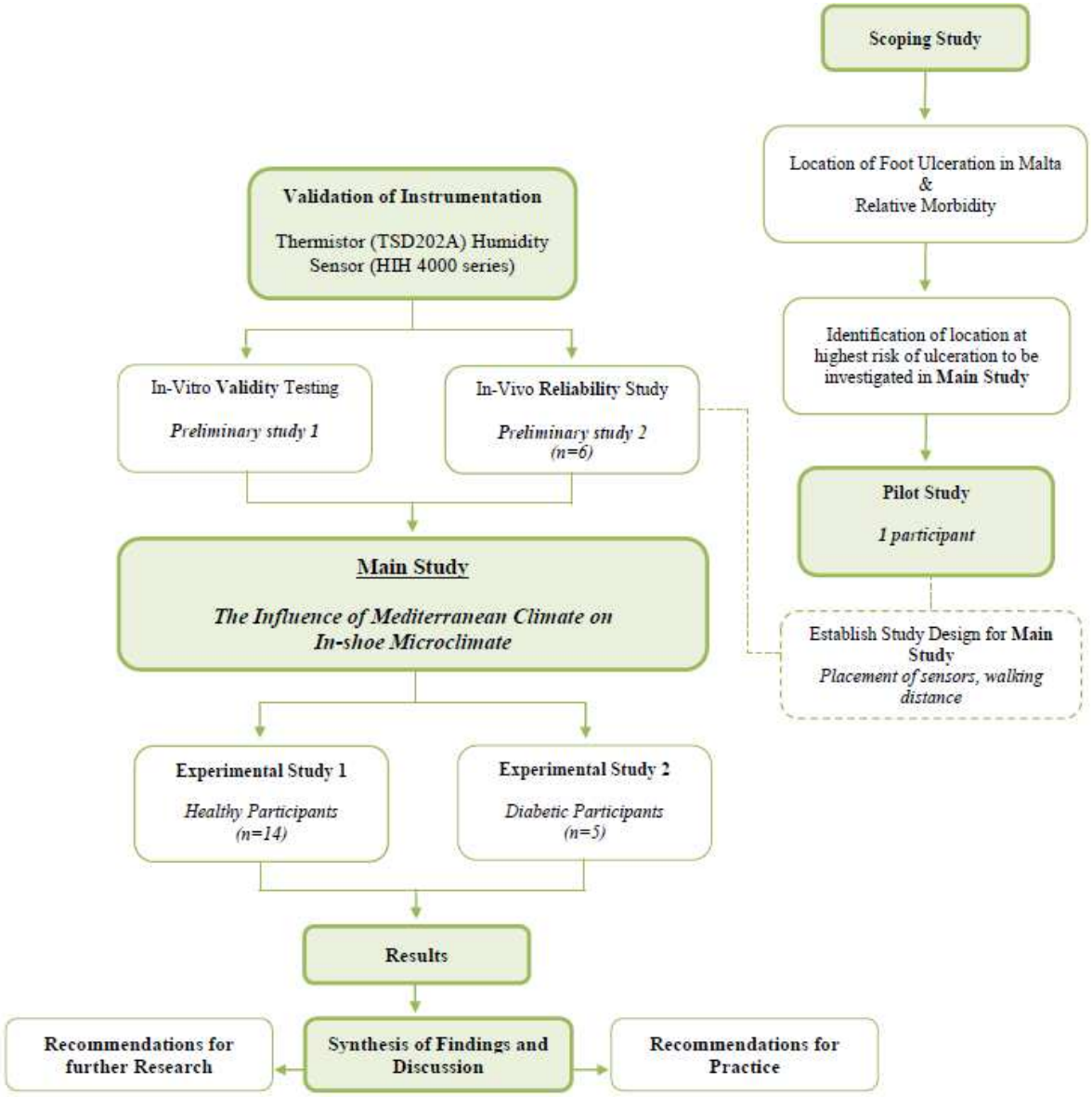


Figure 1.3: Organizational pathway employed within this thesis

1.5 Structure of the Thesis

This thesis has eight chapters. **Chapter 1** has introduced the research context related to diabetic foot ulceration, including the local picture. Research question, aims and objectives of the study were also presented.

Chapter 2 entails a thorough and critical review of the literature. This includes an overview of the pathway of diabetic foot ulceration with particular emphasis on temperature and relative humidity as risk factors in pressure ulceration and their potential involvement in the diabetic foot in-shoe.

Chapter 3 explains methodological considerations and describes methods and processes applied for this research. The first part discusses methodological understanding and philosophical perspectives, which provide epistemological and methodological insights for this research. The second part describes the research design, research methods and research processes used for this research.

Chapter 4 presents a detailed description of each instrument used during data acquisition which includes technical considerations and the justification for the choice of such equipment. A detailed protocol that was used in all of the subsequent experiments was presented.

Chapter 5, Chapter 6 & Chapter 7 contain the experimental chapters designed to meet the objectives of this thesis. These chapters are written as independent manuscripts which include a formal introduction, methods, results and discussion. The content of these chapters range from describing validity and reliability studies on specific sensors (Chapter 5) to the application of these sensors in healthy (Chapter 6) and diabetic participant groups (Chapter 7).

Chapter 8 presents a discussion of findings and associated clinical implications. It also proposes directions for future research, study limitations and concludes this thesis.

Chapter 2

Literature Review

2.1 Introduction

The foot is an efficient anatomical structure, bearing mechanical loading from the body weight during gait, thereupon causing the skin of the foot to be subjected to continuous stress even during sitting or lying (Gefen 2003, Gefen 2010), making it susceptible to injury. Such injuries can lead to ulcerations which precede 85% of lower extremity amputations, establishing diabetic foot ulceration (DFU) as one of the most serious and costly complications of diabetes (Boulton, Vileikyte et al. 2005, Prompers, Huijberts et al. 2008a, Driver, Fabbi et al. 2010). Diabetic foot complications are a major economic concern not only in Malta but worldwide, and having a publicly funded national healthcare system, the Maltese government has recently put Diabetes and its complications as priority concern due to its economic and social impact (Directorate for Health Information and Research 2014). In particular, diabetic foot complications have major repercussions on the quality of life of the patient due to loss of mobility, loss of work, frequent hospital visits or admissions and reduction of social functions (Nabuurs-Franssen, Huijberts et al. 2005). This was evidenced in a local study (Galea, Springett et al. 2009) where the highest incidence of re-ulceration was among individuals of a working age (55-64 years) in whom foot deterioration can have a profound impact on the quality of life causing a huge burden on the family, economy and social welfare (Apelqvist, Ragnarson-Tennvall et al. 1995, Ragnarson Tennvall, Apelqvist 2004).

Once ulceration occurs, intensive care is required for healing as ulcers may become complicated by infection, gangrene and are a frequent cause of amputation associated with diabetes, after which, prognosis is poor with a 68% mortality rate after 5 years (Jeffcoate, Harding 2003, Larsson, Agardh et al. 1998). Current thinking is that prevention is key and relies on early identification of the risk factors to foot ulceration, possibly before the onset of clinical symptoms, in order to the avoid any triggering components such as abnormal mechanical loads during walking, which predispose the foot to tissue injury (Wu, Driver et al. 2007).

Diabetic foot ulceration is a complex, multi-factorial process which has been studied extensively over many years in an attempt to provide an understanding of the mechanism leading to tissue breakdown (Jeffcoate, Harding 2003). This is evidenced with the increased number of publications seen in recent years, where literature related to DFU has increased significantly over the past twenty-five years (Boulton 2008). Despite significant advances in research related to DFU risk factors and their prevention, high re-ulceration rates are still evident both locally (Galea, Springett et al. 2009) and internationally (Maciejewski, Reiber et al. 2004, Reiber, Smith et al. 2002), indicating the need to further explore the current understanding of the mechanism underpinning the development of DFU.

Diabetic foot ulceration has been central to a significant amount of research with the aim of improving the understanding behind the risk factors that contribute to tissue breakdown (Boulton 2008). While epidemiological study designs or case control studies are often employed, empirically derived causal pathways have been suggested to be more representative of the assembly of essential component factors which act together to result in diabetic foot ulceration (DFU) and possible amputation (Reiber, Vileikyte et al. 1999). Causal pathways include interactions between important component risk factors and critical exposures that may be difficult to identify in other study designs. An important example of this is an episode of minor trauma as a precedent to ulceration, which would be more difficult to identify in epidemiological or case control designs than in causal pathway studies. This approach also incorporates synergistic factors that may act concurrently and not sequentially in the pathway to foot ulceration and represents a more realistic view of both the causative component factors and the contributory factors in DFU. Therefore, in order to achieve a better understanding of the complex mechanisms leading to DFU, literature investigating causal pathways leading to ulceration or amputation was explored and analyzed.

2.2 Search Strategy and Selecting Relevant Literature

In this thesis, in depth searches of the literature were undertaken relevant to the topics covered. This section presents the search strategy that was used to find relevant literature related to diabetic foot ulceration and in-shoe microclimate, which is the main area of interest of this work. Although not presented, similar search strategies were applied to the other relevant areas discussed in this review.

A literature search in several electronic databases [Medline, CINAHL, Cochrane Library, Current Controlled Trials, Science Direct EMBASE and Pubmed] was conducted. Google scholar was also accessed as a public site for research. These databases were chosen since they offer a broad coverage of subjects relevant to life sciences, behavioral sciences, chemical sciences, and bioengineering, needed by health professionals and researchers engaged in clinical care and public health. The research was not limited to publishing time or study design in order to find all the relevant papers up to July 2016. Key words were selected by first identifying a list of terms relevant to the research topics. The search terms were chosen from both the researcher's experience in the specialised area, personal communication with medical colleagues and from preliminary background reading in this specific subject area. Synonyms, broader and narrower terms were then identified for each of the key words. A number of key words with Boolean terms (Table 2.1) were used to guide the search.

Table 2.1: Key words, Search Terms with combination of Boolean Terms
In-shoe AND temperature
In-Shoe AND humidity
In-shoe AND temperature AND diabetes
In-shoe AND temperature AND ulceration
In-shoe AND temperature AND tissue breakdown
In-shoe AND humidity AND diabetes
In-shoe AND humidity AND ulceration
In-shoe AND humidity AND tissue breakdown
Temperature AND humidity AND footwear
Temperature AND humidity AND therapeutic footwear
Temperature AND foot ulceration AND amputation
Humidity AND foot ulceration AND amputation

The search was limited to databases commonly used in the medical and health fields, and relevant to Malta: English, Maltese and Italian. Consulting primary sources was a priority; yet secondary sources were also utilised when necessary. It is recognised that relying on electronic databases will not accurately identify all the relevant studies, despite excellent search criteria or capabilities (Garrard, 2011). Therefore, references included within identified journal articles were also used to select any related articles that were not extracted in the search process. Current textbooks on diabetic foot ulceration, footwear, temperature and humidity, provided supplementary information on definitions, footwear/materials utilised as a preventive measure to diabetic foot ulceration (DFU). Other information particularly related to guidelines and international clinical recommendations were retrieved from relevant resources such as; Diabetic Foot Study Group (DFSG), International Diabetes Federation (IDF), International Working Group on the Diabetic Foot (IWGDF) National Institute for Health and Clinical Excellence (NICE), Diabetes UK and World Health Organisation (WHO).

Grey literature was identified by searching through personal databases of science research accrued over the years including unpublished material such as presentations, dissertations and pre-publication manuscripts. After the identification of the various relevant databases, key words were searched either singly or in combination to find the most appropriate and relevant literature. Broader Boolean terms were used when the search yielded very few references. This process resulted in a list of titles of journal articles, most containing abstracts and some full texts.

2.2.1 Inclusion and Exclusion Criteria for Information Sources

The titles and abstracts were screened for eligibility. The titles of the references were individually screened and placed in three general categories:

a) literature that is clearly relevant

b) literature that is clearly irrelevant

c) literature that may be relevant if more information is obtained

For this particular search example, the criteria (Table 2.2) were set to ensure a subject population that will enable the investigation of the set objectives stipulated in this literature review. Clearly set criteria also provides equal opportunity for inclusion and not to exclude any relevant research on the subject area and also ensures unbiased inclusion of literature. For this reason, only research investigating in-shoe microclimate was considered.

Table 2.2: Inclusion and Exclusion Criteria for Literature Review	
Inclusion Criteria	**Exclusion Criteria**
Diabetes Mellitus ➢ ulceration ➢ amputation In-shoe Microclimate ➢ temperature ➢ humidity Footwear Outcome measures: ➢ ulcer prevention ➢ ulcer healing ➢ comfort	Research related to: ➢ RA ➢ Charcot foot ➢ Non-human in-vivo Studies

Once the literature search was completed, duplicate articles were removed. The rest of the articles were screened through the title and abstract. Literature that was identified as irrelevant was immediately discarded while full-text articles of the remaining references was used as a second screening method. These were carefully read for more detail on the specifics of the article and its relevance for this thesis, as summarised in a literature matrix presented in Appendix I. This strategy yielded eight articles which were critically analysed in section 2.6. This process is illustrated in a flow chart overleaf (see Figure 2.1).

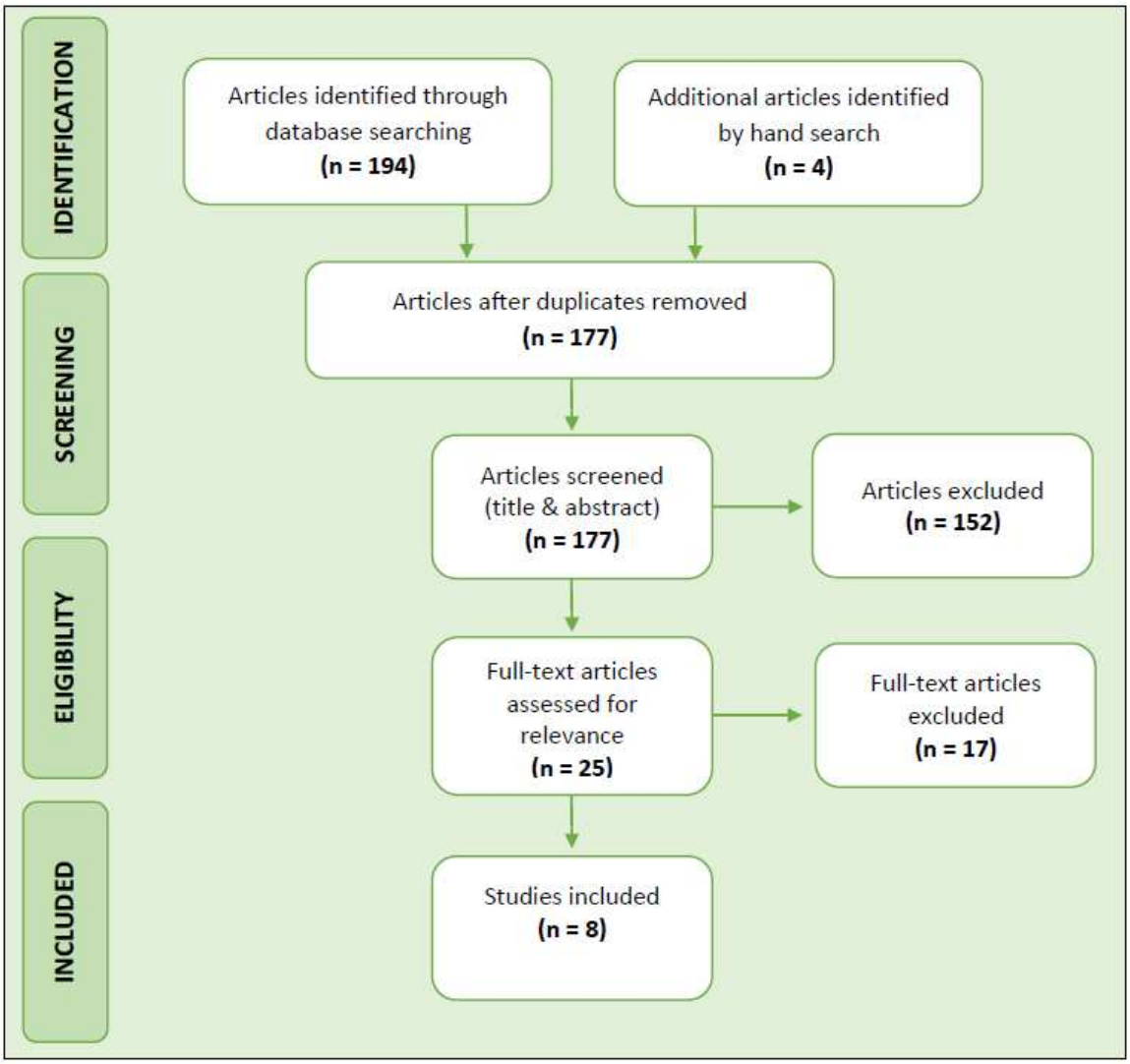


Figure 2.1: Flow diagram of the selection process for including articles in review. Adapted from Moher at al. (2009).

2.3 Causal Component Factors for DFU

When exploring the literature associated with DFU pathway (Reiber, Vileikyte et al. 1999, Jeffcoate, Harding 2003, Pecoraro, Reiber et al. 1990, Boulton 2013), a general consensus related to the most important causative factors and their interaction leading to DFU, can be observed. This pathway was proposed in the literature around 25 years ago (Pecoraro, Reiber et al. 1990), and has remained largely unchanged over subsequent years (Boulton 2013), suggesting that the DFU causal pathways that were proposed at that time, still hold. It was suggested that diabetic foot ulceration invariably occurs as a consequence of the interaction of several contributory factors together with specific abnormalities in the lower extremity acting in conjunction with environmental hazards, such as trauma from ill-fitting footwear (Figure 2.2).

The most common causes that have been identified in this pathway include peripheral neuropathy, foot deformity, external trauma and peripheral vascular disease (Boulton, Armstrong et al. 2008). These components commonly occur in the presence of one or more secondary contributing factors which may also have an impact but which are thought to be less prevalent in the pathway to DFU - such as elevated blood glucose, diabetes duration, oedema and knowledge of foot care (Reiber, Vileikyte et al. 1999, Boulton, Armstrong et al. 2008). For the purpose of this chapter, which is to underpin the current evidence relating to the factors which cause DFU, the causative and contributory components will be individually expounded below. It should be noted that literature reviewed to highlight the current knowledge associated with the pathway to DFU is derived from literature pertaining to diabetic foot ulceration. While both temperature and humidity have been shown to have important contributory effects within this pathway (evidenced in literature derived from other sources), such as a decrease in skin resilience to pressure with increased humidity and an unmet increase in metabolic demand with an increase in temperature associated with microangiopathy, these parameters notably appear to be overlooked in DFU research. Therefore, in order to highlight this knowledge gap, this chapter first presents the current known factors implicated in the pathway to DFU as

published in diabetic foot research, while the influence of temperature and humidity on ulcer development, derived from literature pertaining to other sources, namely pressure ulceration, is discussed in further detail in later sections (see Section 2.5) in this review.

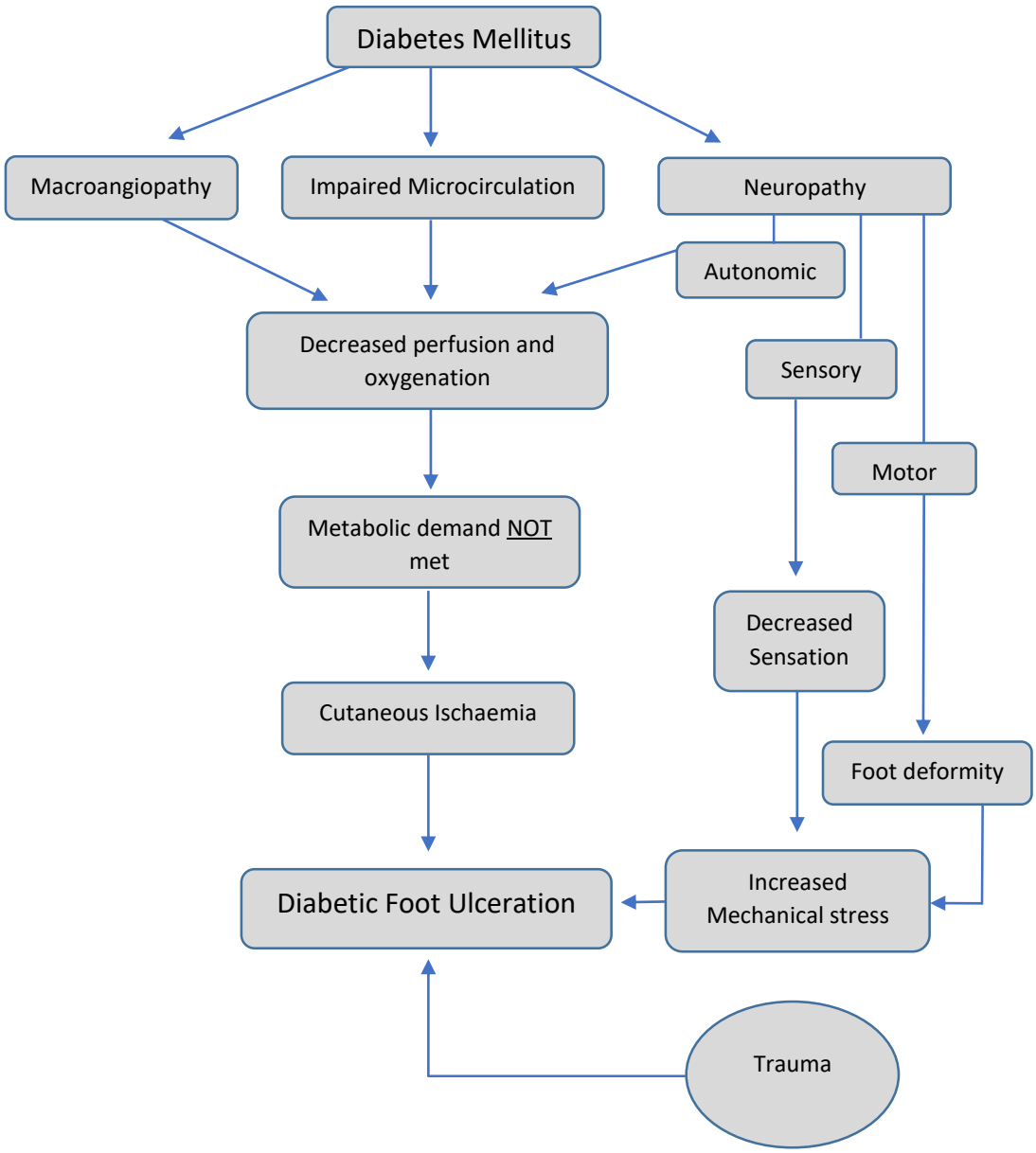


Figure 2.2: Pathogenesis of diabetic foot lesions adapted from Frykberg et al (2006).

2.3.1 Peripheral Neuropathy

Diabetic peripheral neuropathy is defined as peripheral somatic or autonomic nerve damage attributable solely to diabetes mellitus (Pinzur 2011). The two predictors for the development, severity and progression of diabetic peripheral neuropathy are; duration of diabetes and metabolic control (Tesfaye, Stevens et al. 1996). Although the mechanism for the development of peripheral neuropathy in diabetes remains unclear, the proposed process is thought to result from a combination of vascular disease, occlusion of the vasanervorum, endothelial dysfunction, deficiency of myoinositol- altering myelin synthesis and diminishing sodium-potassium adenine triphosphate activity, chronic hyperosmolarity and effects of increased sorbitol and fructose (Boulton, Kirsner et al. 2004). This process is relatively slow and is associated with chronically elevated serum levels of glucose which results in the irreversible binding of high levels of blood glucose to various proteins, producing glycated proteins or glycosylated haemoglobin that precipitate in the walls of small arterioles (Pinzur 2011).

Symptoms of diabetic neuropathy are predominantly sensory and typically include altered temperature sensation, paraesthesia and hyperaesthesia in a stocking-like distribution (Kazamel, Dyck 2015). Diabetic peripheral sensory neuropathy is present in approximately 60% of patients with diabetes (van Dieren, Beulens et al. 2010) and 80% of patients with diabetic foot ulcers. It has long been recognised as the most important component cause for foot ulceration (Reiber, Vileikyte et al. 1999) due to the associated increase in vulnerability to physical and thermal trauma (Singh, Armstrong et al. 2005). This was evidenced by Young et al (1993) who investigated neuropathy as a risk factor for foot ulceration. Using a neurothesiometer, the authors defined that those patients with a baseline threshold above 25V were 7 times more likely to develop a foot ulcer. It is also estimated that 45% to 60% of diabetic foot ulcers are mainly due to neuropathy, while 45% of ulcers result from combined neuropathy and ischaemic factors (Boulton, Kirsner et al. 2004, Reiber, Vileikyte et al. 1999, Boyko, Ahroni et al. 1999, Abbott, Carrington et al. 2002).

It is widely accepted that peripheral sensory neuropathy leads to a lack of protective sensation and areas of trauma in the foot remain unnoticed by the patient due to insensitivity to pressure stress. This results in continued mechanical stresses, commonly due to repetitive minor trauma over bony prominences rubbing against ill-fitting footwear and persistent walking on the affected foot (Iraj, Khorvash et al. 2013). The continued unnoticed stresses due to peripheral neuropathy will trigger tissue response to mechanical stress which may be altered in diabetic patients as further detailed in section 2.4.4 below.

While sensory neuropathy causes reduced peripheral sensitivity, motor neuropathy has been associated with small muscle wasting and absence of ankle reflexes resulting in altered biomechanics of the foot and the development of foot deformity (Bus, Haspels et al. 2011). These structural changes have been shown to have an important role in the development of foot ulceration due to their strong association with plantar pressure increase (Ledoux, Shofer et al. 2005, Boyko, Ahroni et al. 1999, Bus, Maas et al. 2005, Mueller, Hastings et al. 2003). Additionally, diabetic autonomic neuropathy may cause sudomotor dysfunction resulting in abnormal sweating and dry skin (Amin, Doupis 2016). Autonomic neuropathy is also indirectly linked with DFU risk (Vinik, Maser et al. 2003) due to its association with thermoregulatory dysfunction and abnormal tissue perfusion. In this regard microvascular dysfunction is discussed in section 2.4.3.

2.3.2 Structural Foot Deformity

While advances in imaging techniques used in diabetic foot research have contributed to the understanding of bony configurations and plantar soft tissue structures, the pathogenesis of many foot deformities in diabetes is not well understood (Bus 2008). Structural foot deformity in diabetes has been thought to occur due to neuropathic changes causing decreased proprioception and intrinsic muscle weakness leading to flexion deformities of the toes and prominent metatarsal heads (Delbridge, Ctercteko et al. 1985,

Reiber, Vileikyte et al. 1999). Foot structure abnormalities have been associated with an increase in foot pressure and development of foot ulceration (Reiber, Vileikyte et al. 1999, Boyko, Ahroni et al. 1999). Hammer toe deformities and clawed toes were both associated with an increased risk of ulcer occurrence in the affected toes due to an associated increase of plantar pressure at the metatarsal heads (Ledoux, Shofer et al. 2005). However, the involvement of hallux valgus in the development of foot ulceration is still not clear despite its common occurrence in diabetes (Boyko, Ahroni et al. 1999, Ledoux, Shofer et al. 2005). Overall, these findings clearly indicate that foot structure changes in diabetes have clinical importance due to their implication with increased mechanical stress in DFU. In view of this, identification of foot deformity is key in the prevention of DFU and provision of accommodative closed footwear is recommended to avoid diabetic foot lesions caused by mechanical factors (Bus, Armstrong et al. 2016) which can be precursors to DFU as further explained below (see section 2.3.3). However, wearing closed footwear in warm climates may give rise to additional parameters, such as increased in-shoe temperature and humidity. These parameters may also need to be considered due to their influence on tissue tolerance to mechanical stresses within the shoe occurring over bony deformities (see section 2.5).

2.3.3 Mechanical Factors

Mechanical stress has long been established as a major component in the pathway to DFU and has been associated with foot deformity (Lavery, Armstrong et al. 2003, Bus, Maas et al. 2005), limited joint mobility (Birke, Franks et al. 1995, Zimny, Schatz et al. 2004) and altered biomechanics (Boulton 2013, van Schie 2005). Several researchers have explored mechanical stress in terms of plantar pressure and diabetic foot ulceration, reporting increased pressures in ulcerated feet both retrospectively (post ulcer formation) and prospectively (Pham, Armstrong et al. 2000, Veves, Murray et al. 1992). It has been suggested that a pressure of 355 kPa in the forefoot denotes increase ulceration risk when

in conjunction with other contributory factors namely neuropathy and foot deformity (Fawzy, Arafa et al. 2014), although others concluded that there is no optimal cut-point of peak pressure for clearly screening patients for ulceration risk (Armstrong, Peters et al. 1998). Other authors (Yavuz, Botek et al. 2007, Pai, Ledoux 2012) have also investigated shear stress, indicating that shear locations may also be at a high risk of ulceration. However, the inherent difficulty and lack of technology available to measure shear in-shoe (Perry, Hall et al. 2002, Davis, Perry et al. 1998) make it difficult to clearly define the distinct contributions between shear and pressure in the development of DFU.

The role of mechanical stress in the pathway of foot ulceration has been explained by the following mechanism: when mechanical stress is applied to the skin, such as the continuous in-shoe stress sustained by the skin of the foot during activity, transient local ischaemia may occur due to mechanical occlusion of the microvasculature of the skin (Jan, Shen et al. 2013a). In the healthy individual, a protective response to such repetitive mechanical stress is reactive hyperaemia, which is mediated by myogenic responses inherent of vascular smooth muscles (Rossi, Bertuglia et al. 2005, Wong, Wilkins et al. 2003, Brienza, Geyer et al. 2005). While the mechanism is not clearly understood, it has been suggested that reactive hyperaemia in patients with diabetes is impaired, resulting in transient functional ischaemia. This is expressed as an impaired ability in microcirculatory function to vasodilate in response to stress or injury. This impairment of microvascular reactivity to mechanical stress has been demonstrated as a main cause of ischemia of the diabetic foot (Boulton, Armstrong et al. 2008, Burns, Jan 2012, Schramm, Dinh et al. 2006, Chao, Cheing 2009, Arora, Pomposelli et al. 2002).

Abnormal patterns of pressures and forces under the diabetic foot have been recognised as an important component cause of DFU and have been thoroughly investigated, particularly to identify methods of off-loading susceptible areas with the use of insoles to prevent repetitive stresses which may lead to ulceration (Singh, Armstrong et al. 2005, Murray, Young et al. 1996, van Schie 2005). These stresses often described in terms

of shear, pressure and friction may result in specific tissue response which is explained in further in detail later in this chapter.

2.3.4 Minor Trauma

In literature investigating the pathway to DFU, minor trauma, which may be caused by repetitive stress refers to areas where the foot is exposed to mechanical forces over time (Reiber, Lipsky et al. 1998). It is an important component cause and was found to be present in 77% of ulcer pathways (Reiber, Vileikyte et al. 1999) and 81% of causal pathways leading to amputation (Pecoraro, Reiber et al. 1990, Reiber, Smith et al. 2002). The mechanisms by which repetitive stress results in tissue breakdown is further explored later in this chapter as it involves complex tissue response mechanisms which may be altered in diabetes. Shoe-related minor trauma has been reported to be a frequent event leading to foot ulceration and amputation (Apelqvist, Larsson et al. 1990, Edmonds, Nicolaides et al. 1986), in the casual pathway developed thirty years ago. This pathway is still accepted today (McBride, Hacking et al. 2016) although the added risk of altered biomechanics of the foot and skin tissues, coupled with altered microvascular function in diabetes, as established in more recent studies (Schramm, Dinh et al. 2006) are known to make areas of minor trauma or stress at an increased risk of tissue breakdown. These contributing factors which predispose the skin to ulceration when subjected to minor repetitive stresses such as those experienced in-shoe, are explained in further detail later in this chapter. Footwear advice and therapeutic footwear are therefore recommended in an attempt to mitigate stresses within the shoe although their efficacy in reducing ulceration has not yet been established (Reiber, Smith et al. 2002, Maciejewski, Reiber et al. 2004, Bus, Valk et al. 2008).

In Malta, anecdotal evidence and clinical experience have shown that despite the use of recommended footwear, areas of increased mechanical stresses often develop blister-like macerated lesions, more commonly observed during warm summer months.

These areas of minor trauma often precipitate ulceration increasing the risk of amputation, as seen in figure (Figure 2.3) below.

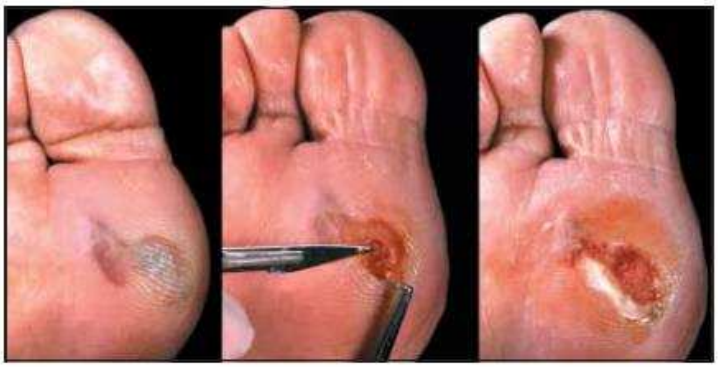


Figure 2.3: Blister formation with underlying ulceration (Edmonds, Foster 2006)

2.3.5 Peripheral Vascular Disease

Peripheral vascular disease has been identified as an important component cause in the development of DFU and this has been attributed to two systems (Hsu, Su et al. 2013) – Macroangiopathy, large vessel disease due to atherosclerosis, which is not specific to diabetes and microangiopathy, which is diabetes-specific. In macroangiopathy, the natural progression of atherosclerosis is similar between diabetics and non-diabetics, with difference occurring only in occlusion site, where involvement of infra-geniculate and tibial arteries are mostly evidenced in diabetes (Alexandrescu, Hubermont 2011). However, the cause of the site difference remains unresolved (Gabbay, Gabbay et al. 2014). Small vessel disease (microangiopathy) refers to alterations in structure and function of the microvascular system which has been implicated in tissue injury and increased risk of diabetic foot ulceration (Korzon-Burakowska, Edmonds 2006, Fiordaliso, Clerici et al. 2016, Flynn, Tooke 1992). Authors suggested that structural alterations in the small vessels, namely, thickening of basement membrane together with functional alterations predominantly attributed to microvascular dysregulation are implicated in the role of

peripheral arterial disease as a risk factor to DFU, which is further explored in the sections later in this review (see Section 2.4.3).

Peripheral vascular disease has been shown in 60% of diabetic foot ulcers cases (Trepman, Nihal et al. 2005). Large epidemiological studies have confirmed that peripheral vascular (PVD) disease is common in patients with diabetes, showing an incidence rate of 5.5 per 1000 type 1 diabetic patients and 13.6 per 1000 type 2 diabetic patients (Selvin, Erlinger 2004, McAlpine, Morris et al. 2005, Norman, Davis et al. 2006). Notably, literature suggests that PVD and neuropathy typically exist concomitantly with other contributing factors and DFUs are frequently neuroischaemic in nature (Ndip, Jude 2009). In the Eurodiale study investigating patients attending 14 different European hospitals in 10 different countries, findings suggest that ischaemia is increasingly more common in the pathogenesis of foot ulceration and neuroischaemic ulcers are the most common type to be seen (Prompers, Huijberts et al. 2008b).

2.3.6 Poor Glycaemic Control and Diabetes Duration

In literature investigating causal pathways to DFU, both poor glycaemic control and diabetes duration have been identified as contributory factors rather than direct causes, increasing the risk of ulceration (Mayfield, Reiber et al. 1998; Reiber, Lipsky et al. 1998). Patients presenting with diabetic foot ulcerations are associated with a history of prolonged diabetes combined with poor health condition. The average age of these patients is commonly over 65 years with diabetes duration of more than 10 years. Additionally, uncontrolled diabetes and an increased level of HbA1c have been associated with increased ulcer risk (Prompers, Huijberts et al. 2007).

Poor glycaemic control has been associated with the incidence of neuropathy (Selby, Zhang 1995) which in turn is a component cause as discussed above. Diabetes duration of 10 years or more has also been associated with DFU and was present in 36.8% of patients with foot ulceration. Also, a long term history of diabetes for more than 20 years has been associated with a six fold increase in the risk of foot ulceration compared to patients with a history of diabetes of nine years or less (Rith-Najarian, Stolusky et al. 1992). Additionally uncontrolled hyperglycaemia and duration of diabetes have been associated with risk of peripheral arterial disease, which has been evidenced as a main component cause in the pathway to foot ulceration (Amin, Doupis 2016).

Lower extremity complications in individuals living with diabetes have become an increasingly significant public health concern. These foot complications, commonly initiating with minor trauma and neuropathy in combination with other factors, predispose the foot to ulceration due to the resultant increase in mechanical stresses. Once mechanical stresses occur, a tissue response which is influenced by the mechanical properties of the tissue involved, is triggered as a natural reaction within the human body. This response is known to be altered in the diabetic foot as discussed in more detail below.

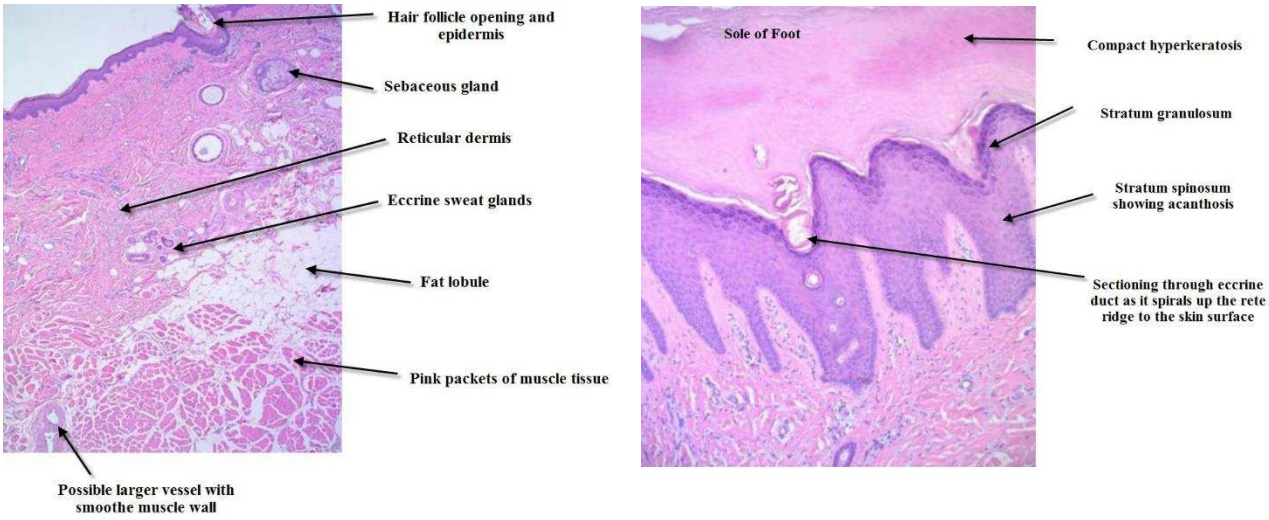
2.4 Behaviour of Skin Tissue in Response to Mechanical Stress

The mechanical properties and the response to mechanical stress of plantar soft tissues have been found to be altered in the diabetic foot (Pai, Ledoux 2010), and this has been proposed as an additional predictive factor of foot ulcer development in combination with other pathological conditions such as diabetic neuropathy and impaired micro vascular reactivity (Jan, Shen et al. 2013b).

A sound knowledge of normal skin structure and function is required for understanding the skin mechanical behaviour associated with the mechanism of injury and foot ulceration in diabetes and is therefore presented in the next section. The human skin is a complex tissue consisting of several distinct layers (refer to Figure 2.4), each having different properties influencing the biomechanics of the particular tissue (Sandby-Moller, Poulsen et al. 2003). A fundamental understanding of microvascular function in the skin are also important considerations as they may precipitate consequent clinical implications which are presented later in this section. This section will therefore focus on the understanding of tissue mechanics and response to stress within the context of diabetic foot ulceration whilst also acknowledging the role of in-shoe parameters which may be implicated in the mechanism of tissue injury.

2.4.1 Anatomy of Skin Morphology and Microcirculation

The skin of an adult human being is the largest organ of the human body with a total average surface area of $2m^2$ and weighing about 4kg (Edwards, Marks 1995). Human skin is multifunctional and consequently is an extremely complex structure, serving as a large protective barrier that protects the human being from environmental hazards such as ultraviolet (UV) radiation, temperature extremes, toxins, bacteria and mechanical forces (Misery 1997).



(a) **(b)**

Figure 2.4: Images of skin histopathology. (a) Hairy skin; (b) Glabrous plantar Skin

Skin is multilayered, mainly composed of the epidermis, dermis and hypodermis and the dermis often has indistinct interlayer boundaries with varying properties depending on function and location on the body (Smith, Holbrook et al. 1982, Ramshaw 1986); figure 2.4). There are two main kinds of human skin. Glabrous skin (non-hairy skin), is thick skin, with its characteristic dermatoglyphics, and is found on the palms and soles. It is characterized by a relatively thick epidermis (up to 1.5 mm), lack of hair follicles, and a dermal layer, purposely designed to provide cushioning and shock absorption particularly in the forefoot and heel regions, protecting the underlying bone and soft tissues during daily locomotion activities (Saltzman, Nawoczenski 1995). It has a high density of eccrine sweat glands and like palmar skin it is devoid of sebaceous glands. Hairy skin – thin skin (Figure 2.4a), on the other hand, covers most of the rest of the body including the dorsum of the foot, and it characterised by a thin epidermis and hair follicles with attached sebaceous glands and eccrine sweats glands (Springett, White 2002).

The epidermis is the outer layer (approximately 75-150 µm in thickness) consisting of keratinized stratified squamous epithelium, important in determining the mechanical strength of the skin, differentiated in four layers, the basal cell layer, the squamous cell layer, the stratum granulosum, and the stratum corneum. Keratinocytes moving from the basal layer, undergo the process of keratinisation (differentiation) as they progress through the different epidermal layers which results in continuous renewal of the skin surface through eventual shedding of small skin scales - desquamation (Marks, Barton et al. 2012).

Underneath the epidermis lies the dermis, also important in establishing the mechanical strength of the skin, determined by a dense structure of fibrous proteins, namely collagen, elastin and reticulin, forming the major mass of the skin with a total thickness of 1-4mm (Smith, Holbrook et al. 1982) and contributes to a 15-20% of the total body weight. The epidermis also contains Langerhans cells (antigen-presenting immune cells), a few nerve endings and pressure-sensitive mechano-receptors, Merkel cells, which are present in higher densities in hairless skin and play an important role in the provision of protective sensation from mechanical injury and stresses in the foot (Fradette, Godbout et al. 1995). These stresses and forces acting on the skin can occur as a result of external loading or from internal loading by bony prominences. In general, a load will act over a definite area, and the ratio of the magnitude of load to area is termed ‘pressure’.

The epidermis has no blood supply with nutrition provided by the papillary layer in the dermis. The dermis of hairless skin (glabrous skin) contains a large number of highly innervated arteriovenous (AV) shunts and plays a role in thermoregulation. These AV shunts are less dense in non-glabrous skin and blood flow plays a predominant nutritive role (Vinik, Erbas et al. 2001). The integrity of the capillary circulation has an impact on the viability of the skin, as exchange of nutrients and metabolites between blood and tissues occurs at the capillary level (Figure 2.5). Alterations and increased AV shunting has been evidenced in patients with diabetes and is suggested to have an important role in tissue breakdown due

to induced tissue hypoxia, since blood is shunted away from nutritive cutaneous capillary network (Fagrell, Jorneskog et al. 1999, Gabbay, Gabbay et al. 2014).

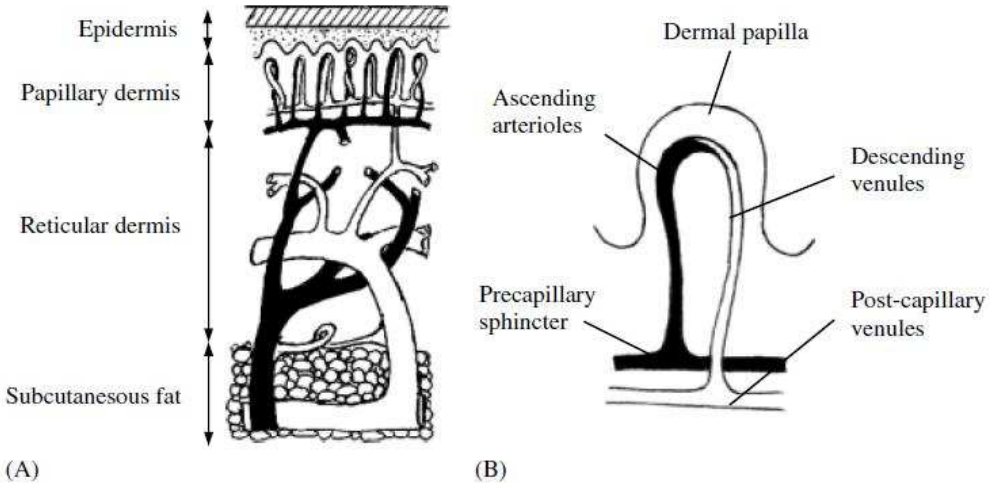


Figure 2.5: Schematic representation of the microvasculature in human skin. (A) Upper nutritive capillary loops and a lower thermoregulatory arteriovenous shunt circulation in the dermal layer of the skin (B) A single capillary loop inside a dermal papilla (Chao, Cheing 2009)

The normal structure and function of the skin are important features required to maintain skin resilience that enable it to withstand mechanical forces such as those sustained by the foot skin in-shoe during walking. However, there is ample evidence suggesting that diabetes mellitus is associated with alterations in skin biomechanics which have an effect on skin resilience, making it more susceptible to injury. These alterations are discussed in more detail below.

2.4.2 The Effect of Diabetes Mellitus (DM) on the Structure and Function of the Skin

The skin has unique biomechanical properties that allow it to protect and conform to the body. Mechanical properties of the skin vary considerably according to body site, age, race and gender (Hussain, Limthongkul et al. 2013). Mechanical skin properties also vary according to the rate of application of stress and length of time over which the stress is maintained and are very sensitive to ambient conditions (such as temperature and humidity), age and disease, including diabetes (Zhong, Xing et al. 2006). The following sections will discuss the altered biomechanical properties of skin and skin microvascular system in diabetes and explore their relevance with regard to diabetic foot ulceration.

2.4.3 Altered Microvascular Function in DM

The literature reports some differences in the microvascular system between healthy and diabetic individuals, although most of the microcirculatory differences are attributed to functional alterations rather than structural (Schramm, Dinh et al. 2006). While no significant histological differences exist in the skin microvascular density between healthy individuals and diabetic patients (Jaap, Shore et al. 1996), thickening of the capillary basement membrane in the microvasculature of the feet and reduced vasodilation has been evidenced in patients with diabetes (Schramm, Dinh et al. 2006, Dinh, Scovell et al. 2009).

Early research into wound healing in the diabetic foot suggested that thickening of the capillary basement membrane due to endothelial proliferation in arterioles, delayed both entry of essential nutrients into the wound and clearance of metabolic by-products (Siperstein, Unger et al. 1968). However, research that emerged in the ‘80s (Boulton et al., 1982; Edmonds et al., 1982) demonstrated evidence of hypoxia and capillary ischaemia in the presence of normal local blood flow. While causes of this clinical contradiction are not

fully understood, there is mounting evidence making a link between the dysfunction of the microvascular system (Khan, Elhadd et al. 2000, Jan, Shen et al. 2013b) with capillary and cutaneous hypoxia (Fiordaliso, Clerici et al. 2016, Gabbay, Gabbay et al. 2014).

Functional alterations causing impaired vasodilation are evident in both type 1 (Khan, Elhadd et al. 2000) and type 2 (Morris, Shore et al. 1995) individuals with diabetes. Diabetes mediated alterations have been evidenced by a few studies which have investigated skin vascular response and an extensive review of the altered cellular mechanisms associated with hyperglycaemia have been described by Gary Sibbald and Woo (2008). These alterations include the absence of C-Peptide produced in pancreatic islet β cells which is known to have nitric oxide dependent role in microvascular vasodilation (Forst, Kunt 2004), impairment in endothelium-dependent and endothelium-independent vasodilation even in the pre-diabetic state (Caballero, Arora et al. 1999, Gomes, Matheus et al. 2008, Khan, Elhadd et al. 2000) and reduced nitric oxide bioavailability required for vasodilation (Martina, Bruno et al. 1998). These changes have been strongly associated with poor glycaemic control and hyperglycaemia (Chan, Vallance et al. 2003, Rodríguez-Mañas, López-Dóriga et al. 2003).

Impaired vasodilation can also be manifested as a delayed vasodilatory response during thermoregulation (Kenny, Sigal et al. 2016). The microvascular system in the skin has important regulating mechanisms which have been extensively reviewed (Hodges, Johnson 2009, Charkoudian 2010, Holowatz, Thompson-Torgerson et al. 2010), stating that a complex regulatory system of the microcirculation that accounts for whole body and local regulation is mediated via long descendent autonomic fibres that include local reflexes within the skin, central reflex control and short reflex arcs through the spinal cord (Vinik, Erbas et al. 2001). The regulation of reflex and local vasomotion is controlled by humoral factors, responsible for local regulation of microvascular blood flow and neural factors responsible for vasodilation in 75 to 90% of the body (Charkoudian 2010). Humoral factors are produced in the endothelium and include nitric oxide, prostacyclin and endothelium

derived hyperpolarizing factor, responsible for vasodilation and thromboxane A2 and endothelin-1 responsible for vasoconstriction (Epstein, Vane et al. 1990).

The microvascular system has an important role both in body temperature regulation and in maintenance of skin integrity. When the skin is under mechanical stress causing temporary mechanical occlusion of the capillaries, reactive hyperaemia by vasodilation follows, to restore the required nutrients in healthy individuals. It has been suggested that due to impaired vasodilation this process might be impaired, contributing to the development of DFU (Stirban 2014).

2.4.4 Altered Biomechanical Skin Properties in DM

Diabetes Mellitus induces various forms of pathophysiologic changes in the skin derived from an impaired skin homeostasis in the dermis and epidermis thought to be caused by either secondary diabetic complications (neuropathy and/or vasculopathy) or abnormal metabolism (Sakai, Endo et al. 2003). Insulin resistance and hyperglycaemia in diabetes have a confirmed involvement in impaired structure and biomechanical properties in various tissues, altering their behaviour. In the case of skin, diabetes has been associated with collagen glycation (Kennedy, Baynes 1984) and inhibited keratinocyte proliferation (Wertheimer, Spravchikov et al. 2001) which have been implicated in causing the changes in skin biomechanical properties inhibiting the wound healing process (Paul, Bailey 1996, Yoon, Baik et al. 2002). Knowledge of the exact mechanism of deficient biomechanical properties of the skin in diabetes before ulceration (baseline) is limited since the majority of studies that investigated the biomechanics of the skin in diabetes have examined it in the ulcerated state (Bermudez, Herdrich et al. 2011). The few studies that have addressed baseline mechanical properties in diabetic human skin demonstrated significant changes when compared to the properties of healthy tissue, both at the epidermis and dermis (Bermudez, Herdrich et al. 2011, Hashmi, Malone-Lee et al. 2006).

The dermis has important biomechanical functions and most of the mechanical characteristics of skin are conferred by tough collagen-rich dermis. Its micro-structure is a three-dimensional complex network of collagen and elastin fibres (Alberts, Johnson et al. 2002) which provides two dimensional isotropic bulk properties. However, disease, age and exposure to sunlight may affect some of these physical properties in one way or another.

Solar radiation, which is particularly relevant in Mediterranean countries, induces extrinsic aging leading to a decrease in skin extensibility, elastic recovery (Springett, White 2002) and a marked decrease in collagen content (56% less) when compared to naturally aged skin (Hussain, Limthongkul et al. 2013). Even in healthy tissue, human skin undergoes major changes in its mechanical properties due to natural (intrinsic) aging alone. These include atrophy of the dermis due to loss of collagen by approximately 6% per decade (Diridollou, De Rigal et al. 2007), degeneration in the elastic fibre network and loss of hydration (Hussain, Limthongkul et al. 2013) which are similar changes observed due to diabetes (Hashmi, Malone-Lee et al. 2006). Since the prevalence of diabetes increases with age and continues to increase steadily as more people live longer and grow heavier (Cowie, Rust et al. 2009), in this population, ageing and diabetes play a combined role in the alteration of collagenous tissue properties.

In both diabetes and aging, evidence shows that the dermis undergoes a marked reduction in thickness due to significant loss of collagen fibres (Hussain, Limthongkul et al. 2013). The remaining collagen fibres become stiffer and thicker (Andreassen, Seyer-Hansen et al. 1981a), while diabetes further induces a haphazard cross linking process (Andreassen, Seyer-Hansen et al. 1981b), decreasing the ability of the skin to withstand shearing forces, which are particularly significant in the foot during gait. In addition, elastin fibres, which provide the skin with elasticity, decrease in number and become fragmented, resulting in increased fragility and loss of elastic recoil (Hashmi, Malone-Lee et al. 2006, Aoki, Yazaki et al. 1993). These alterations in skin structure make the skin of the foot less resilient to the normal mechanical forces it is subjected to during gait, as they result in altered behaviour

of the skin in response to external forces. Decrease in collagen and elastin, and stiffer collagen fibres lead to progressive loss in the elastic properties of the skin in the area of small stress, and progressive increase in the time required for viscoelastic recovery from great stress (Bus, Haspels et al. 2011). The epidermis, also has an important role in skin resilience and alteration in structure due to DM may have an important role in the development of DFU.

The mechanical properties of the epidermis are mostly attributed to its geometric form and the intrinsic properties of its components, mainly, the elastic high modulus keratin fibres embedded in a viscoelastic matrix of lower modulus (Hashmi, Malone-Lee et al. 2006). This is of significant importance in the foot where the keratin in the epidermis provides the skin with resilience and flexibility which is necessary during joint and muscle movement in gait.

The keratin filaments in the epidermal cells provide mechanical integrity to the cells (Fuchs 1995) which is critical for the plantar epidermis which has to withstand shear, compression and torsion stresses (Edwards, Marks 1995) during contact with the ground or the shoe. However, in diabetes, epidermal mechanics are altered principally due to dehydration induced by autonomic neuropathy which results in clinically anhidrotic skin in the foot (Hashmi, Malone-Lee et al. 2006). With reduced water content in the stratum corneum, the interaction between keratin non-helical regions and water extractable materials between keratin fibres is decreased, resulting in diminished elastic and viscoelastic properties of the epidermis (Jokura, Ishikawa et al. 1995). However, this does not fit with the clinical observation of the diabetic skin of the foot when the shoe is taken off particularly during the summer. Although there is a paucity evidence in the literature associated with in-shoe maceration in DM patients, through personal experience and informal discussions with other health professionals, the skin of the diabetic foot is observed to be moist as soon as the shoes and hosiery are removed but quickly dries to the dry state noted for hairy skin (Crăciun, Moldovan et al. 2012).

This observation is mostly noted particularly when the climate is hot and humid, typical of Mediterranean spring and summer months, during which patients with diabetes are advised to wear closed footwear following guidelines published by various bodies, aiming to protect the insensate skin from trauma which may lead to skin breakdown (Veves, Murray et al. 1992, Bakker, Apelqvist et al. 2012). An increased hydration of the skin has been associated with altered tissue response to mechanical stress making it more susceptible to injury as observed in studies (Keller, Wille et al. 2002) on bedridden patients, where the skin is moist and subjected to pressure, analogue to in-shoe environment in hot Mediterranean climates. However, this clinical scenario is not well addressed in literature related to DFU. A further insight into the skin response to stress when exposed to such a climate, is required to bring to light any possible influencing factors in this mechanism thus providing a better understanding of the development of DFU in a population living in a Mediterranean climate, which is currently not clear in the literature.

2.4.5 Skin Behaviour in Response to Stress

During weight bearing activities, the feet are exposed to large forces particularly when the activity is dynamic, such as walking or running. During each step the entire weight bearing plantar skin surface is subjected to dynamic forces greater than the body weight (Ledoux, Blevins 2007). Even to healthy tissue, such forces lead to tissue deformation and several studies (Cavanagh 1999, van Deursen 2004, Chen, Lee et al. 2010) demonstrated a relationship between force applied to the skin and deformation sustained, known as the stress-strain relationship. Apart from eliciting localised changes and tissue deformation, dynamic activity also imposes mechanical and thermal stress on the microvascular component of the skin (Jan, Shen et al. 2013b). Literature available related to these localised responses to mechanical stress during activity will be critically reviewed and presented below.

2.4.6 Tissue Deformation in Response to Mechanical Stress

Stress or pressure (Table 2.3) perpendicular to the tissue surface leads to a compressive strain (deformation). Stress parallel to the tissue surface (shear stress) will lead to shear strain in the presence of friction, particularly if the surface coefficient of friction is large as in damp skin against footwear. The specific behavioural response of the plantar skin to these forces has important implications relevant to diabetic foot ulceration.

Mechanical tests (Zeng, Liu et al. 2004, Meijer, Douven et al. 1999) show that the human skin has a non-homogeneous, anisotropic, nonlinear viscoelastic behaviour (refer to Table 2.3 below). The viscoelastic properties of human skin are conferred by the combination of elastin, collagen, reticulin, the ground substance and the extracellular fluid in the skin. Studies of the viscoelasticity of skin tissue reveal that its stress-strain relationship depends on strain rate, loading rate, the period of loading and on the preconditioning stress history, and that it exhibits considerable hysteresis in cyclic tests, as well as stress relaxation under constant strain (Pai, Ledoux 2010, Pai, Ledoux 2012).

Table 2.3: Definitions of Biomechanical Terms (Hussain, Limthongkul et al. 2013)	
Term	**Definition**
Stress	Pressure within materials that arises from externally applied forces
Strain	Amount of deformation an object experiences compared with its original size or shape; may be expressed as a ratio of lengths or percentage change
Creep	Tendency of a solid material to slowly move or deform under influences of stress
Friction	A force resisting the relative motion of solid surfaces, fluid layers, and material elements sliding against each other
Elasticity	Ability of a deformed material to return to its original shape and size when forces causing the deformation are moved (e.g. metal spring)
Anisotropy	Exhibiting properties of different values when measured along axes in different directions
Viscoelasticity	Combines elastic and viscous behaviour; application of stress causes temporary deformation if stress is quickly removed (elastic) but permanent deformation if it is maintained (viscous)
Non-homogeneous	Nonlinear relationship whereby a force causes a change, but as the force is removed, the reversal in change is not as much as the initial.
Hysteresis	a retardation of an effect when the forces acting upon a body are changed

The amount of deformation (strain) in response to stress depends on the characteristic of a tissue expressed by a stress strain curve (van Deursen 2004). If the force applied to skin (stress) is plotted against the change in length (strain), a graph similar to figure 2.6 is seen. The stress-strain behaviour of healthy skin is composed of three phases (Annaidh, Bruyère et al. 2012). Initially, a small force produces a large change in length

(Payne 1991). This is primarily due to linear alignment of the elastin fibres in the dermis, and the coiled structure of elastin and collagen which plays a role in the initial attenuation of mechanical stress during loading (Silver, Seehra et al. 2002, Green, Mansfield et al. 2014). A greater force is required to deform the skin, which corresponds to a change in orientation of collagen fibres and displacement of extracellular matrix as it is squeezed out between the fibres. Once the collagen fibres are at maximal length, the force required to produce further strain increases markedly. Furthermore, the effect of deformation on the skin is time-dependent. A slowly applied constant force will result in a hyperplastic response in skin and skin expansion (Payne 1991). This property is called 'creep'. The corollary of creep is 'stress-relaxation', which is where a force required to keep skin at a certain length decreases with time. However, if a force is applied too rapidly then the collagen fibres will rupture.

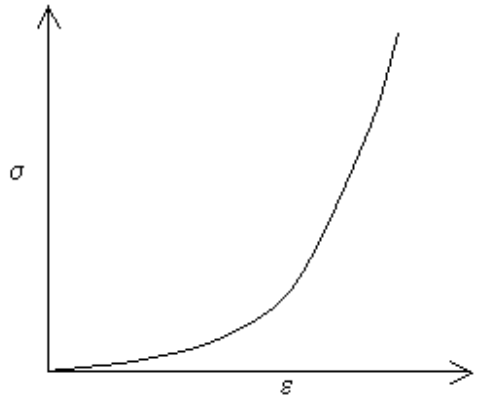


Figure 2.6: Stress Strain Curve – ***δ*** Stress (force) ***σ*** Strain (change in time)(Annaidh, Bruyère et al. 2012).

It has been shown that healthy skin has the ability to adapt to progressive loading with increase in collagen fibre diameter and reorganisation of collagen matrix (Sanders, Goldstein 2001). However, diabetic skin has been shown to have inferior biomechanical properties and altered behaviour.

Diabetes-induced stiffness characterised by additional cross-links between collagen molecules along the entire triple helix occur very slowly and in several steps (Figure 2.7), the most critical of which is the formation of a substance called an advanced glycation end product (AGE). In collagen, the most common cross-link is called glucosepane (Sell, Biemel et al. 2005, Sjöberg, Bulterijs 2009) and is formed from high glucose, or blood sugar, reducing the ability to change collagen fibre orientation, hindering matrix reorganization and other processes required for adaptive stretching in response to mechanical stress (Maluf, Mueller 2003).

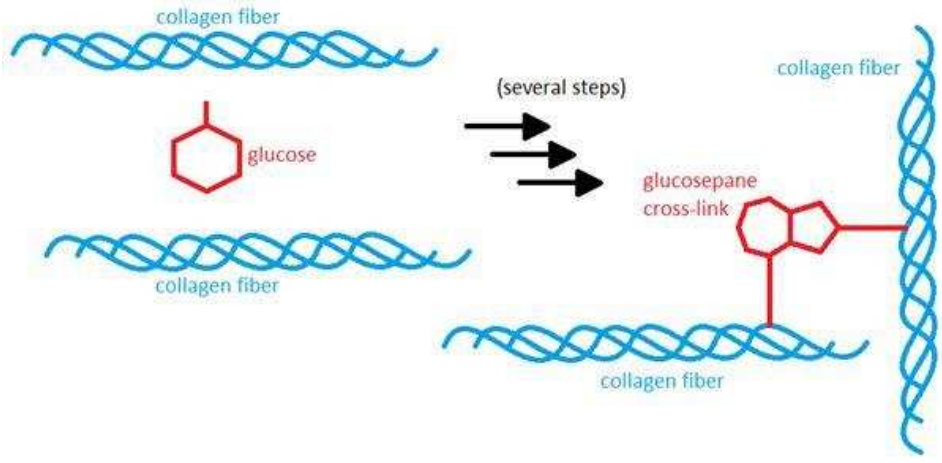


Figure 2.7: illustrating a cross-link which may form between two collagen fibres, after several reactions over a period of time (Sjöberg, Bulterijs 2009).

Following this mechanism, the tissue beneath the metatarsal heads has been found to be less elastic and less able to distribute pressure through deformation in diabetes (Gefen, Megido-Ravid et al. 2001, van Schie 2005). Evidence shows that the amount of stress required to cause mechanical yielding (tensile stress) of diabetic skin was found to be significantly less compared to that required in non-diabetic skin (4.14 MPa ± 1.88 vs. 6.52 MPa ± 1.71 p=0.03) (Bermudez, Herdrich et al. 2011). This is particularly relevant to the skin of the foot when cross linking of adjacent collagen fibrils and non-enzymatic glycosylation of keratin cause stiffening of the affected tissues when subjected to mechanical stress (Andreassen, Seyer-Hansen et al. 1981b, Jørgensen, Ahrensberg et al. 2001). Consequently, the pathologic stiffening of the diabetic plantar soft tissue may cause stress concentrations, which could lead to micro tears during load bearing, and could be exacerbated in the neuropathic foot due to undetected or abnormal mechanical forces especially when improper footwear is used (Gefen 2003).

The processes described above, derived from a review of literature related to diabetic foot ulceration and tissue mechanics in diabetes, describe the mechanism of injury leading to tissue breakdown occurring when the skin of the foot is subjected to mechanical stresses, which is further complicated by impaired tissue resilience at baseline. The risk factors which are evidenced in the literature as being implicated in DFU have also been highlighted, indicating that a complex mechanism leading to increased mechanical stresses is key and current research is aimed at mitigating these stresses through offloading and specialised footwear. However, the high recurrence rates evidenced in the literature (Maciejewski, Reiber et al. 2004, Reiber, Smith et al. 2002), despite advances in research and health provision, could indicate that the current understanding in the mechanisms underpinning ulcer development in the diabetic foot may need to be reconsidered. One such consideration, relevant to this work is the influence of change in coefficient of friction on interstitial shear stress. Research related to wound prevention in other areas of the body, predominantly related to pressure ulceration, suggests that the skin's ability to withstand mechanical stresses can be affected by environmental factors, namely

temperature and relative humidity (Gefen 2011, Yusuf, Okuwa et al. 2015). Despite similar wound characteristics acknowledged between DFU and pressure ulcers (Vowden, Vowden 2016, Boulton 2013), this notion has been relatively overlooked in DFU research. Therefore, in order to explore this literature gap, research investigating risk factors associated with the development pressure ulceration was reviewed.

2.5 Environmental Influences on Pressure Ulceration

Like DFU, a pressure ulcer is a multi-factorial phenomenon involving varied intrinsic and extrinsic factors, with pressure, shear and sensory deficits being main etiologic factors (Posada-Moreno, Elena Losa Iglesias et al. 2011). In addition, the tolerance of tissue to external mechanical forces, like DFU, depends on intrinsic factors influencing multiple mechanisms, such as circulatory disturbances and collagen synthesis as evidenced in diabetic patients (Linder-Ganz, Gefen 2009), discussed earlier in this chapter. The mechanism of injury in pressure ulceration was described briefly as external mechanical forces which decrease blood flow, leading to tissue ischaemia and eventual necrosis if the pressure in not relieved, with loading over a bony prominence as a well-recognised etiologic factor (Thorfinn, Sjöberg et al. 2006), similar to accepted DFU mechanism of injury in the literature (Reiber, Vileikyte et al. 1999). Moreover, in pressure ulcer development, the level of humidity at the skin-support surface interface, in association with other factors, is known to predispose the patient to ulceration (Wu, Ahn et al. 2009, Donovan, Dinh et al. 1993, Maklebust, Sieggreen 1996) while an increase in temperature is also postulated to have an influence on the occurrence of pressure ulcers (Knox 1999, Donovan, Dinh et al. 1993). The influence of temperature and RH on tissue breakdown as evidenced in literature emerging predominantly from studies related to pressure ulcers in bed ridden or wheelchair bound patients is further discussed below (see Section 2.5.1 and 2.5.2).

2.5.1 Relative Humidity and Pressure Ulceration

Increased relative humidity has been suggested to be a primary risk factor for pressure ulceration, together with shear, immobility and activity in hospitalised patients (Magalhaes, Gragnani et al. 2007). In a highly cited document related to the prevention of pressure ulceration, Clark et al (2010) state that temperature and humidity, termed as 'microclimate' between the skin and the supporting surface, are important extrinsic factors implicated in ulcer development, together with pressure and shear. This was confirmed in a mathematical study (Gefen 2011), which demonstrated that an increase in humidity levels around the skin, decreases skin tolerance to superficial pressure. The authors stated that an increase in RH by 25%, decreased skin tolerance by 24%, making it more vulnerable to pressure ulcer formation. In the mechanism of pressure ulcer development it is thought that while perspiration hydrates the skin, it softens the stratum corneum and dissolves the molecular collagen cross-links in the dermis, reducing the strength of skin tolerance to pressure and shear forces (Reger, Ranganathan et al. 2007). In pressure ulceration therefore, a conceptual framework which includes the impact of high pressure over bony prominences, leading to internal damage (Frykberg, Zgonis et al. 2006), while influence of increased RH leads to a decrease in skin tolerance, resulting in superficial skin problems, is acknowledged (Yusuf, Okuwa et al. 2015). Appropriate moisture conditions are therefore recommended to prevent or reduce pressure ulceration in bed-ridden patients (Clark, Romanelli et al. 2010). The clothing (and the bedding) system play an important role in moderating liquid and moisture to maintain a healthier climate near the skin surface (Reddy, Gill et al. 2006). This also includes maintenance of adequate temperature which was also identified to be implicated in pressure ulcer development as further explored below.

2.5.2 Temperature and Pressure Ulceration

Elevated skin temperature has been associated with pressure ulcer development in several studies (Kokate, Leland et al. 1995, Posada-Moreno, Elena Losa Iglesias et al. 2011, Rapp, Bergstrom et al. 2009, Sae-Sia, Wipke-Tevis et al. 2005). Any condition that increases skin temperature is suggested to increase the susceptibility to tissue breakdown (Knox 1999). While the mechanism is still not well understood (Kenny, Sigal et al. 2016), it is thought that an increase skin temperature is associated with pressure ulceration formation because higher temperatures increase metabolic demand by the tissues and this may not be met by the blood perfusion in the area (Donovan, Dinh et al. 1993). Increased skin temperature may also increase pressure ulcer risk by increasing oxygen consumption, metabolic waste products and $C0_2$ production in an area already compromised by pressure induced tissue ischaemia (Sae-Sia, Wipke-Tevis et al. 2005, Angelidis, Lidman et al. 2009). In a study conducted over 30 years ago (Fisher, Szymke et al. 1978), still cited in more recent literature (Yusuf, Okuwa et al. 2015, Clark, Romanelli et al. 2010), it has been estimated that a 1^{o}C increase in body temperature increases metabolic demand by approximately 10%. Where skin, subcutaneous tissue and muscle perfusion are already compromised, any increased metabolic activity may give rise to ischaemia and subsequent tissue damage faster and at lower levels of pressure or shear than if the body temperature was normal (Knox, Anderson et al. 1994).

This was shown in a study by Sae-Sia et al. (2005) who observed that the incidence of pressure ulcer formation was significantly higher in those patients with an increase in temperature by 1.2^{o}C. This was further evidenced in a mathematical modelling study investigating microclimate factors (Gefen 2011). Gefen (2011) reported that an increase in skin temperature and ambient temperature (i.e. at the interface between the skin and support surface) consistently decreased skin tolerance to superficial pressure, reporting a substantial decrease of 19% of skin resilience to pressure when the temperature increased from 35^{o}C to 36^{o}C. While minor differences of a degree may not have a direct clinical impact on the individual, it may have an important effect on skin resilience in the compromised

area, making it more likely to develop injury (Donovan, Dinh et al. 1993, Posada-Moreno, Elena Losa Iglesias et al. 2011). Additionally, body extremities such as the foot, have been hypothesized to be more influenced by external factors such as temperature, particularly in the presence of poor thermoregulation (Crossley, Holdcroft 1999). In the human being, an increase in temperature can occur either by exposure to a hot and/or humid environment or by physical activity, both of which increase the mean body temperature (Kenny, Sigal et al. 2016).

2.6 Microclimate and Diabetic Foot Ulceration

The literature review above has demonstrated that environmental factors influence skin resilience in relation to pressure ulcer development and efforts to maintain a favorable microclimate by using specific support surface materials (Hermans, Weyl et al. 2014) and reduce skin temperature (Lachenbruch 2005a) are central in the prevention of pressure ulceration. In the diabetic foot, efforts to prevent ulceration include the use of adequate footwear, commonly understood by clinicians as being closed leather shoes. However, the possible influence of temperature and humidity does not appear to have been addressed in literature evaluating footwear efficacy in preventing diabetic foot ulceration. Personal clinical experience and anecdotal evidence from colleagues and patients have shown that increased in-shoe temperature and humidity is experienced when patients use recommended footwear particularly during the warm months, suggesting that ambient climate (hot Mediterranean Summer) may have an influence on in-shoe microclimate. Despite limited evidence regarding efficacy in DFU prevention (Maciejewski, Reiber et al. 2004, Reiber, Smith et al. 2002, Bus, Haspels et al. 2011), footwear recommendations are based on literature emerging mainly from studies carried out in cool temperate climates (Reiber, Smith et al. 2002, Edmonds, Blundell et al. 1986), where ambient climate is not comparable to that experienced in a Mediterranean Summer. The few studies evaluating footwear efficacy published from warm climates (Viswanathan, Madhavan et al. 2004) also

fail to address the possibility of the influence of in-shoe temperature and humidity in DFU development when using closed footwear. Some studies conducted in the USA (Boulton 2013, Wrobel, Mayfield et al. 2001, Sargen, Hoffstad et al. 2013, Margolis, Malay et al. 2011), demonstrated evidence of geographical variability in prevalence of lower extremity amputation due to diabetes, illustrating higher amputation rates in the South Eastern coastal regions, namely; Texas, Oklahoma, Louisiana, Arkansas and Mississippi (Margolis, Malay et al. 2011); See Figure 2.8). Although authors tried to link traditional factors such as socio-economic status, race and co-morbidities to the reported variation in amputation rate, they concluded that such variation was based on geographical location. While the authors stated that the reason for this geographical variation in the prevalence in lower extremity amputations is still unclear, the possibility of climate influence was not addressed. It should be noted that the mentioned locations generally exhibit a humid climate with long, hot summers and short mild winters. Therefore, while the geographical variability in amputation prevalence has been acknowledged, the possibility of the influence of ambient climate associated with the geographical variability in amputation has been overlooked.

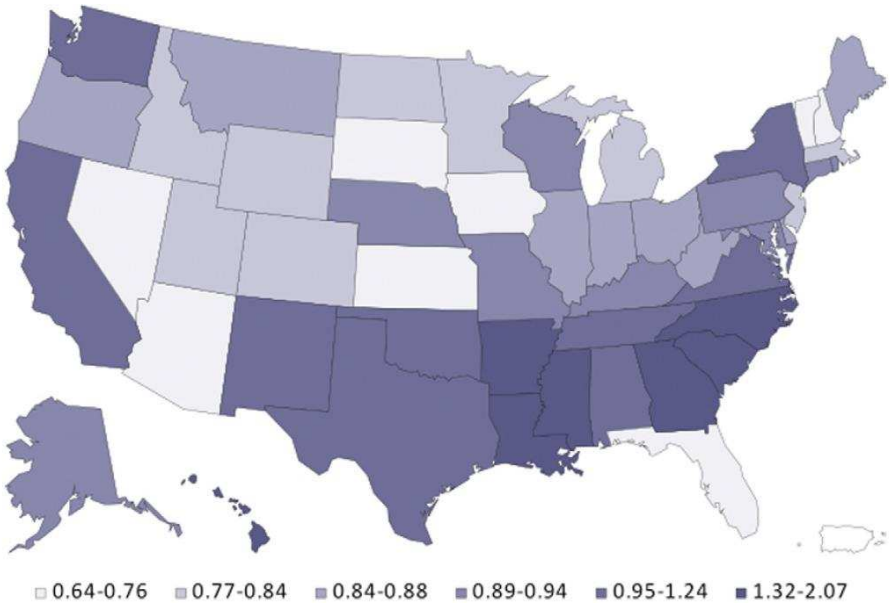


Figure 2.8: Geospatial map showing the ratio of rates of lower extremity (LE) amputation per state with the national average in the USA (Jones, Patel et al. 2012)

The study of the influence of ambient climate and in-shoe microclimate in relation to the diabetic foot is limited in the literature. Very few studies (Kang, Hoffman et al. 2003, Maluf, Mueller 2003, Foltyński, Mrozikiewicz-Rakowska et al. 2014, Nardin, Fogerson et al. 2010) have investigated microclimate in-shoe during walking with differing ambient climates being reported.

In their study, Maluf et al (2001) demonstrated the applicability of an in-shoe system to measure in-shoe microclimate and pressure. However, their report focuses on the validity and reliability of the system rather than on the actual temperature and humidity recordings. Therefore, information related to in-shoe microclimate cannot be compared with the current work.

Nardin et al. (2010) investigated the influence of seasonal variation on foot skin temperature patterns as they varied over a 32-hour period, indoors and outdoors, in 39 healthy individuals. In their study, data of both foot skin temperature in-shoe and also during sleep un-shod, was reported as they occurred during spring, summer, autumn and winter. The participants were different for each season and inter-participant activity during data acquisition also differed, hence making it difficult to determine in-shoe temperature kinetics during walking from their study. Nevertheless, their observations report that a higher ambient temperature is associated with higher foot skin temperature. In their study, in winter, when ambient temperature was -4.5^{0}C, the lowest foot temperature recorded during activity was 15.9^{0}C and in summer at an ambient temperature of 33.9^{0}C, the highest mean foot skin temperature was 35.5^{0}C. However, it is important to note that their study was conducted in Boston, USA and the ambient climate studied by Nardin et al. (2010) is that experienced in a North-eastern American region which has a continental climate, according to the Köppen climate classification. In this North-eastern American city, summer months are warm with July being the hottest month of the year, having an average temperature of 23^{0}C and winter being cold to freezing and having a mean temperature of -

1.7^0C, which is notably different from the Mediterranean winter and summer seasons which are the main interest in the work of this thesis.

Foltinsky et al. (2014) also reported a correlation between ambient temperature and foot skin temperature, although they did not specifically investigate different climates or seasons. In their study of 20 neuropathic and 10 healthy participants, the authors measured foot skin temperature over 24 hours. Their investigation of different ambient temperatures was derived from incidences of lower or higher temperatures due to varying locations such as indoors or outdoors during their data collection. As with the previously discussed study by Nardin et al. (2010), direct comparison of in-shoe temperature kinetics cannot be made between these studies as they were not reported in an analogous way. Additionally, their study was conducted in Warsaw which also has a continental climate, with cold winters and mild summers, differing distinctly from the Mediterranean climate.

In a similar study, also conducted in Boston, Kang et al (2003) investigated foot skin temperature over 24 to 48 hour recordings in 4 healthy and 12 neuropathic participants. While like Foltinsky et al. (2014), they did not explore different climates or seasons, the authors stated that foot temperature in healthy subjects was not correlated with ambient temperature. This disagreement can be explained by the method of ambient temperature recording where ambient temperature was measured by placing the sensor in a box inside the sock. This method may have influenced their interpretation of findings, since being in such close proximity to the body, ambient temperature readings may have been affected by body heat, resulting in falsely higher ambient temperature recordings, which may be the cause of the lack of correlation in their findings.

While the above studies investigated foot skin temperature fluctuations over several hours and including different activities, some recent works have investigated in-shoe temperature kinetics during walking but reporting only one controlled climate without

comparing the influence of different climates (Shimazaki, Matsutani et al. 2016, Reddy, Cooper et al. 2016, Sandoval-Palomares, Yáñez-Mendiola et al. 2016, Shimazaki, Murata 2015).

In a recent study by Sandoval-Palomares et al. (2016), in-shoe microclimate was evaluated in 2 healthy participants in the context of assessing the applicability of a portable system for monitoring microclimate in diabetes. In a controlled ambient climate at 23^0C and 50% RH, the authors reported an average increase of in-shoe temperature of approximately 6^0C after 40 minutes of treadmill walking, reaching a maximum of 29.4^0C and 29.3^0C in their 2 studied participants. However, the temperature sensors were placed in the insole rather than attached to the skin, thus not measuring actual skin temperature.

A similar study evaluating an in-shoe temperature measurement system was also recently published by Reddy et al. (2016.) They measured in-shoe temperature at different walking speeds in 14 participants but only 5 participants completed the whole trial. While in their study temperature sensors were also embedded in the insole (like the study discussed above), rather than attached to the skin, their participants did not wear socks to maintain skin contact although the authors stated that this method resulted in sensor displacement and occasional non-contact. The authors also failed to report data on ambient temperature studied.

In another study Shimazaki and Murata, (2015) investigated in-shoe temperature kinetics at different walking speeds in 17 healthy individuals in a controlled ambient climate (28.6^0C and 72% RH). Temperatures were recorded on eight different sites over the foot by applying thermocouples while participants walked for 50 minutes on a treadmill. While temperature readings are not clearly reported, the results indicate that temperature increased over time. The authors concluded that sites with higher impact such as the heel and big toe resulted in higher temperatures.

In a recently published study, Shimazaki et al. (2016) evaluated in-shoe temperature kinetics during four different treadmill walking speeds in a controlled climate chamber in 7 healthy males. Thermocouples were attached to several points on the participants' feet. Their ambient climate was controlled at 25^0C and 50% RH, while participants walked for 30 minutes on a treadmill. Similar to their previous work, although reports on actual in-shoe skin temperature measurements are not very clear, the results illustrate that in-shoe skin temperature increased over time reaching a maximum varying from 36^0C to 39^0C. The authors concluded that foot movement, metabolism and footwear ventilation influenced energy balance for temperature variations in the foot.

Therefore, while most of the previously published studies reported a significant correlation between foot temperature and ambient temperature, in both healthy and individuals with diabetes, none of the studies discussed above measured in-shoe RH or seasonal variation in Mediterranean climates.

Others (Rutkove, Nie et al. 2007) measured foot temperature to study thermoregulation in diabetic neuropathy during sleep and wakefulness or during exercise (Zontak, Sideman et al. 1998) without reference to relative humidity or DFU. There is some previous research investigating in-shoe microclimate available but it was predominantly aimed at assessing footwear comfort in military footwear (Uedelhoven, Kurz et al. 2002), sports footwear (Rebay, Arfaoui et al. 2008) or in extremes of temperature such as in ski boots (Hofer, Hasler et al. 2014) or in fire fighter protective footwear (Irzmańska 2015), with no relation to footwear in diabetes. Nevertheless, these studies report an increase in relative humidity levels and temperature while running in extreme climatic conditions, indicating changes in-shoe microclimate occurring during exercise. However, to date, it is not known whether such information is relevant to the diabetic foot in-shoe and therefore it is still not specified in the literature whether knowledge related to the influence of microclimate on skin resilience in pressure ulceration could also be applicable to the diabetic foot in-shoe. The inherent difficulties of measuring these parameters during

ambulation are probably the cause of a paucity of literature in this area of study in the diabetic foot and a knowledge gap is therefore highlighted. The need to fill this gap and improve the understanding related to the evolution of in-shoe microclimate, particularly for patients living in warm climates, has been elucidated.

The current research is therefore aimed at filling this knowledge gap which has been highlighted in the literature related to DFU. It is hoped that any new evidence gained from this research will add to the body of knowledge related to in-shoe microclimate and can directly or indirectly inform or augment existing therapeutic approaches.

2.7 Summary

The impact of DFU to the person suffering from it and to the health care systems worldwide has driven researchers to investigate component causes of ulceration with the aim of identifying means of prevention of this important complication of diabetes. While significant technological advances have been achieved over the years, allowing researchers to measure key known factors implicated in diabetic foot ulceration, such as in-shoe mechanical stresses, knowledge related to in-shoe microclimate in relation to the diabetic foot is still limited.

The work of this thesis was undertaken to fill this knowledge gap by providing an original contribution to the body of knowledge in-forming footwear prescription, taking into account environmental conditions relevant to Mediterranean countries. Therefore, the aim of the work of this thesis is to investigate environmental factors specific to the Mediterranean climate, particularly temperature and humidity as these are known to modify tissue response to mechanical stresses which may lead to ulceration. Information gained from this work could mean fewer foot complications, a better quality of life to all

patients living with diabetes mellitus in this specific Mediterranean country and reduced expenditure within the Maltese Health Care system. A summation of the research questions leading to the research objectives of the current thesis are presented in Chapter 1.

Chapter 3

The Philosophical and Methodological Approach to the Work of this Thesis

3.1 Introduction

The overall purpose of this thesis was to increase knowledge associated with in-shoe microclimate in relation to seasonal variation during ambulation, which may help in better understanding in-shoe temperature and RH kinetics, implicated to be detrimental in pressure ulcers. This chapter describes the research methodology underpinning the work of this thesis. It presents the theoretical framework and philosophical perspective within which research decisions were made.

The theoretical framework of a research project relates to the philosophical basis on which the research project takes place and forms the link between the theoretical aspect and practical components of the investigation undertaken. The theoretical framework, therefore, 'has implications for every decision made in the research process' (Mertens 2014).

Once the research question was formulated (see Chapter 1, Section 1.3), the process whereby this question might be answered provided justification for the methodologies and methods. This was implemented as suggested by Crotty (1998). Methodologies relate to the research question, plan of action, strategy, design, or process supporting and linking the choice of methods to desired outcomes (Crotty 1998). The justification of the choice of methodology, relates to the identification of underlying assumptions about the reality and understandings of human knowledge that the researcher brings to the research. Therefore the theoretical framework includes the methods, methodologies and exposes the underlying philosophical assumption about the researcher's view of the human world (Ontology) and the philosophical basis, nature and limits of human knowledge (Epistemology) underpinning the research (Crotty 1998).

3.2 Ontology and Epistemology

3.2.1 Ontology

As a philosophy, the researcher's view of the human world, is concerned with assumptions about the variety of phenomena in the world. It is a theory of the nature of reality (Delanty, Strydom 2003); it is a theory of being and is concerned with issues of what exists and also refers to the claims that a particular paradigm makes about reality or truth (Hitchcock, Hughes 1995). In simpler terms, ontology is about what exists, what it looks like, what components make it up and how the components interact with each other (Willis, Jost et al. 2007).

From an ontological perspective, the researcher thinks about issues such as 'whether the world exists independently of your perceptions of it' (Greener 2011). The researcher's ontological position therefore begins to shape the methodological decision-making, dependent on whether the researcher sees an external, independent reality or a constructed reality based on social or individual human conception (Creswell 2013). Crotty (1998) notes that an ontological stance implies a specific epistemological stance and vice versa. The complimentary nature of the terms is highlighted when he cites the ontological notion of realism, which postulates that reality exists independent of the mind and its compliment objectivism, which is an epistemological notion that meaning exists in objects without the interference of the mind. If one ontological stance is adopted, so is its compliment epistemological notion (Crotty 1998). In this thesis, this notion is exemplified in the validation part of the study (Validity and calibration Chapter 5) where I felt that objectivity was important and strict control of variables and statistical analysis were required in order to achieve scientific and objective data.

3.2.2 Epistemology

Epistemology, concerns the philosophical study of knowledge and 'the grounds upon which we believe something to be true' (Oliver 2010) – in other words, 'what counts as knowledge and how is it obtained' (Crookes 2013). Epistemology is concerned with how phenomena can be made known to the researcher (Walker, Evers 1988). According to Brewerton & Millward (2001), the term refers to the inquiry of what differentiates defensible belief from opinion. Epistemology can sometimes also have a major impact on the data collection choices as well as on the methodology in a research process (Willis, Jost et al. 2007). The ontological and epistemological perspective taken will affect whether a quantitative approach is necessary to fit an objective and measurable study, a qualitative approach to encompass a subjective and interpretative study or a mixed-methods approach. Over the years, there was the recognition of different epistemologies with the application of different paradigms and the application of a variety of methodologies and methods (Jacob 1988, Wiersma, Wiersma 1985, Torbert 1999).

Earlier research was governed by the dominant empirical analytical methodologies and the regular law-like relationships characterised by the 'hard or natural sciences' (Grant, Giddings 2002). However, in the latter half of the 20th century, there was a growing recognition of the appropriateness of alternative approaches creating the recognition of alternative paradigms, alternative epistemologies and the application of a variety of methodologies and methods. From this debate, two broad and contrasting theoretical perspectives emerged (Table 3.1): objectivism (positivism) that holds that there is an independent reality and constructionism that assumes that reality is the product of social processes (Neuman 2002). These philosophical perspectives can be placed on an epistemological continuum. While the perceived dichotomies between positivism (deduction-objective approach) and constructionism (inductive-subjective approach) described in Morgan (2007), still continue today, other researchers have chosen to integrate these methodologies (Tashakkori, Teddlie 1998, Johnson, Onwuegbuzie et al. 2007) creating the 'third methodological movement' (Teddlie, Tashakkori 2003) known as pragmatism.

Table 3.1: The Philosophical perspectives and respective methods (Onwuegbuzie and Turner, 2007; Onwuegbuzie et al. 2009)			
Paradigm	**Positivism**	**Pragmatism**	**Constructivism**
Methods	Quantitative	Quantitative + Qualitative	Qualitative
Logic	Deductive	Deductive + Inductive	Inductive
Epistemology	Objective point of view. Knower and known are dualism	Both objective and subjective point of view	Subjective point of view. Knower and known are inseparable
Axiology	Inquiry is value free	Values play a large role in interpreting results	Inquiry is value bound
Ontology	Naïve realism	Accept external reality. Choose explanations that best produce desired outcomes	Relativism

3.3 Philosophical Perspective

A research philosophy within which a thesis is grounded, is a belief about the way in which data about a phenomenon should be gathered, analysed and used (Oliver 2010). The research questions and methods of the current thesis are framed within a post-positivist philosophical perspective as are the data collection and analysis. Coming from a positivistic background and surrounded by colleagues and research entrenched in this philosophical underpinning, I felt comfortable in applying positivistic principles in my research approach to answer the research question. The primary research question emerged during clinical practice from patients' complaints of using closed footwear in hot summer months in Malta and from ulcer recurrence in the diabetic population when using such footwear. While I

acknowledge that an interpretive phenomenological approach investigating patients' personal experiences regarding this may help me understand their views and my position as a clinician, I felt that it was important to first establish scientifically, the in-shoe parameters causing this perception and feeling of discomfort while at the same time exploring whether these may influence tissue integrity and place the foot more at risk to ulceration. Following several discussions with colleagues and supervisors and exploration of the literature associated with in-shoe parameters I felt more convinced that this approach was right for this area of investigation as further gaps in the current knowledge which required positivistic enquiry were discovered.

3.3.1 Positivism/Post-Positivism

Positivism is the belief that reality is stable and can be observed and described from an objective viewpoint (Levin 1988) and can be studied by applying methods and principles of natural sciences and scientific inquiry. Positivism maintains that "the object of study is independent of researchers; knowledge is discovered and verified through direct observations or measurements of phenomena; facts are established by taking apart a phenomenon to examine its component parts" (Krauss 2005). "Positivism has a long and rich historical tradition. It is so embedded in our society that knowledge claims not grounded in positivistic thought are simply dismissed as ascientific and therefore invalid" (Hirschheim 1985). Following criticism of this paradigm, a more contemporary paradigm developed known as post-positivism. While post-positivists also believe in a singular realty, they acknowledge that reality can never be fully known but its understanding is limited due to the human beings intellectual and sensory limitations (Guba 1990).

Research embedded within the post-positivistic philosophy often involves manipulation of reality with variations in only a single independent variable to identify regularities and form relationships between elements of interest. Like positivism, post-

positivism allows predictions to be made based on previously observed realities and their inter-relationships and post-positivists also strive to be neutral and objective to ensure that findings fit with the existing knowledge base (Crossan 2003). This view is often also adopted within clinical research as this approach has been found to be employed in empirical studies (Alavi, Carlson 1992) due to its successful association with the physical and natural sciences.

The research questions and methods of the current thesis are framed within a post-positivist philosophical perspective as are the data collection and analysis. The nature of the research question and the objectivity and manipulation of variables required for data collection supported the notion that a quantitative enquiry within the post-positivism paradigm was best suited to answer the research question in its entirety.

The knowledge constructed within this thesis is built from objective learnings where objective information was sought in experimental methods. This research primarily investigates in-shoe parameters, namely temperature and humidity and tests hypotheses for statistical significance rather than generating hypotheses. It aims to add to the understanding of these parameters in relation to seasonal variation, essentially by measuring them using specific instrumentation to generate knowledge that has practical implications. These parameters warranted a quantitative approach to data collection where it was important that variables investigated were totally independent from the researcher's influence, by applying strict study protocols for data acquisition as described further below (see Section 4.4).

3.4 Research Design

The aim of this thesis is to find out whether different ambient climates (summer, winter) result in different in-shoe temperature and RH levels and kinetics in people with diabetes in Malta. In order to achieve this, of particular importance was the control of several variables. Since ambient climate was the manipulated independent variable (intervention), this left the in-shoe microclimate parameters as dependent variables (outcome). In order to minimize the confounding variables in such an experiment (e.g. individual physiological differences), a single group of participants was measured repeatedly. Further detail of the experimental procedure is presented in chapter 4 (see Section 4.4.1, on page 90).

The main approaches of this thesis can be best described as Quasi-experimental, where the research designs test the effect of an intervention on an outcome (Ryan, Consumers 2013). Quasi-experimental studies encompass a wide range of non-randomised intervention studies. This study design is frequently used in medical studies where it is not logistically feasible or ethical to conduct a randomised control trial (RCT). However, in a similar way to randomised trials, quasi-experiments aim to demonstrate causality between an intervention and an outcome (Harris, McGregor et al. 2006). The lack of random assignment of participants is the major weakness of the quasi-experimental study design.

Quasi-experimental designs identify a comparison group that is 'as similar as possible' to the treatment group in terms of baseline characteristics, so that the observed differences can be attributable largely to the intervention. In the current study, the participants acted as their own controls and strict control of confounding variables was employed (such as the type of footwear used, and walking speed) as further explained in chapter 4 (see Section 4.4, on pages 85-93). While it is acknowledged that RCTs provide a higher level of evidence when compared to quasi experimental studies, conclusions from quasi experimental designs are thought to be valid and are generally-well accepted (Ryan,

Consumers 2013). However, it is important that assurance of the quality (validity) of data is provided and the limitations of the study and how they may affect the results, are made explicit.

3.5 Research Approach

The research approach refers to the method of data collection that is best suited to the research question (i.e. whether it is a quantitative or qualitative method). Different researchers (Creswell 2013, Lynch 1983) use the terms quantitative and qualitative in fundamentally different ways, describing quantitative data as including numbers, whereas qualitative data include words, symbols, pictures and other non-numeric data. This is the common understanding of these terms in texts that broadly review research design (Johnson, Christensen 2008) and in evaluation (Patton 1990, Newcomer, Hatry et al. 2015).

3.5.1 Qualitative Research and Quantitative Research

Qualitative research is often observed in social science research, where the interest of the researcher is often aimed at exploring abstract constructs such as attitudes, behaviours, experiences and views (Creswell 2013). Qualitative research infrequently tests a theory but rather generates theoretical insights arising from their findings. In contrast, quantitative research generates data that can be analysed in terms of numbers. It tends to emphasize relatively large-scale representative sets of data and is based on the traditional objective-scientific method with controlled study designs and statistical analysis with hypothesis testing, commonly observed in scientific empirical research (Cohen et al., 2000). These two methodological approaches differ distinctly in their data collection methods and the analysis of information where qualitative research often employs the use of focus

groups, questionnaires and in-depth interviews and tends to focus on exploring, in as much detail as possible, instances or examples which are seen as being interesting or illuminating, and aims to achieve `depth' rather than `breadth' (Ruane 2005). Quantitative methods on the other hand commonly employ experimental methods with manipulation of variables and draw heavily on statistical analysis techniques to examine the data collected (Creswell 2013).

The use of study controls and manipulation of independent variables are key design considerations in scientific inquiry as adopted in the current thesis. As is the case in many quantitative studies, it is necessary to ensure validity of the measurement tools and techniques that are being employed to quantify the effects of an intervention. An investigation of validity is best suited for this purpose. These methods also ensure that there is a 'distance' between the subjective bias of the researcher and the objective reality of the measures to be made. This generally involves hypothesis generation and testing: resulting in the supporting or refuting of the hypothesis. The concepts of reliability and validity in quantitative studies are related, but will be discussed separately in the next section (section 3.6) explaining the chosen methodology for the validity studies.

Once the philosophical perspective and methodological approach were identified, the concept of validity of the measurement tools were assessed, as it is a critical component of rigorous research and refers to the degree to which evidence and theory support the interpretation of measurement (Quinn 2002).

3.6 The Concept of Validity

The concept of validity was developed within the positivist tradition and a rich literature highlighting its complexity has emerged. Likewise, a concern for validity is held with equal seriousness by practitioners of the interpretive tradition who have claimed their own unique paradigms with corresponding validity criteria (Whittemore, Chase et al. 2001, Quinn 2002).

Validity is a construct developed to assess the true value of inferences made from study measurement and findings. The validity of a method is a critical component of rigorous research. Gareth Morgan (1983) has convincingly argued that the criteria for judging the quality of a research method is derived from the paradigm that underpins that method. Therefore, quantitative criteria are used to judge quantitative inquiry (Hammersley 2013).

Within the context of the temperature and humidity sensor validation studies in the current work (see Chapter 5), validity is concerned with whether the instruments used for measurement are actually measuring what they are supposed to be measuring and whether they are accurate (Winter 2000, Czaja, Blair 2005, Dunn, Roberts 1999, Ruane 2005). Types of validity are discussed in greater detail in the next section (Section 3.6.1). Therefore, in the validation study of this thesis, 'validation' is concerned with the accuracy and consistency (precision) of experimental sensors which were used to measure temperature and humidity at the interface between the skin and the shoe. The validation of the instrumentation (temperature and humidity sensors) used, was of critical importance in this thesis because the findings may be used to discuss clinical implications related to in-shoe microclimate as an influencing factor leading to tissue breakdown, in, for example the diabetic foot. In studies involving measurement, confidence in what the instruments are actually measuring and how well they do so, is usually established (Validity). Furthermore, to be beneficial, a measuring instrument must accurately quantify a given parameter and

do so with consistency (Reliability). Both aspects are critical, as one without the other is quite ineffectual (Krug 2008, Ruane 2005).

3.6.1 Validity, Repeatability and Reliability in the Quantitative Studies

In quantitative studies, quality of an experiment is described in terms of reliability and validity. These parameters are therefore important aspects to be considered during the study design. Validity, repeatability and reliability are common terms used to designate test accuracy and precision and are important aspects of measurement (Bartlett, Frost 2008). Reliability refers to the reproducibility of a measurement, validity refers to the agreement between the value of a measurement and its true value, while repeatability is a measurement of precision, which denotes the absolute difference between a pair of repeated test results. In the next sections accuracy and precision are discussed. Different types of validity and reliability will be explored to provide justification for most appropriate methods that are suited for each component of the work of this thesis.

3.6.1.1 Validity

According to the classical test theory, scores obtained during the measurement process are composed of the 'true' and the 'error' scores (Crocker, Algina 1986). The true score is essentially the score a researcher would receive if the measurement instrument is perfectly accurate, which in real life is virtually impossible. Therefore, validating a measurement instrument is largely focused on identifying and reducing error in the measurement process. According to Crocker and Algina (1986), the researcher has a responsibility to "identify the sources of measurement error that would be most detrimental to useful score interpretation".

Most literature identifies several different kinds of validity based on scope, relevance, predictive quality, and association of the work being undertaken. Depending on the selection of the accepted reference (criterion or gold standard), the primary types of validity most commonly used include Test (Criterion) Validity, Internal Validity and, External Validity (Goodwin 1997, Kraemer, Adams et al. 2002, AERA 1999, Molenberghs, Laenen et al. 2007, Viswanathan, Madhavan et al. 2004, Hand 2004). These types of validity tests emerged from the positivist conceptualisation of validity

(i) Test (Criterion) Validity

There are various types of Test Validity, which is often termed content validity, construct validity, criterion-related validity, and face validity (Czaja, Blair 2005, Ruane 2005, Dunn, Roberts 1999, Wright, Stone 1999, Galvan 2006, Muijs 2004). Of these, criterion validity is the concept most commonly used in studies using physical measures. Content, construct and face validity on the other hand are often used in social science research to measure intangible constructs such as attitudes, behaviours, emotions, or personalities commonly used in qualitative research designs.

Criterion validity is the degree to which scores of a measuring instrument are an adequate reflection of a "gold standard". Most validation studies of physical measurements use criterion validation techniques. The method of choice is often comparing a new method of measurement to an existing gold standard measurement method – criterion concurrent validity. Criterion concurrent validity assesses whether scores on the instrument agree with, or concur with scores on the established gold standard instrument. Concurrent validity was the type of criterion validity method of choice for the current study where the temperature and humidity sensor readings were compared with gold standard measurements of both humidity and temperature within a controlled environment (Chapter 5, Section 5.4). In these types of studies, researchers seldom need to use any other approaches to validation. The statistical methods of sensitivity and specificity

for a categorical standard or limits of agreement for a continuous standard can be used, together with the usual statistical methods for comparisons of groups and relationships between continuous variables, such as t tests and regression (Kwiecien, Kopp-Schneider et al. 2011).

(ii) Internal Validity

Gay and Airasian (2000) describe internal validity as "the condition that observed differences on the dependent variable are a direct result of the independent variable, not some other variable." Efforts are made to establish a one-to-one relationship between enquiry and reality by using study controls and manipulation of independent variables or using control groups. In the work of this thesis, efforts made to ensure internal validity of the measurement tools included strict control of any identified confounding variables where possible (Chapter 4, Section 4.4).

(iii) External Validity

External validity refers to the ability to generalise findings to or across populations, locations, settings and times. With regards to the validity of the temperature and humidity sensor readings, agreement of scores was assessed both in the lab and also in the field (in-shoe) which potentially improves the external validity of the sensors.

Once the methodological approach pertaining to the type of validity was identified, the specific concepts of validation needed to be determined as they are important guiding features leading to method selection.

3.6.1.2 Accuracy, Precision, Repeatability and Reproducibility

Accuracy is defined as “freedom from mistake or error” or “conformity to truth or to a standard” or “degree of conformity of a measure to a standard or a true value.” Precision is defined as “the quality of being exactly or sharply defined” or “the degree of refinement with which a measurement is stated.” The subtle difference between these two terms may lie in whether a truth or a reference standard is required or not.

Historically, accuracy has been used to measure systematic bias while precision has been used to measure random error around the expected value. The deviation of the mean from the true value, (i.e., systematic bias), serves as the measure of accuracy while “precision” refers to the closeness of agreement between test results under the prescribed conditions. The key phrase “under the prescribed conditions” is important since precisions are only comparable under the same conditions (Barnhart, Haber et al. 2007). These can also be referred to as repeatability or reproducibility. Repeatability and reproducibility are two special kinds of precision under two extreme conditions and they should not be used interchangeably. Repeatability (Chapter 5, Section 5.5) assesses pure random error due to “true” replications, whereas reproducibility assesses closeness between observations made under condition other than pure replication, e.g., by different labs or observers. The use of accuracy for measuring the systematic bias, and precision for measuring random error, is commonly encountered in the literature pertaining to medical and statistical research (Bland, Altman 1999, Bland, Altman 1986, St. Laurent 1998, Bland, Altman 1995, Dunn 2004).

3.6.1.3 Reliability

Reliability has been defined as the consistency of measurements, or of an individual’s performance, or a test; or ‘the absence of measurement error’ (Atkinson, Nevill 1998, Martin, McPoil 2005). In rigorous research, any new measuring device requires reliability testing to be performed. A measure is said to have a high reliability if it produces

similar results under consistent conditions. The variation in the repeated measures will determine the degree of measurement error and the confidence in the measures taken.

The design of a reliability study permits such errors to occur so that they can be quantified. Following this rationale, knowledge of the measurement error of the sensors, particularly when used to measure parameters at the interface between the skin and the shoe, was important as this influenced data interpretation when used in the participant group. There was therefore a need to conduct a reliability study, to determine the instrument measurement errors, before using this instrument in the main study involving the participant group (see Chapter 5, Section 5.4)

3.6.1.4 Types of Reliability

Within the framework of classical test theory, there are several types of reliability coefficients based on the source of the random errors (Webb, Shavelson et al. 2006). The types of reliability discussed below are split-half reliability and test retest reliability.

(i) Split-half Reliability

This strategy involves the development of parallel or equivalent forms of a test that measure the same phenomenon and administer them within several days of each other. The internal consistency or homogeneity of a test may also indicate reliability. Using split-half reliability, the items on a test would be split and correlated with one another. Whether assessed through test-retest, equivalent forms or internal consistency procedures, reliability is expressed as the coefficient alpha and represents the true score variance divided by observed score variance.

(ii) Test re-test Reliability (Repeatability)

Another strategy for assessing reliability includes test-retest reliability, where the same test is administered in the same group of people twice and the results are correlated with one another. The (ISO 2004) defined repeatability is the closeness of agreement between independent test results under repeatable conditions that are as constant as possible. This is where independent test results are obtained with the same methods, on identical test items, in the same laboratory, performed by the same operator, using the same equipment, within short intervals of time. Therefore, in the work of this thesis, test re-test repeatability was the best choice for assessing reliability of the sensors where the test was repeated under the same conditions on two different days in order to assess day-to-day variability. A measurement will be deemed to be repeatable when this variation is within a predetermined acceptable limit.

As with validity, once the methodological approach to reliability was identified, the measures to be assessed needed to be specified. In studies investigating reliability of new measurement methods, Weir (Weir 2005) recommends the assessment of absolute and relative reliability which are discussed below.

3.6.1.5 Measures of Reliability

The test re-test reliability and the concepts of absolute and relative consistency will be reported as suggested by Weir (2005). Absolute consistency refers to the consistency of scores of participants, whereas relative consistency refers to the consistency of the participants in the group relative to the others. Absolute consistency is quantified using 'typical error' and relative consistency is measured using reliability coefficients called Intra-class Correlation Coefficient (ICC).

(i) Relative Reliability

The Pearson correlation coefficient is commonly used to test reliability as it is acceptable for two trials. However, it overestimates the true correlation for small sample sizes when they are less than 15 (Hopkins 2000a). Also, for the purpose of this study, Pearson's correlation coefficient is an inappropriate measure of reliability because the strength of linear association, and not agreement, is measured and it is possible to have a high degree of correlation when agreement is poor (Rankin, Stokes 1998). A better measure of the test re-test correlation is the intra-class correlation coefficient (ICC). It does not have a bias with small samples. Therefore, following recommendations by Weir (2005) Intra-class Correlation Coefficient (ICC) was used for this study (Chapter 5, Section 5.4.6) as it is a standard test providing an estimate of relative reliability for consistency of measurement in a heterogeneous population. In this context reliability (relative consistency) is formally defined as:

$$\text{reliability} = \frac{\text{between subjects variability}}{\text{between subjects variability + error}}$$

Error in the ICC to be used, refers to random error and not systematic error as there are no learning effects or 'fatigue' in this study.

(ii) Absolute Reliability

The absolute index of reliability is provided by the 'typical error', sometimes also referred to SEM – standard error of measurement, which quantifies the precision of individual scores on a test and has the same units as the measurement of interest (Hopkins 2000a). Most references estimate the 'typical error' as follows:

$$SEM = SD \sqrt{1 - ICC}$$

3.7 Summary

In order to justify the methods used, careful consideration of the philosophical approach and research design was employed. The choice of philosophical approach (Section 3.3) was informed by careful evaluation of the best methodological strategy required to best answer the research questions posed. The use of close study controls and manipulation of independent variables are key design considerations for the best protection against threats to validity when using instruments (Funk 1992). Therefore, the validity and reliability studies for both the temperature and humidity sensors were designed within this framework and comprised the first quantitative study of this thesis. The methods employed are presented in the following chapter.

Chapter 4

Equipment

and

General Methods

4.1 Introduction

The purpose of this chapter is to present a detailed description of each instrument used during data acquisition which includes the technical considerations and the justification for the choice of such equipment. A description of the experimental methodology that was utilized throughout all of the subsequent experimental chapters will also be presented in this section. Methods and procedures specific to single experiments are described where appropriate in the relevant chapters.

4.2 Equipment

In the field of footwear and ambulatory in-shoe microclimate measurement, only limited research has focused on measuring in-shoe temperature and in-shoe RH at the interface between the skin and the shoe during ambulation. Therefore, identifying the right sensors entailed a lengthy process due to the specific application of the sensors. A substantial amount of time was first dedicated exploring and searching for the best commercially available temperature and relative humidity (RH) sensors that could be placed on the skin in-shoe. Several meetings with biomedical engineering colleagues were organized to discuss the advantages and disadvantages of each sensor, including operating ranges, accuracy, cost, stability, sensitivity and ease of use. A justification of the selected instruments over other methods is given below.

4.2.1 In-Shoe Temperature Sensor

There were three types of sensors which could be used for measuring temperature in-shoe (Table 4.1). Thermocouples, resistance temperature detectors (RTDs) and thermistors are temperature transducers that have been used to measure skin temperature in both clinical and exercise physiology investigations (Nybo, Secher et al. 2002, Imrie, Hall 1990).

1. **Thermocouples,** are based on the junction between two different metals, which produces a voltage which increases with temperature. They are inexpensive, commonly used temperature transducers, which cover a wide temperature range, but they are the least accurate of the three temperature transducers (Cigoy 2007).

2. **Resistance temperature detectors (RTDs),** employ the property that the electrical resistance of metals varies with temperature. They are positive temperature coefficient (PTC) sensors whose resistance increases with temperature. They are more expensive than thermocouples but offer greater accuracy and stability.

3. **Thermistors,** are made from certain metal oxides whose resistance decreases with increasing temperature. Because the resistance characteristic falls off with increasing temperature they are called negative temperature coefficient (NTC) sensors. They are less expensive than RTDs, more accurate than thermocouples, and offer excellent sensitivity. Although they operate over a fairly small temperature range, thermistors are commonly used in human environment temperatures measurement, from 0^0C to 30^0C (Cigoy 2007). Like an RTD, a thermistor changes resistance as temperature changes. The thermistor offers higher sensitivity than RTDs, meaning that the thermistor resistance will change much more in response to temperature changes than an RTD (Roveti 2001, Cigoy 2007).

Since, for the purpose of the experimental trials conducted in this thesis, it was anticipated that the temperature measurements would fall within this small range, the characteristics of the thermistors were ideal and best suited, due to their low cost, high sensitivity and temperature-measuring range. The use of thermistors overcomes the limitations observed in other methods of in-shoe temperature monitoring as it can offer direct information about skin temperature during exercise-related conditions.

Table 4.1: Comparison of Three Common Temperature Transducers

	RDT	**Thermocouple**	**Thermistor**
Temperature Range	-260 to 850 ^{0}C (-436 to 1562 ^{0}F)	-270 to 1800 ^{0}C (-454 to 3272 ^{0}F)	-80 to 150 ^{0}C (-112 to 302 ^{0}F)
Sensor Cost	Moderate	Low	Low
System Cost	Moderate	High	Moderate
Stability	Best	Low	Moderate
Sensitivity	Moderate	Low	Best
Linearity	Best	Moderate	Poor
Specify for:	✓ General purpose ✓ Highest accuracy ✓ Temperature averaging	✓ Highest temperatures	✓ Best sensitivity ✓ Narrow ranges (e.g. medical) ✓ Point sensing

Some of the other advantages of thermistors over other sensors include; fast response time (due to their very small size), registering of temperature changes quickly and ease of set up and operation. For the measurement of in-shoe temperature a thermistor, TSD202A (Figure 4.1) was used for the purpose of this study, due to its relevant

characteristics. The TSD202A is a small (1.7mm diameter; 5mm long) and a fast-response (0.6sec) temperature probe, making it appropriate for measuring skin temperature within the shoe, even during shod gait.

Table 4.2: Temperature Sensor Product Specifications (thermistor TSD202A, BIOPAC, Biopac Systems Inc., Goleta, CA, USA)	
Response Time	0.6 sec
Size with housing	1.7 mm (diameter) x 5 mm (long)
Sensor only	10 mm sensing diameter, 1.4 mm sensor thickness
Interface:	SKT100C
Nominal Resistance	2252 Ω at 25° C
Maximum operating temperature	60° C (when used with SKT100C)
Accuracy and Interchangeability	0.2° C

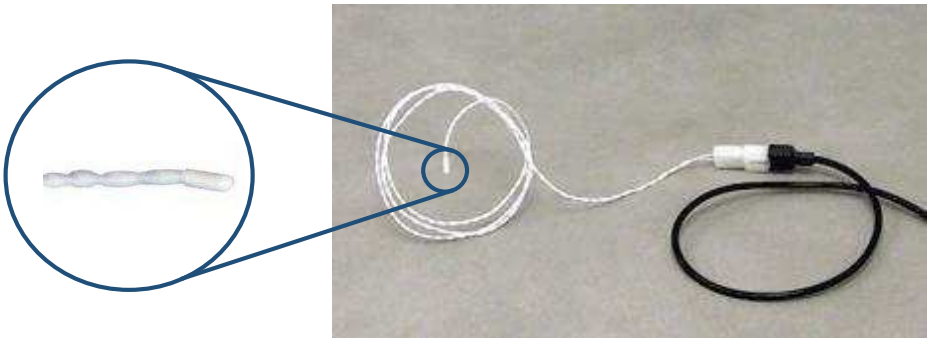


Figure 4.1 – Temperature probe, TSD202A model used in this study

All temperature measurements were recorded in real time using a physiological data monitoring system (Biopack Systems, Inc. USA). The thermistor TSD202A was connected to the PC via an amplifier module SKT100C, which is designed specifically for skin temperature measurement and has been utilised in previous studies involving psycho-physiological

investigations and sleep studies (McGinnis 1999, Karkalic, Jovanovic et al. 2015, Kawakami, Sato et al. 2015). System details and sensor connections are presented in further detail below (see Section 4.2.4).

4.2.2 In-shoe Humidity Sensor

A humidity sensor is a device with an integrated circuit that allows precise relative humidity measurement. Relative humidity refers to the ratio (stated as a percent) of the moisture content of air compared to the saturated moisture level at the same temperature and pressure. Relative humidity is usually expressed in percent (%), and can be computed from psychrometric data. There are three main sensing technologies, resistive, capacitive, and thermal conductivity, each offer distinct advantages (Roveti 2001).

1) **Resistive** S**ensors,** measure the change in electrical impedance of a hygroscopic medium such as a conductive polymer, salt, or treated substrate**.** They are interchangeable, usable for remote locations, and cost effective.

2) **Capacitive Sensors,** consist of a substrate on which a thin film of polymer or metal oxide is deposited between two conductive electrodes. They provide wide RH range and condensation tolerance, and, if laser trimmed, are also interchangeable.

3) **Thermal Conductivity Sensors,** measure the absolute humidity by quantifying the difference between the thermal conductivity of dry air and that of air containing water vapor. They perform well in corrosive environments and at high temperatures. For most applications, therefore, the environmental conditions dictate the sensor choice.

Humidity sensors, for the purpose of measuring in-shoe relative humidity during gait, are not commercially available. A thorough search was undertaken to identify commercially available humidity sensors with the relevant characteristics required for the purpose of the experimental studies presented in this thesis. Of particular relevance and importance was the integration of sensitivity and compact dimensions of the device so that it could be safely used during shod gait without causing damage to the skin or to the sensor itself. Identification of such a device was challenging since commercially available sensors with such characteristics are very limited. This process involved a significant amount of research on the subject area, both in the clinical and industrial fields and in-depth discussions with biomedical engineers.

The Honeywell HIH-4000 Series (Figure 4.2) was deemed to be best suited due to the compact dimension of the integrated circuit, making it ideal to be placed inside a shoe. This relative humidity (RH) sensor is a laser trimmed, thermoset polymer capacitive sensing element with on-chip integrated signal conditioning. This sensor is designed for simple and quick installation, making it ideal for the work of this thesis. Manufacturer specifications include, operating temperature of -40^0C to 85^0C, an operating humidity range of 0% RH to 100% RH and with a repeatability of ±0.5% RH (Table 4.3).

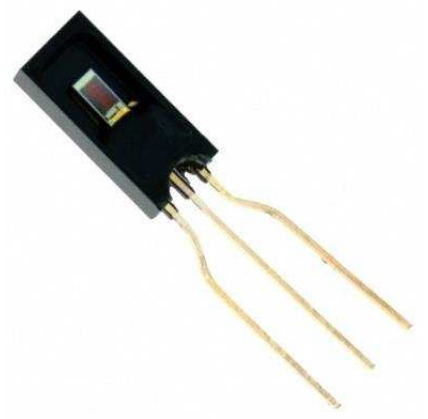


Figure 4.2: HIH 4000 series Humidity Sensor

Table 4.3: Humidity Sensor Product Specifications (HIH 4000 series, Honeywell International Inc., MN, USA)	
Operating Temperature	-40 °C to 85 °C [-40 °F to 185 °F]
Operating Humidity Range	0% RH to 100 %RH
Interchangeability	0 %RH to 59% RH ±5 %RH, 60 %RH to 100 %RH ±8 %RH
Hysteresis	±3 %RH
Response Time	15 s 1/e in slow moving air
Repeatability	±2 % RH
Settling Time	70 ms max.
Long-term Stability (Drift)	±1.2 %RH for five years; ±0.25 %RH each year
Stability at 50% RH	±1.2 %RH
Output Signal	Analog voltage

4.2.3 Ambient Climate Measurement

A calibrated and certified humidity and temperature data logger CEM DT-172 (Figure 4.3) was used to monitor ambient relative humidity and temperature during the experimental studies. The data logger has a manufacturer certified temperature range of -40 to 70^{0}C (accuracy of± 1^{0}C) and a humidity range of 0 to 100% RH (accuracy of ±2% RH). The data logger was placed within 50cm of the participant during ambulation in the laboratory, in order to capture ambient temperature and RH close to the participant, while avoiding falsely higher ambient temperature recordings due to body heat interference.

Figure 4.3 – Temperature and Humidity Datalogger (CEM DT-172)

4.2.4 Sensor Connections

The Honeywell HIH-4000 Series Humidity Sensors for relative humidity capture and TSD202A BIOPAC thermistors for temperature data capture were connected to a PC via a BIOPACK ® MP150A-CE system. System amplifiers SKT100C and HLT100C modules were required to connect the mains powered external equipment (BIOPACK ® MP System – Figure 4.4) to the thermistor and RH sensors. Three of the thermistors were connected to the HLT100C module through an amplifier while the fourth temperature sensor was directly connected to the SKT100C BIOPAC module. Data captured through the mentioned BIOPAC modules was recorded through the BIOPAC Acq*Knowledge*® software package at a sampling rate of 1 KHz. The voltage values obtained from the temperature and humidity sensors were converted to temperature and humidity values through a linear function pre-set in the Acq*Knowledge*® software according to the calibration parameters of the sensors under the re-scaling setup as discussed in Chapter 5. A biomedical engineer was present to ensure that the connectivity of the sensors, related hardware and software (AcqKnowledge 4.3+), worked seamlessly before and during data capturing. The captured data was then processed using MATLAB (The Math-Works Inc, Natick, Massachusetts).

Figure 4.4: BIOPACK ® MP150A-CE system, with adjacent SKT100C and HLT100C modules

4.3 Measurement Tools

4.3.1 Borg 6-20 Rating of Perceived Exertion (RPE) Scale®

The Borg 6-20 RPE Scale® (Rating of Perceived Exertion) is designed to describe perceptions of physical exertion during exercise. It is based on the perception of physical sensations that a person experiences during exercise, including increased heart rate, increased respiration or breathing rate, increased sweating, and muscle fatigue. Although this is a subjective measure, a person's exertion rating may provide a fairly good estimate of the actual heart rate during physical activity (Borg 1990). The Borg (RPE) scale consists of a numbered scale (6-20) and descriptors which range from 'very, very light' to 'very, very hard' (Figure 4.5). Before the start of the experimental studies where this scale was used, participants were asked to keep to a level of 'moderate' exertion (an RPE level of 12 to 13) during exercise.

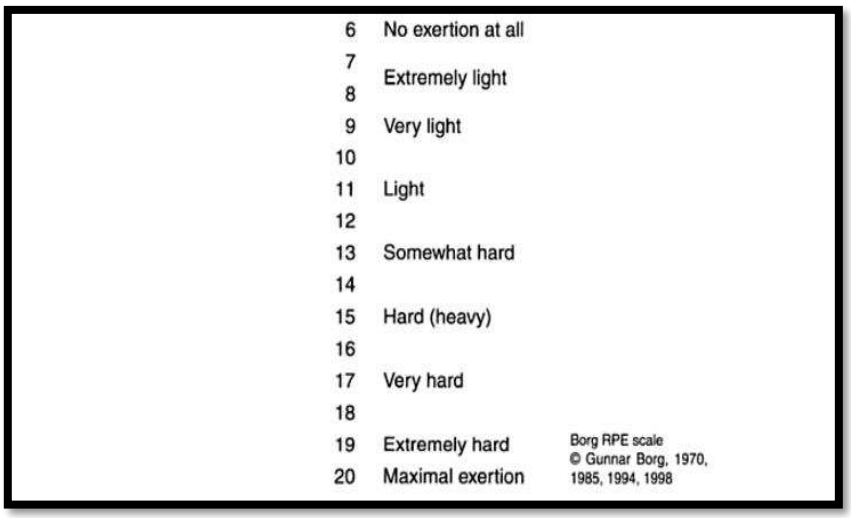


Figure 4.5: Fifteen-category Borg Rating of Perceived Exertion Scale (Borg, 1998 cited in Haile et al., 2014)

4.4 Protocol for Data Acquisition

The work of this thesis comprised of two preliminary studies (Chapter 5) and two experimental studies (Chapter 6 and 7) – as illustrated in the study design flowchart overleaf, figure 4.6. The preliminary studies were undertaken to establish the validity and reliability of the sensors and study protocol. For this, an in-vitro study (*preliminary study 1*) and an in-vivo study (*preliminary study 2*) were first undertaken and are presented in Chapter 5. Two experimental studies followed, where each study evaluated the effect of season on in-shoe microclimate in a specific cohort. Therefore, *study 1* investigated the influence of seasonal variation on in-shoe parameters in healthy participants, where the same study was undertaken twice (in summer and in winter). The same exact protocol was repeated in *study 2*, where in-shoe parameters were measured in participants living with diabetes. Below is a description of the protocol followed during the in-vivo (preliminary study 2) and experimental studies (study 1 and study 2) explaining those procedures which were common between the studies. Any differing procedures applied within each individual study are outlined in the specific experimental chapter (e.g. see Chapter 6, Section 6.5.2).

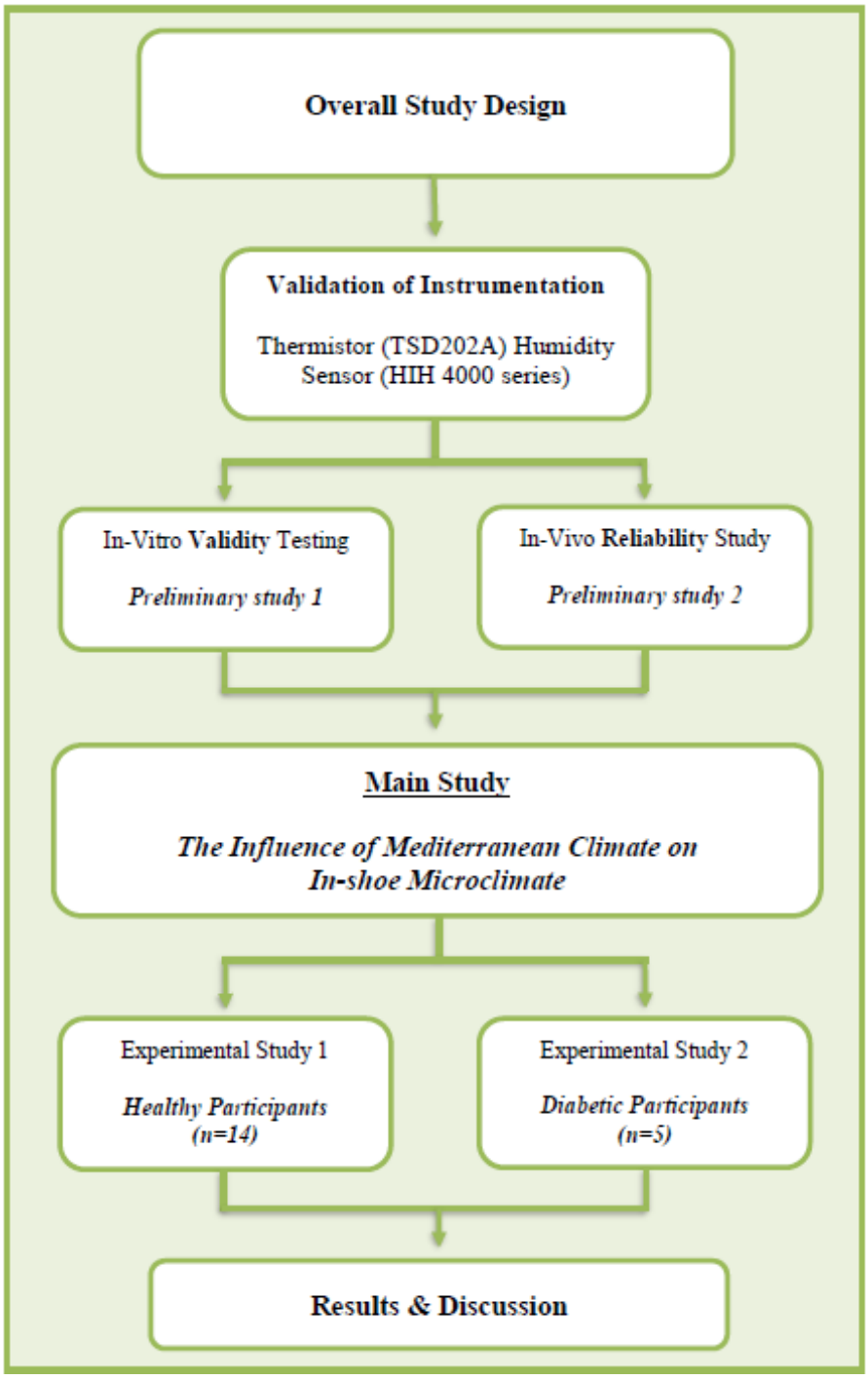


Figure 4.6: Study design employed

In each of the studies, since the primary aim was to assess the variability of ambulatory in-shoe microclimate between summer and winter, the same strict protocol was maintained during both seasons (trial 1 and trial 2), in order to minimize measurement errors (random & systematic errors) that may interfere with the interpretation of the results. These included, same sample population, identical conditions for both trials (such as shoes, socks and participant physical exertion level), accurate instrumentation, acclimatisation and placement of sensors.

1. Same sample population – The same sample population was used for both trials, in summer and winter. Also, the same time slot was kept for each participant in both experimental trials in order to minimize individual physiological variation. Also, participants were asked to refrain from consuming caffeine, alcohol or nicotine up to eight hours before the experiment, and not to perform any vigorous exercise up to 24 hours prior to data collection.

Caffeine: can decrease cerebral blood flow (Cameron, Modell et al. 1990) as well as antagonise A_1, A_{2A} and A_{2B} adenosine receptors in blood vessels, thereby reducing adenosine-mediated vasodilatation and consequently decreasing myocardial blood flow (Namdar, Schepis et al. 2009).

Alcohol: Consumption of alcohol 24 hours prior to exercise has also been shown to reduce aerobic performance by 11% (O'Brien, Lyons 2000).

Nicotine: Although the results are conflicting and some authors report increases in cutaneous blood flow and skin temperature (Usuki, Kanekura et al. 1998), others report a decrease in cutaneous blood flow and subsequent decline in skin temperature associated with nicotine consumption (Sørensen, Jørgensen et al. 2009, Leow, Maibach 1998).

2. Identical conditions for Trial 1 and Trial 2 –

   a. **Hosiery** – participants were supplied with identical socks to ensure the use of the same material for the two trials amongst all participants. The socks that were used were made of 75% cotton, 23% Polyamid and 2% Elasthane.

      Participants were asked to bring with them the shoes they normally use for daily walks. Most participants presented with normal trainers composed of rubber material for the outsole, EVA and Polyurethane composing the midsole with synthetic leather/mesh materials for the uppers.

   b. **Treadmill Speed** – Participants were asked to self-select their walking speed so that it reflected 'moderate' exertion, using the Borg RPE scale (see Section 4.3.1) (Borg 1990), in order to control for participant fatigue during the experimental trials. Indices of perceived exertion (RPE) is measured using the 6-20 Borg scale, a standard scale for experimental studies of this nature. This scale allows subjects to easily determine quantitatively the level of physical exertion that they are experiencing, and is relevant for the low level exercise asked of participants in this study. The numbers in the lower end of the scale (i.e. 6 and 7) represent "very, very easy", while the higher numbers (i.e. 19 and 20) indicate "very, very hard". The level chosen by each participants for experimental trial 1 was recorded and the same speed was kept during experimental trial 2.

3. Accurate Instrumentation - The limits of agreement of the temperature and humidity thermistor (when compared to gold standard instrumentation measuring the same variables) can be found in detail in Chapter 5, section 5.4.7.

4. Acclimatization - In scientific research involving measurement of skin temperature, acclimatisation in a controlled environment is an important part of the study design. Acclimatisation may be defined as the time necessary to achieve adequate stability in the participant's blood pressure and skin temperature. For this study, the protocol for acclimatization recommended by Roy et al. (2006) was followed. The authors recommend waiting 15 minutes for the optimal stabilization of skin temperature, with a minimum of 10 minutes. They also noted that, when acclimatization exceeds 30 minutes, temperature oscillation can occur, creating an asymmetry between the left and right sides of the subject. Therefore, participants were asked to lay in a supine position for 15 minutes on an experimental couch inside the data collection room, barefoot and without any surface contact on the plantar aspect of the foot. During this time, each participant had time to adapt to the room temperature, reaching stable skin temperature.

5. Placement of Sensors - Thermistors and humidity sensors were placed in close proximity at two distinct anatomical positions - between the hallux and second digit and below the navicular (Figure 4.7) as employed by Purvis and Tunstall (2004). These are clinically suitable locations, and this also enables data comparison with these authors' data (Purvis, Tunstall 2004). All sensors were placed by the researcher, who is also a clinician, to ensure uniformity. Each sensor was labelled so that same sensor was placed in the same anatomical position in every trial.

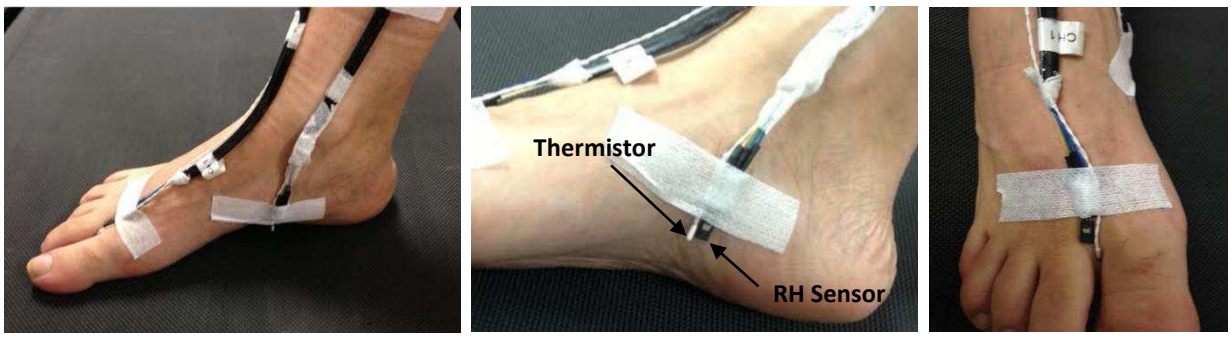


Figure 4.7 – Placement of sensors

4.4.1 General Experimental Procedure

All participants were assessed in an interview room adjacent to the experimental room, by an experienced podiatrist for any foot conditions that might interfere with data collection, such as skin abrasions and inflammation. Vascular and neurological status was also noted. Using a Doppler for assessing vascular status, only participants with tri-phasic and bi-phasic wave forms at the dorsalis pedis and posterior tibial artery, indicating good-to-satisfactory peripheral perfusion, were recruited. Moreover, peripheral neuropathy was assessed with a 10-gram Semmes Weinstein Monofilament. Participants unable to perceive the 10-gram force on any of the four sites – hallux, 1st MTPJ (metatarsophalangeal joint), 3rd MTPJ and 5th MTPJ indicated signs of peripheral neuropathy (Singh, Armstrong et al. 2005) and were excluded from the study. A list of the inclusion and exclusion criteria pertaining to each individual study are tabulated in their respective experimental chapters (e.g. see section 6.5.1).

Adherence to instructions mentioned in the information sheet (Appendix IV), such as having refrained from strenuous exercise, alcohol, caffeine and tobacco for 24 hours was verified. Demographic data was recorded. Also, height and weight measurements were recorded, from which the body mass index (BMI) was calculated. Once participants were deemed to be suitable candidates to take part in the research project they were asked to go to the experimental room, change into comfortable gear and prepare for acclimatization.

Once the clinical assessment was completed, each participant was asked to lay in a supine position, barefoot without any plantar surface contact for 15 minutes inside the data collection room (Roy et al, 2006), which was set to reflect ambient climate of summer and winter during the respective studies. Details explaining how this was achieved are presented in the specific experimental chapters (see Section 6.5.2 and 7.5.2). During these

15 minutes, each participant had time to acclimatize to the room temperature, reaching stable skin temperature.

In all experimental trials, four calibrated sensors (2 thermistors and 2 RH sensors) were placed on each foot directly on the skin by the researcher on the anatomical positions as shown in figure 4.7. Each sensor was fastened to the skin, using medical adhesive tape (Mefix® self-adhesive fabric tape) to reduce risk of movement during the test. The wires were secured at the participants' ankles and knees before being threaded up through the waist band of their shorts for safety. After it was ensured that all sensors were securely fastened to the skin, socks and shoes were worn and wiring was fastened to a belt at the hip area before participants started the trial. At this point it was ensured that sensors and wiring were comfortable and that they posed no hindrance to activity. Participants were then asked to walk on a treadmill at a comfortable self-selected speed for 38 minutes, representing moderate physical exertion as previously described in this section.

At this point it was ensured that sensors and wiring were comfortable and that they posed no hindrance to activity – figure 4.8 overleaf.

Figure 4.8 – Sample trial demonstrating experimental setup including connection wiring to Biopack.

4.5 Data Treatment

All data from the sensors was uploaded through the BIOPACK onto a laptop for statistical analysis. Data was first median filtered to smooth out any spikes and subsequently down sampled to 1 sample/min. The resulting humidity data was then adjusted according to temperature using the temperature compensation formula as stated in the datasheet for the humidity sensors. Every temperature sensor was physically paired with a humidity sensor for data collection using tape (see Figure 4.7 above), and the temperature value used for the humidity adjustment calculation was taken from the temperature sensor with which the particular humidity sensor was paired.

In data capturing sessions where a particular sensor was recording highly fluctuating values – probably due to a fault in the sensor, the recorded data for that particular sensor was eliminated. Moreover, the temperature and humidity plots were then visually inspected and any artefactual spikes with more than 5^{o}C/min or 5% RH/min, were eliminated. This is an improvement over the method employed by Keppler et al (2016) who replaced abnormal spikes by values derived from a linear interpolation procedure or by values derived from averaged data from a larger time span. With this method the authors created data points which were not really measured, possibly influencing final results.

4.6 Ethical Considerations

All studies received ethics approval from the local ethics committee (Appendix III), University of Malta, after a formal proposal was presented and were conducted in accordance with the Declaration of Helsinki principles. All participants were fully verbally briefed on the nature and purpose of each study, were familiarized with all procedures involved and received a detailed written explanation and description of the study in which

they were about to participate and an informed consent (in the language of their choice - Maltese/English), stating that they have fully understood the process and their involvement, before participation. Both the information sheet and the consent form were back-translated between Maltese and English and the wording was checked with bilingual speakers to make certain that the meaning was not lost in translation before information was used (Appendix IV).

All participants were informed, both verbally and in writing, that their name and personal information would remain strictly confidential, would not be identifiable in any way and would only be known and accessed on the computer by the researcher. To confirm this, participants were coded with a number. It was also made clear that if at any given time they wished to withdraw from the study they could do so without giving any justification and this would not affect them in any way.

Ethical concerns related to participants' physical and mental tiredness with repeated walking were also addressed. Clinical judgement on the quality of gait and specific, gentle enquiry at timed intervals during data collection were conducted. Furthermore, each participant was informed that if, at any time, they felt distressed and/or experienced any discomfort or pain for any particular reason, their participation would be terminated immediately. The environment of the experimental room where the trials were conducted, was risk-assessed as part of routine University practice. In addition, participants' safety was ensured by inspecting the study environment before each trial, eliminating any obstacles which might induce the risk of falling or tripping. In view of the vulnerability of the participants due to diabetes in one of the studies (Chapter 7), additional care was given in order to eliminate any possible known risks to injury. These have been discussed in more detail in its respective chapter (section 7.4).

Chapter 5

Validation, Calibration

and

Reliability Studies

5.1 Introduction

Measurement in research and other disciplines is critical for assessment in clinical trials and experimental decision-making (Jones, Manly et al. 2011). It is known that measurement is not perfect and some level of error needs to be accepted. However, an acceptable limit needs to be set in the magnitude of error associated with a new method of measurement, and is critical to understanding the limitations of this method. When using a new method of measurement, validity and precision are important concepts to be considered and are best evaluated using a standard reference or true value (Barnhart, Haber et al. 2007). As discussed in Chapter 3, section 3.4 every effort was made to identify the best methodological approach to be employed. Also, because of the relationship between temperature and humidity, as presented in Chapter 2 section 2.5, the need to control ambient temperature and humidity in such investigations was critical for the study to be valid (van Marken Lichtenbelt, Wouter D, Daanen et al. 2006a).

After several discussions and meetings with colleagues from various specialties, medical/biomedical engineers, two-possible methodological approaches were identified; (i) the use of an environmental chamber, or (ii) the use of 'gold standard' equipment located at the Standards and Metrology Institute Malta (MCCAA). A substantial amount of time was first dedicated in researching both medical and industrial fields. However, no environmental chambers where both temperature and RH can be manipulated were available in Malta for research purposes. Therefore, validity testing was conducted at the Standards and Metrology Institute Malta, where the Dew Point Mirror Humidity Generator (Figure 5.1) was used as the gold standard instrument for RH and the Platinum Resistance Thermometer (PRT) was used as a gold standard instrument for temperature.

This study established the validity and reliability of temperature and relative humidity measurements measured by thermistors and RH sensors respectively. These instruments were also calibrated against standard instruments.

5.2 Aims of the Validity, Reliability and Calibration Study

The aim of the studies presented in this chapter, was to establish the validity and reliability of the RH and temperature sensors to be used in the novel context of measurement of in-shoe variables at the interface between the shoe and the skin, and to calibrate these against standard instruments. Therefore, a series of in-vitro (laboratory-based) and in-vivo (on the skin of healthy human participants) studies were undertaken. University of Malta Research Ethics approval (Appendix III) was obtained for the in-vivo part of this study. Therefore, the aims were to determine:

i. Measurement error, if any, of the RH sensors and thermistors by comparing them to a gold standard instrument – Section 5.4, ***Preliminary Study 1***.

ii. Reliability and repeatability of the RH sensors and thermistors– Section 5.5, ***Preliminary Study 2***.

5.3 Study Design

In order to accomplish the stated aims, the work presented in this chapter, was conducted in two separate studies. The first study (***preliminary study 1*** – section 5.4) examined the accuracy and precision of the devices by comparing them to an established gold standard instrument - validity. Following this, individual corrections were applied to each sensor and rescaling parameters were adjusted. The second study (***preliminary study 2*** – section 5.5) examined the reliability of measures on human skin during walking by placing the sensors in different pre-defined areas of study on the foot - reliability.

5.4 **Preliminary Study 1** – Calibration, Validity and Reliability of the Relative Humidity Sensor and Thermistor.

The accuracy of the experimental sensors was determined by calculating the difference in scores obtained with the two methods of measurement, representing the 'bias' of the new instrument relative to the gold standard one. The accuracy (bias) estimates for this study of ±2% RH and ±0.2^{0}C were established a priori as the maximum parameters that will indicate acceptable agreement between RH sensors and thermistors respectively and the gold standard instrumentation. These values reflect respective inherent errors as stated by the manufactures of the sensors (Chapter 4, Equipment and General Methods). Each sensor was calibrated against standard instrumentation as detailed below.

5.4.1 Location of Data Collection

Sensor validity and calibration tests were undertaken at the Standards and Metrology Institute, Malta (MCCA), where separate experiments tested different sensors for measurement of temperature and relative humidity against standardised equipment that is routinely calibrated. The sensors, together with related hardware and software, were setup in the lab at the site of data collection (Figure 5.3).

No ethical approval was required since no humans or animals were involved in this study.

5.4.2 Aim of this Study

As noted above, the aim of this study was to determine the measurement error, if any, of the RH sensors and thermistors using a gold standard instrument for calibration, and demonstrating validity and reliability.

Assessment of the extent of agreement (Bland, Altman 2007) of the commercial sensors with standard instrumentation was undertaken as follows:

- humidity sensor (HIH 4000 series, Honeywell International Inc., MN, USA) with a Dew Point Mirror Humidity Generator - gold standard humidity measure
- thermistor (thermistor TSD202A, BIOPAC, Biopac Systems Inc., Goleta, CA, USA) with a Platinum Resistance Thermometer (PRT).

5.4.3 Hypotheses

Hypothesis 1:

H_0 - The null hypothesis states that there are no significant differences between the measurements obtained by the humidity sensors (HIH4100 series) and the Dew Point Mirror Humidity Generator.

H_1 - The alternate hypothesis states that there are significant differences between the measurements obtained by the humidity sensor (HIH4100 series) and the Dew Point Mirror Humidity Generator.

Hypothesis 2:

H_0 - The null hypothesis states that there is no significant difference between the measurements obtained by the thermistor TSD202A and the Platinum Resistance Thermometer (PRT).

H_1 - The alternate hypothesis states that are significant differences between the measurements obtained by the thermistor TSD202A and the Platinum Resistance Thermometer (PRT).

5.4.4 Methods

This section presents the methods employed to assess differences and agreement between the four RH sensors (Honeywell HIH-4000 Series) and the four thermistors (TSD202A) with the gold standard equipment. Physical principles, technical considerations and other details of the sensors used are provided in chapter 4. It was anticipated that, following these results, an adjustment process would be applied to the sensors before they were used to measure in-shoe microclimate. The process involved a first calibration which included a set of operations under specified conditions (see Section 5.4.5). Following this, when required, adjustment was applied to ensure that the instrument values are correct within specified limits. The sensors were then subjected to a second calibration to re-establish the difference between the measured values of the sensor and the corresponding values of the standard equipment. In this section, the Honeywell HIH-4000 Series and the TSD202A are referred to as the 'experimental' sensors (RH_{new} and T_{new} respectively).

The reference standard precision hygrometer used during calibration was a MBW 373 Dew Point Mirror (figure 5.1) held at the Malta Competition and Consumer Affairs Authority (MCCAA), the indicants of which are traceable to national standards and thus to international realizations of the S.I. units. The 373 Dew Point Mirror is a precision

hygrometer, satisfying the highest requirements in the measurement of humidity used as a true laboratory reference instrument, relying on proven optically detected chilled mirror techniques. In this chapter, the MBW 373 Dew Point Mirror will be referred to as the 'gold' standard equipment (RH_{ref}).

Figure 5.1: Dew Point Mirror Humidity Generator

A platinum resistance thermometer (PRT), known as a precision temperature-measuring device (due to its accuracy, stability and linearity), was used as a temperature reference during the calibration process (see Figure 5.2). It is generally accepted as the most accurate temperature measuring instrument available (Childs, Greenwood et al. 2000). The PRT consist of a fine platinum wire wound on an electrical insulator and connected to copper leads, in which the principle of measurement is the variation in the resistance of a platinum wire as a function of temperature (Michalski, Eckersdorf et al. 2002).

Figure 5.2: Fast response Platinum Resistance Thermometers (PRTs)

The humidity generator used during the calibration was a Thunder Scientific 2500 Benchtop "Two-Pressure" humidity generator (Figure 5.3).

Figure 5.3: Thunder Scientific 2500 Benchtop

A foam access port and plug on the side of the 2500 chamber (Figure 5.4) was removed and the hygrometer's sampler, the platinum resistance thermometer (PRT) and the ‘experimental’ sensors were inserted through the two-inch port and set-up in the humidity chamber as close as possible to each other in such a way to occupy a small volume of chamber space as possible (Figure 5.5). It was also ensured, in accordance with the European Cooperation for Accreditation of Laboratories (EAL-G31 1997), that the experimental sensors remained separated and did not touch the inner walls of the chamber or one another. This minimised as much as possible, the recorded humidity and temperature variations generated throughout the chamber space, which was set at predetermined levels as presented in section 5.4.5.3.

Figure 5.4: Access port plug of the 2500 Humidity Chamber

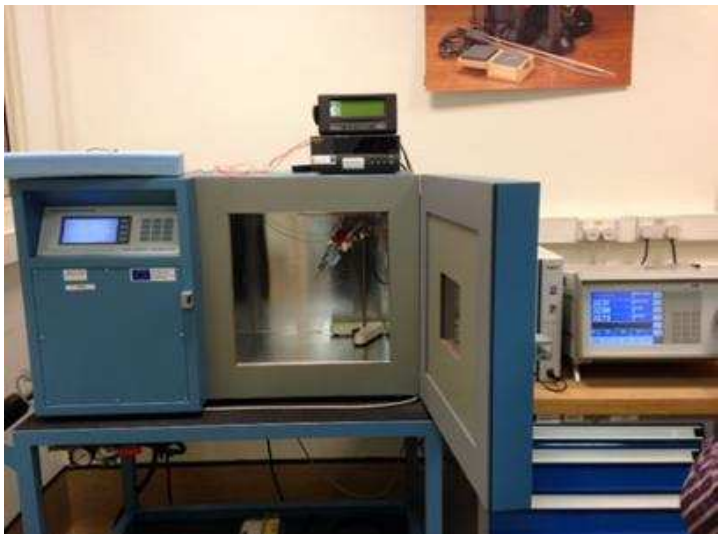


Figure 5.5: Sensors placed in the humidity chamber as close as possible to each other

All experimental sensors were individually connected to the data acquisition and analysis system - BIOPACK® MP150A-CE (Figure 5.5), which was interfaced with a laptop via USB. A biomedical engineer was present to ensure that the connectivity of the sensors, related hardware and software (AcqKnowledge 4.3+), worked seamlessly before and during

data capturing. The RH sensors and thermistors were programmed to record at a sample rate of 0.5 seconds. The raw data output recorded from the RH sensors and thermistors were in volts (V). The conversion of the 'experimental' sensor output signals into humidity and temperature units was effected by means of their transduction characteristics as set by the parameters under the re-scaling setup, consistently with the sensors' conditions of use by the formula below:

Humidity [%] = m x (Output) [V] + n

Temperature [^{0}C] = m x (Output) [V] + n

Figure 5.6: BIOPACK ® MP150A-CE hardware

5.4.5.1 Calibration Process for Humidity sensor, Honeywell HIH-4000 Series

The calibration process was initiated by setting the generator through a drying stage, maintained for 6 hours at 20% RH at a 23°C state. After this stage, the test process was initiated at pre-determined relative humidity states of 50%, 65%, 80% and 95% at a pre-set isotherm. For this study, the process was repeated at the pre-determined isotherms of 20^0C, 30^0C, 40^0C & 50^0C, as explained in section 5.4.5.3. Each selected relative humidity state was maintained for about 4 hours for each isotherm (to ascertain that a significant plateau by the sensors is reached and well sampled). Finally, the generator was set again to the drying phase, after which the generator was set at 50% RH at a 23°C state - the ambient and conclusive state. For each isotherm the process always started from the drying state and went through the whole process as described above.

After calibration, the sensor readings were adjusted (re-scaled) as part of the calibration process (see results section 5.4.7). After adjustment, the calibration process described above was repeated, always starting from the drying stage, then set to the pre-set RH states (50%, 65%, 80% & 95% RH) at 30^0C. This isotherm point was chosen since it was the closest to the average in-shoe temperature reported during a pilot study. The process ended with the drying phase.

5.4.5.2 Calibration Process for Thermistors TSD202A

For the thermistors, the same process of calibration was repeated as per humidity sensors, starting with the drying stage. The chamber was then set at a constant humidity level of 50% at pre-determined isotherm points - 20^0C, 30^0C, 40^0C & 50^0C, maintained for four hours per isotherm. Following this, thermistor readings were adjusted (re-scaled) as part of the calibration process. After adjustment, the calibration process was repeated at 50%, 65%, 80% & 95% RH at 30^0C.

5.4.5.3 Justification of Pre-Determined RH and Temperature Levels

The temperature and relative humidity levels that were selected reflect what is stated to be Malta's climate norm (see Section 1.2). The overall variation in temperature is, to a large extent, due to the regional weather patterns in the Central Mediterranean and the influence from the surrounding sea, which has a warming influence in winter, and a cooling influence in summer (Galdies 2011). Therefore, taking into consideration that in Malta the lowest mean temperature is 5^0C (winter) and the highest mean temperature is 37^0C (summer), which may increase in-shoe during walking, the ranges identified for testing the thermistor were set at 4 different temperatures; 20^0C, 30^0C, 40^0C and 50^0C. Similarly, since Malta's ambient RH varies from a minimum of 61% to max of 87% (Galdies 2011) the ranges identified for the validity test of the humidity sensor was set at 4 different levels of RH: 50%, 65%, 80% and 95% RH.

5.4.6 Data Analysis

In order to assess validity of the RH sensors and thermistors, data were analysed both for correlation and limits of agreement (differences) between each individual 'experimental' sensor and the gold standard equipment; Dew Point Mirror Humidity Generator (RH_{ref}) and the Platinum Resistance Thermometer (T_{ref}). The Intra-class Correlation Coefficient (ICC), which is a global measurement of reliability and Bland & Altman Limits of Agreement method (LoA), which provides information about the distribution of differences of measurements, or a combination of both tests, are the most commonly-used methodologies for assessing agreement in relation to continuous variables (Streiner, Norman et al. 2014, Bland, Altman 1986, Kottner, Audigé et al. 2011). This approach was applied for statistical analysis of the data recorded for this study where the ICC was used to establish correlation and the Bland and Altman test was used to establish limits of agreement (differences) between the 'experimental' sensors and the gold standard equipment. Very low bias, high correlation, low typical error and narrow 95% LoA

demonstrate good agreement between methods of measurement. The following sections provide justification explaining the choice of statistical methods.

Correlation

Intra-class correlation coefficient (ICC), a two-way random single-measure model, was chosen as the statistic of choice following recommendations by Bruton, Conway, & Holgate (2000). An important application of this statistical test is the assessment of consistency (correlation) and/or agreement of quantitative measures between two measuring devices (Bruton, Conway et al. 2000). When establishing correlation, the ICC reports values between 0 and 1, based on analysis of variance techniques. It is close to 1 when the differences between paired measurements is very small compared to the differences between subjects (Giavarina 2015). When compared to other procedures, such as the t-test and Pearson product moment correlation coefficient, the intra-class correlation coefficient is considered to be a more plausible statistical test because it can be closer to 1 only if there is no bias and the paired measurements are in good agreement (Kottner, Audigé et al. 2011). However, agreement measures of ICC are less reliable than those obtained by the LoA, due the intrinsic dependence of ICC on variance (de Vet, Terwee et al. 2006), therefore, ICC was used mainly to establish correlation.

Limits of Agreement

The Bland and Altman Limits of Agreement statistical test is often used to compare two methods of measurement, or a new method with an established one. It determines whether these two methods can be used interchangeably or the new method can replace the established one. This method of analysis is documented in a series of papers by J. Martin Bland and Douglas G. Altman (2007, 1999, 1986). Various published clinical and laboratory studies have analysed their data by evaluating agreement between two measurement methods using Bland-Altman analysis (Opdam, Wan et al. 2007, Niedhart, Kaiser et al. 2006, Anderson, Sartipy et al. 2007, Button, Weibel et al. 2007). Bland and Altman recommended

the use of plots, which is particularly useful as it allows a visual understanding of the agreement between two quantitative measures (Bland, Altman 1999). The Bland–Altman method calculates the mean difference between two methods of measurement (the 'bias'), and 95% limits of agreement as the mean difference (2 SD) [or more precisely (1.96 SD)] (Bland, Altman 1986). It is expected that the 95% limits include 95% of differences between the two measurement methods. The smaller the range between these two limits, the better the agreement is. For the purpose of this study, this statistical test was the method of choice as it gives a schematic representation of the measurement error between the reference 'gold standard' instrument and the 'experimental' sensors.

Data were analysed using MedCalc version 15.6 (MedCalc®, Meriakerke, Belgium, http:www.medcalc.be/). This is a complete reliable statistical package for Windows designed for biomedical researchers.

5.4.7 Results

The RH and temperature values were analysed for each humidity sensor and thermistor to establish correlation and limits of agreement compared to the 'gold' standard equipment; Dew Point Mirror Humidity Generator and the Platinum Resistance Thermometer (RH_{ref} and T_{ref} respectively, the instruments used and calibrated to international standards by the Maltese Metrological Office). The sections below present statistical tests (ICC and Bland and Altman LoA) applied to the data before and after adjustment. Since there was negligible difference between the data reported for the four RH sensors and the four Thermistors, only the data for RH sensor 1_{new} and Thermistor 1_{new} will be presented and discussed in detail below. Results for RH sensors 2_{new}, 3_{new} & 4_{new} and Thermistor 2_{new}, 3_{new} & 4_{new} are included in Appendix VI.

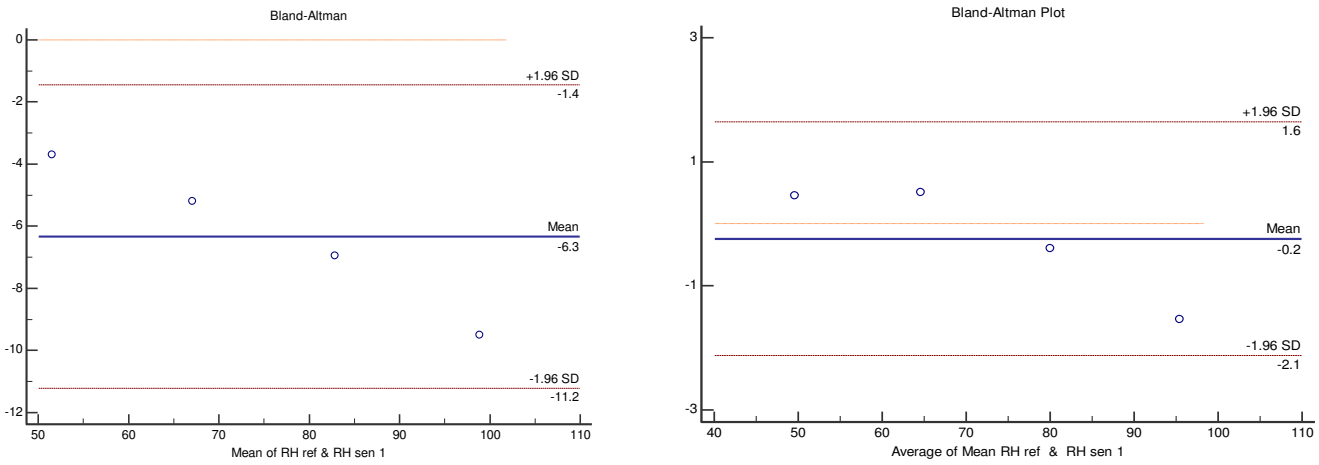
Comparison between RH_{ref} & RH Sensor 1_{new}

The table below presents ICC and Bland and Altman results between RH_{ref} and RH sensor 1_{new}. Raw data for RH sensor 1_{new} BA (*Before Adjustment*) and AA (*After Adjustment*) is presented in Appendix V. Results below show the data as analysed *before adjustment*.

(a) Before Adjustment

Table 5.1: Correlation and Level of Agreement RH Sensor 1_{new} *before adjustment*			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
*Temp. Level @ **20°C***	**ICC** *Single Measures r* 0.99	0.89	1.0
	Bland & Altman *Mean Difference (±SD)* -6.3 (±2.5)	-11.22	-1.44
*Temp. Level @ **30°C***	**ICC** *Single Measures r* 0.99	0.92	1.0
	Bland & Altman *Mean Difference (±SD)* -4.2 (±2.1)	-8.34	-0.04
*Temp. Level @ **40°C***	**ICC** *Single Measures r* 1.00	0.93	1.0
	Bland & Altman *Mean Difference (±SD)* -2.4 (±1.9)	-6.08	1.33
*Temp. Level @ **50°C***	**ICC** *Single Measures r* 1.00	0.98	1.0
	Bland & Altman *Mean Difference (±SD)* -0.2 (±1.0)	-2.12	1.64

Results show that ICC values before adjustment ranged from 0.98 to 1.0 [95% CI 0.89, 1.0] when analysed at the pre-determined isotherms, indicating a very strong correlation between the two instruments. It is generally accepted that an arbitrary cut-off of >0.75 for the ICC indicates good correlation (Chien, Khan 2001, Kramer, Feinstein 1981). This implies that even before adjustment the experimental sensors demonstrated a high correlation with readings obtained from the gold standard equipment.



(i) (ii)

Figure 5.7: Bland and Altman plots for the data presented in table 5.1 at isotherm 20^0C **(i)** & 50^0C **(ii)**

Bland and Altman plots illustrate the level of agreement between the RH_{ref} instrument and RH sensor 1_{new}. Plots for all RH sensors and all isotherms are presented in Appendix VI. The plot presents the mean difference between both instruments providing an estimate of the systematic error or bias and the 95% limits of agreement - random error (Bland, Altman 1986). The solid line in the plot indicates the mean of the paired differences (mean RH_{ref} – mean RH sensor 1_{new}) – its distance from zero provides an estimate of the bias between the reference instrument and experimental sensor. The dashed lines indicate the

estimated limits of agreement and their CI limits. Results indicate that the mean value of measurement (bias) and the standard deviation of this difference were larger at low isotherms [-6.33 ±2.49 SD at 20^0C] – figure 5.7 (i), and decreased linearly with increasing temperature, achieving lowest bias at 50^0C [-0.2418 ±0.9599 SD] – figure 5.7 (ii). Similarly limits of agreement (LoA) were wider at lower isotherms [95% CI -11.2169, -1.4451 at 20^0C], decreasing at higher isotherms [95% CI -2.1231, 1.6396 at 50^0C]. These results imply that *before adjustment* the RH sensor 1_{new} reported poor agreement at 20^oC and 30^0C since SD exceeded ±1.96. However, at 40^0C and 50^0C the RH sensor 1_{new} demonstrated acceptable agreement with the gold standard instrument since SD was less than ±1.96.

Therefore, *before adjustment*, despite a high correlation reported by ICC even at lower isotherms, the Bland and Altman method demonstrated poor agreement at the same isotherms. Furthermore, raw data indicated (Appendix V) that when paired mean relative humidity values were compared across all temperature levels, (*before adjustment*) measurements from the RH sensor 1_{new} were higher than the RH_{ref} (median difference 2.77%; mean difference 3.28%), indicating that the RH sensor 1_{new} consistently reported higher relative humidity readings compared to the gold standard equipment.

(b) <u>After Adjustment</u>

Following the application of adjustment, raw data indicates that mean measurements from the RH sensor 1_{new} were slightly higher than the RH_{ref} (median difference 0.48%; mean difference 0.60%).

Table 5.2: Correlation and Level of Agreement RH Sensor 1_{new} – *<u>Before & After Adjustment</u>*					
Before Adjustment			After Adjustment		
Statistical Test	95% CI		Statistical Test	95% CI	
	Lower Limit	Upper Limit		Lower Limit	Upper Limit
ICC *Single Measures* *r* **0.99**	0.92	1.0	**ICC** *Single Measures* *r* **1.0**	1.0	1.0
Bland & Altman *Mean Difference (±SD)* **-4.2** (±2.1)	-8.3	-0.0	**Bland & Altman** *Mean Difference (±SD)* **-0.6** (±0.6)	-1.8	0.6

Results indicate that although ICC reported a high correlation between RH_{ref} and RH sensor 1_{new} *before adjustment* [*r* = 0.99; 95% CI 0.92, 1.0], this was further improved indicating perfect correlation [*r* = 1.0; 95% CI 1.0, 1.0] after adjustment.

Before adjustment the Bland and Altman plot (figure 5.8 **i**) indicated poor agreement between the two instruments with a bias of -4.2, a SD (±2.12) exceeding ±1.96 and 95% CI -8.3, -0.0. However, *after adjustment*, Bland and Altman plot (figure 5.8 **ii**) showed improved agreement with a mean difference of -0.6 (±0.61) and 95% CI -1.8, 0.6. It is therefore

expected (with 95% confidence) that *after adjustment*, the difference in the RH reading, as measured by the two instruments, will be between 0.6 and -1.8. These results imply that readings from the RH sensor 1 $_{new}$ are in close agreement with the gold standard instrument, even performing better than the manufacture's specification (±3.5%). Therefore, *after adjustment* the null hypostasis is accepted and the alternative hypothesis is rejected.

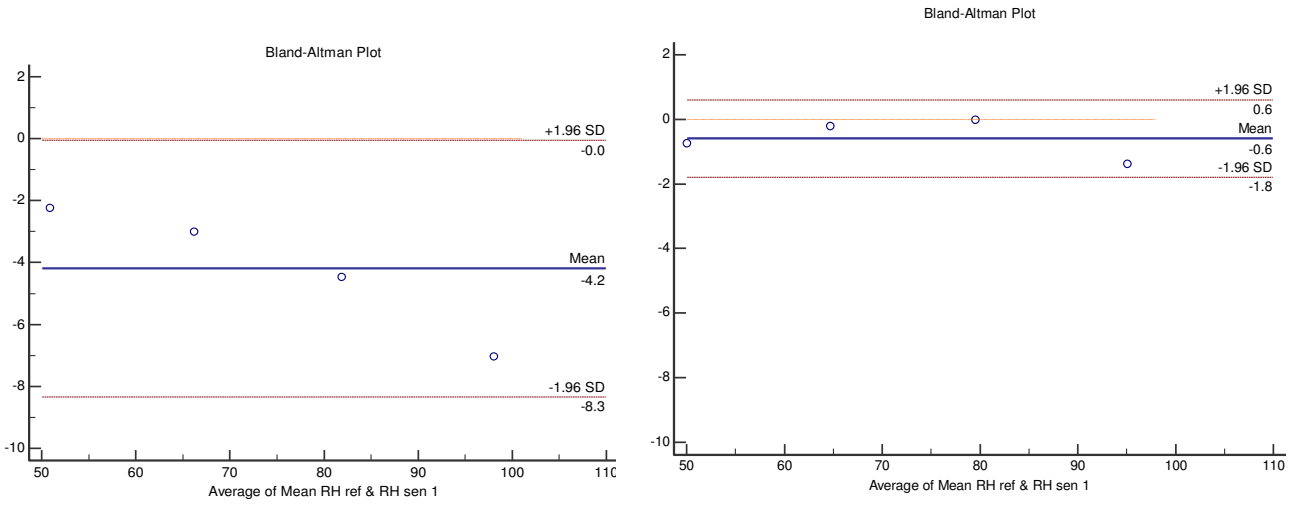


Figure 5.8: Bland & Altman plot illustrating differences in RH measured by RH_{ref} (gold standard instrument) and RH sen_1 against their means at 30^{0}C *before adjustment* **(i)** and *after adjustment* **(ii)**. Solid line represents mean; upper dashed line shows the mean +1.96 SD and lower dashed line the mean -1.96 SD, each with 95% CI.

Comparison between PRT (T_{ref}) & Thermistor 1_{new}

The table below presents ICC and Bland and Altman results when T_{ref} and Temperature sensor 1_{new} were compared. Results below are from data analysed *before adjustment*.

Before Adjustment

Table 5.3: Correlation and Level of Agreement of T_{ref} & Thermistor 1_{new} – *Before Adjustment*

Statistical Test		95% CI	
		Lower Limit	Upper Limit
RH Level @ 50%	**ICC** *Single Measures r* 1.00	0.97	1.00
	Bland & Altman *Mean Difference (±SD)* -0.4 (±0.8)	-2.0	1.1

Results show that ICC values *before adjustment* for thermistor 1_{new} is 1.0 [95% CI 0.97, 1.0] when analysed at the pre-determined isotherms, indicating a high correlation between the two instruments. It is generally accepted that an arbitrary cut-off of > 0.75 for the ICC indicates good correlation (Kramer, Feinstein 1981, Khan, Elhadd et al. 2000). This implies that even before adjustment the experimental temperature sensors demonstrated a high correlation with readings obtained from the gold standard equipment (PRT).

In order to analyse the extent of agreement between the thermistor 1_{new} and the gold standard instrument, Bland and Altman plots were created. As described earlier, differences between the two measuring instruments were plotted against their mean and

the limits of agreement were calculated as the mean difference ±2 SD of the difference. In normally distributed data, 95% of the data will fall between 2 SDs from the mean.

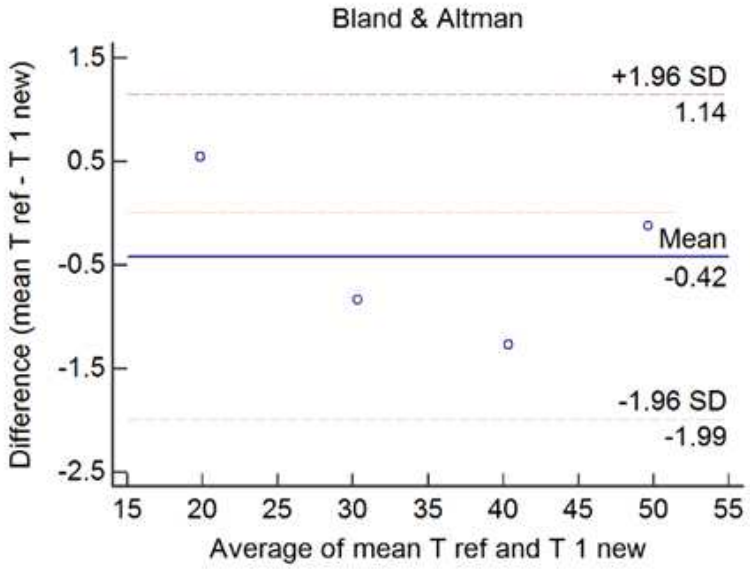


Figure 5.9*:* Bland and Altman plot for the data presented in table 5.3 at 50% RH

Results derived from the Bland and Altman plots are illustrated in table 5.3. *Before adjustment* the Bland and Altman plot (figure 5.9) indicated strong agreement between the two instruments with a bias of -0.42^{0}C, a SD (±0.8) and 95% CI -1.96, 1.14. It is therefore expected (with a 95% confidence) that before adjustment, the difference in temperature readings as measured by the two instruments will be between 1.14 and -1.96 for 95%. These results imply that *before adjustment*, readings from the Thermistor 1_{new} are in close agreement with the gold standard instrument. In view of this, no further adjustment was required and further tests at different RH levels at 30^{0}C were undertaken (see Table 5.4).

Table 5.4: Correlation and Level of Agreement of T_{ref} & Thermistor 1_{new}			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp Level @ 30°C	**ICC** *Single Measures r* 0.91	0.15	0.99
	Bland & Altman *Mean Difference (±SD)* -1.37 (±0.01)	-1.4	-1.3

The estimated reliability between the two methods of measurement is 0.91, with 95% CI [0.15, 0.99] indicating a high correlation between the 'gold' standard and the 'experimental' thermistor with wide CI limits. An alternative way of exploring the reliability between the measurements of the two instruments is the Bland and Altman plot (figure 5.10).

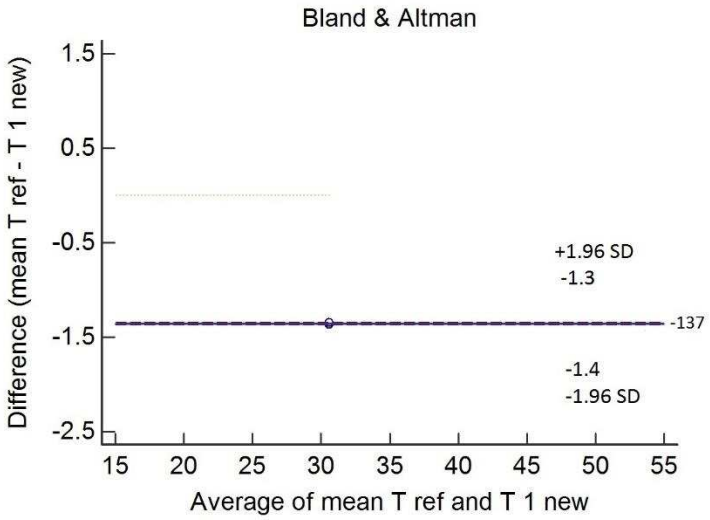


Figure 5.10: Bland and Altman plot for the data presented in table 5.4 at 30^0C

The Bland and Altman plot (figure 5.10) shows good agreement with a mean difference of -1.37^{0}C (±0.01) and 95% CI of -1.3 to -1.4^{0}C. It is therefore expected that the difference in temperature reading as measured by the two instruments would be between -1.3 and -1.4^{0}C for 95% of future measurements. These results imply that readings from the Thermistor 1_{new} are in close agreement with the gold standard instrument, performing better than the manufacture's specifications (±0.2^{0}C). Hence, the alternate hypothesis is accepted and the null hypothesis is rejected.

5.5 **Preliminary Study 2** – Reliability of the 'Experimental' Sensors

Following results obtained from study 1 (section 5.4), a reliability study was designed to assess consistency of the 'experimental' sensors when used to measure in-shoe micro-climate at the interface between the skin and the shoe during ambulation. There are many techniques used to measure reliability. These have been discussed in detail previously (Chapter 3) together with the justification of the method of choice for this study.

5.5.1 Study Design

Test re-test reliability (repeatability) of the experimental sensors, was determined by repeating the same experiment twice on different days to the same set of healthy participants under the same exact conditions using the same protocol, and then correlating the two measurements made at Time 1 and that at Time 2.

5.5.2 Location for Data Collection

The experiment was performed at the Biomechanics Laboratory, Podiatry Department, University of Malta. All equipment was setup with the support of a biomedical engineer who ensured that the connectivity of the sensors, related hardware and software (AcqKnowledge 4.3+), worked seamlessly before and during data capturing. Each sensor (x4 RH sensors & x4 Thermistors) was individually connected to data acquisition and analysis system - BIOPACK® MP150A-CE, set up in close proximity to a motorised treadmill. The environment is risk-assessed and is set up for students' use, often working with patients and other participants so there was no deviation in this study from normal use.

5.5.3 Ethical Approval

Ethical approval was granted first by the Faculty of Health Sciences Ethics Board and consequently by the University of Malta Research Ethics Committee. All participants were healthy adults who volunteered to participate in the study and signed an informed consent after being provided with an information sheet. All relevant documentation is provided in Appendix III.

5.5.4 Aim of the Study

The aim of this study was to assess the reliability of the thermistor (TSD202A) and humidity sensor (HIH4100 series) by determining the correlation between repeated measures (time 1, time2).

5.5.5 Hypotheses

Hypothesis 1:

H_0 - The null hypothesis states that there is no significant difference and a significant correlation exists between the test re-test measurements obtained by the humidity sensors.

H_1 - The alternate hypothesis states that there is a significant difference and no significant correlation exists between the test re-test measurements obtained by the humidity sensors.

Hypothesis 2:

H_0 - The null hypothesis states that there is no significant difference and a significant correlation exists between the test re-test measurements obtained by the thermistors.

H_1 - The alternate hypothesis states that there is a significant difference and no significant correlation exists between the test re-test measurements obtained by the thermistors.

5.5.6 Methods

The aim of this study was to measure in-shoe temperature and humidity of healthy participants during walking in a controlled ambient temperature and humidity laboratory using a pre-devised protocol. Since the test re-test reliability was identified as the method of choice, data collection was organized on two consecutive days (from now on referred to as – 'trial 1' and 'trial 2'). To minimise possible changes in the participant's physiological or physical state due to circadian rhythm, trials were performed at the same time of the day, on separate days. Also, a number of other parameters where taken into consideration by the researcher in order to minimise measurement errors (random & systematic errors) that may interfere with the interpretation of results and ensure internal validity. Therefore, in preparation for the reliability study, confounding variables were identified and addressed where possible, to minimise sources of error as discussed in section 4.4.

5.5.7 Participant Selection and Data Acquisition

A number of inclusion/exclusion criteria were designed for participants to be eligible for this study.

Table 5.5: Inclusion/exclusion criteria for participant in preliminary study 2

Inclusion Criteria	Exclusion Criteria
• Males & females • Aged over 18 years • Not taking any medication • Intact epidermis • No signs of neuropathy/peripheral disease	• Any foot deformity • Foot pain • Diabetes mellitus • History of foot ulceration • Participants showing unwillingness to participate.

Six participants, one male and five females, with a mean age of 33.1 years (±5.6), 68.67 kg (±12.47), 176.13 cm (±11.00), were recruited by convenience sampling through an invitation letter sent to all staff at the Faculty of Health Sciences, University of Malta. This sampling technique (convenience sampling) is used when no specific population is to be investigated. The scope of this study was to investigate the functionality of the sensors rather than participant characteristics, therefore, gender was not relevant since participants acted as their own control. Ethical approval was sought and granted by the University of Malta Ethics Committee. Informed consent was provided by each participant prior to the beginning of the study (Appendix IV). A description of the experimental procedure which was followed during both trials is provided in section 4.4.1.

5.5.8 Data Analysis

In order to establish the reliability of the RH sensors and thermistors in vivo, data were analysed for both relative reliability and absolute reliability, between the two sets of data observed in trial 1 and trial 2. Intra Class Correlation Coefficient (ICC) is a standard test (section 5.4.6) providing an estimate of relative reliability for consistency of measurement in between two consecutive trials. If the test is reliable, and the observed scores will not change from trial 1 to trial 2, then a high value of *r* is expected. Confidence Interval was set at 95% level. Cicchetti (1994) has recommended the following ranges to interpret the reliability of clinical instruments by using ICC: less than 0.40, poor; 0.40 to 0.59, fair; 0.60 to 0.74, good; and 0.75 to 1.00, excellent.

The Paired Sample T-Test was used to determine whether scores for trial 1 and trial 2 differ from each other - Absolute Reliability. Error level set at 0.05.

5.5.9 Results

Of particular relevance to this study was the controlled environment of the data collection room. The ambient RH and temperature were recorded for Trial 1 and Trial 2. Table 5.6 shows that the mean RH for Trial 1 was 61.6% (SD ±6.2) and for Trial 2, mean RH was 65.7% (SD ±4.2). Mean temperature for Trial 1 was 22.7^0C (SD ±0.4) and for Trial 2, mean temperature was 22.4^0C (SD ±0.3). The mean ambient RH and temperature for Trial 1 and Trial 2 were tested for significant difference to investigate whether similar ambient environment conditions were maintained across both trials. No significant difference was found using paired sample t-test for both ambient RH (p = 0.174) and ambient temperature (p = 0.305) between Trial 1 and Trial 2.

***Table 5.6*: Data collection room – Analysis for controlled environment** Paired sample t-test			
Ambient Environment	Mean	Standard Deviation (SD)	Sig. 2-Tailed (Paired Sample T-Test)
Relative Humidity Trial 1	61.6 (RH%)	6.25	**0.17**
Relative Humidity Trial 2	65.7 (RH%)	4.17	
Temperature Trial 1	22.7 (^{0}C)	0.45	**0.30**
Temperature Trial 2	22.4 (^{0}C)	0.26	

In-shoe Temperature Results

As described previously, two thermistors on each foot were placed between the hallux and 2nd toe and below the navicular. For the purpose of ease of understanding, the thermistors will be referred to as T_{Toe} representing reading from thermistor located between hallux and 2nd toe and T_{Arch} representing temperature reading from thermistor located below the navicular.

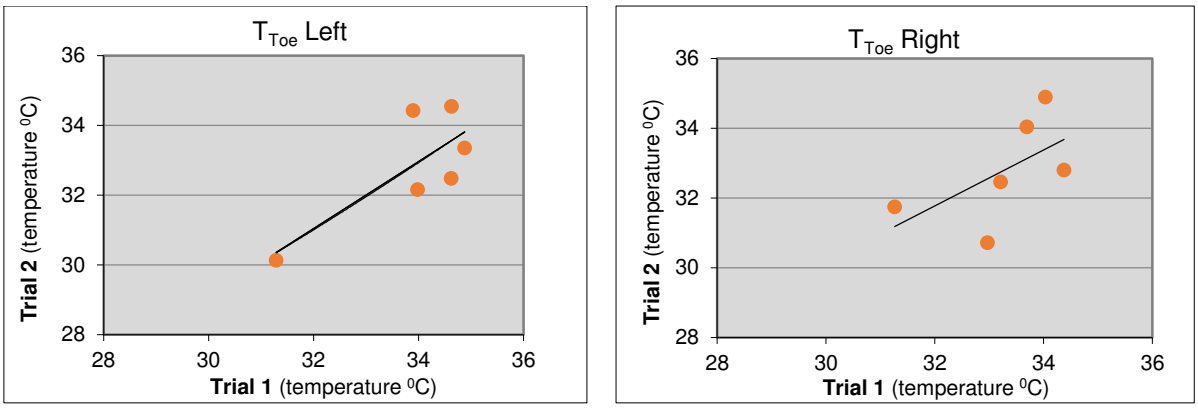
As described in section 5.5.8, the data were analysed to investigate reliability of the sensors using intra-class correlation coefficient (ICC) and paired sample t-test. The correlation between the two sets of measurements from the two trials was calculated. The temperature data from thermistors T_{Toe} and T_{Arch}, left and right from Trial 1 were compared to temperature data recorded for T_{Toe} and T_{Arch} left and right from Trial 2. Table 5.7 shows descriptive measures, intra-individual SD, measurement error, ICC and mean differences for each sensor.

Table 5.7: ICC and Paired Sample t-test (in-shoe temperature) for thermistors located in the forefoot (T_{Toe}) and arch (T_{Arch}) -Trial 1 and Trial 2							
	Mean (n=6)	Change in Mean	Standard Deviation (SD)	Lower Conf. Limit	Upper Conf. Limit	Sig. 2-Tailed (Paired Sample T-Test)	Intraclass Correlation Coefficient (ICC)
Left T_{Toe}	33.4 (^{0}C)	-1.0	1.0	-1.9	-0.17	**0.06**	**0.86**
Right T_{Toe}	33.0 (^{0}C)	-0.48	1.2	-1.51	0.55	**0.39**	**0.69**
Left T_{Arch}	33.6 (^{0}C)	-0.34	0.6	0.16	-0.84	**0.23**	**0.84**
Right T_{Arch}	34.4 (^{0}C)	-0.65	0.7	-1.23	-0.07	**0.08**	**0.88**

Results indicate good to excellent repeatability of measurement between thermistors T_{Toe} and T_{Arch} left and right with ICC ranging from 0.69 – 0.88.

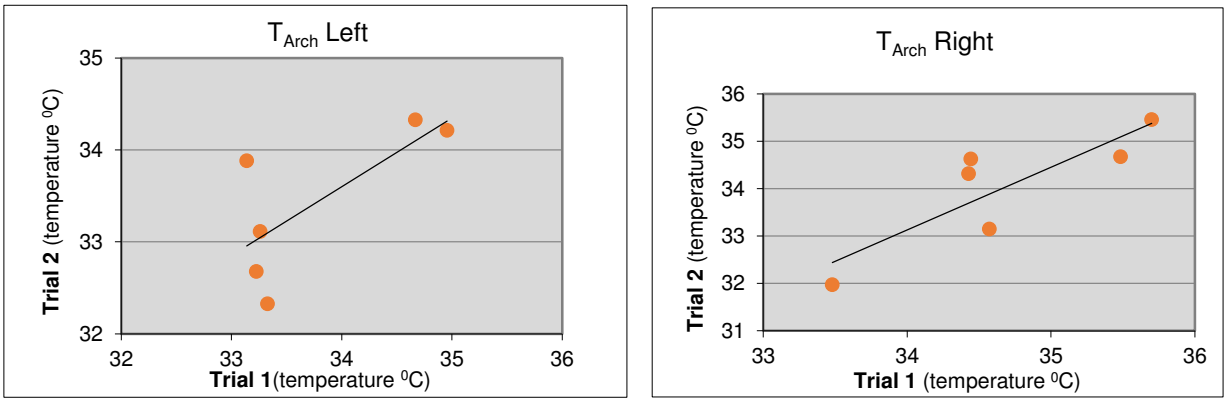
Agreement Between Test and Re-test

Agreement between test and re-test (trial 1 and 2) for each sensor (T_{Toe} left and right / T_{Arch} left and right) are illustrated in a scatterplot below (Figure 5.11 and 5.12). The scatterplot displays trial 1 on the horizontal (x) axis, and trial 2 on the vertical (y) axis. Each participant is identified by a single dot on the graph which is located so that the coordinates of the point (the X and Y values) match the participant's mean temperatures recorded in trial 1 and trial 2. The straight line represents identical temperature readings on re-test. The mean temperatures for trial 1 was subtracted from that of trial 2 giving the change in mean. This ranged from -0.34 to -1.00 [±SD 0.6 to 1.2], which incorporates both the systematic and random change.



(a) **(b)**

Figure 5.11: Scatterplot of test retest for temperature mean scores, **(a)** T_{Toe} left temperature and **(b)** T_{Toe} right temperature.



(a) **(b)**

Figure 5.12: Scatterplot of test retest for temperature mean scores, **(a)** T_{Arch} left temperature and **(b)** T_{Arch} right temperature.

In-shoe Relative Humidity

As described previously in the methods section (Chapter 4, Section 4.4), two humidity sensors on each foot were placed between the hallux and 2nd toe and below the navicular. For the purpose of ease of understanding, the humidity sensors will be referred to as RH_{Toe} representing readings from humidity sensor placed on the forefoot between hallux and 2nd toe and RH_{Arch} representing RH readings from humidity sensor below the navicular in the arch of the foot.

As described in section 5.5.8, the data were explored to assess reliability of the sensors using intra-class correlation coefficient (ICC) and paired sample t-test. A comparison of the reliability of measurements from two trials was performed. The RH data from humidity sensors RH_{Toe} and RH_{Arch}, left and right from Trial 1 were compared to RH data recorded for RH_{Toe} and RH_{Arch} left and right from Trial 2. Table 5.8 presents descriptive measures, intra-individual SD, measurement error, ICC and the mean differences for each sensor.

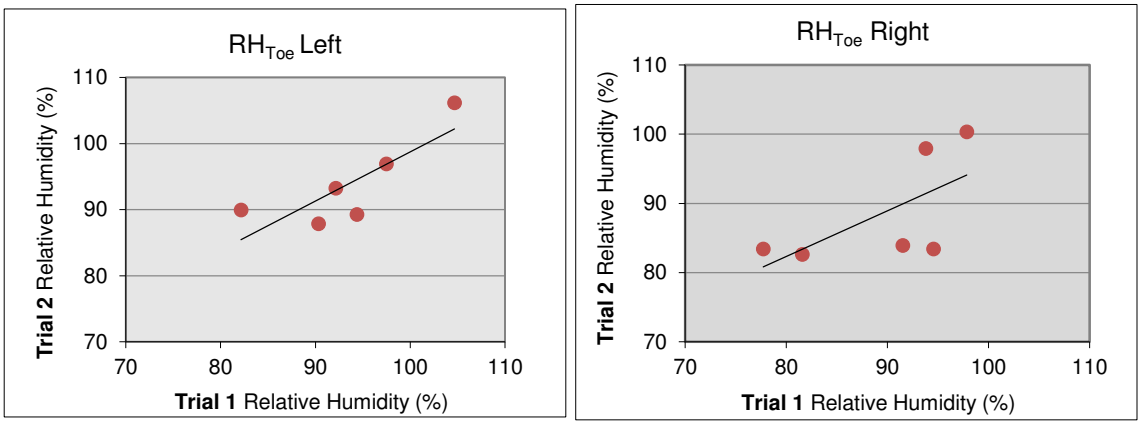
Table 5.8: ICC and Paired Sample t-test (in-shoe relative humidity) for RH sensors located in the forefoot (RH_{Toe}) and arch (RH_{Arch}) -Trial 1 and Trial 2

	Mean (n=6)	Change in Mean	Standard Deviation (SD)	Lower Conf. Limit	Upper Conf. Limit	Sig. 2-Tailed (Paired Sample T-Test)	Intraclass Correlation Coefficient (ICC)
Left T_{Toe}	93.7 (RH %)	0.4	4.4	-3.2	-4.0	**0.86**	**0.91**
Right T_{Toe}	89.1 (RH %)	-0.9	6.8	-6.5	4.7	**0.76**	**0.77**
Left T_{Arch}	77.7 (RH %)	-5.1	7.1	-10.2	0.7	**0.14**	**0.71**
Right T_{Arch}	76.7 (RH %)	-3.0	6.3	--8.1	2.2	**0.30**	**0.90**

Results indicate good to excellent repeatability of measurements of humidity sensors RH_{Toe} and RH_{Arch} left and right with an ICC ranging from 0.71 to 0.91. The data for RH_{Arch} left and right foot were analysed in the same way and readings from Trial 1 were compared to those from Trial 2 and tested for reliability

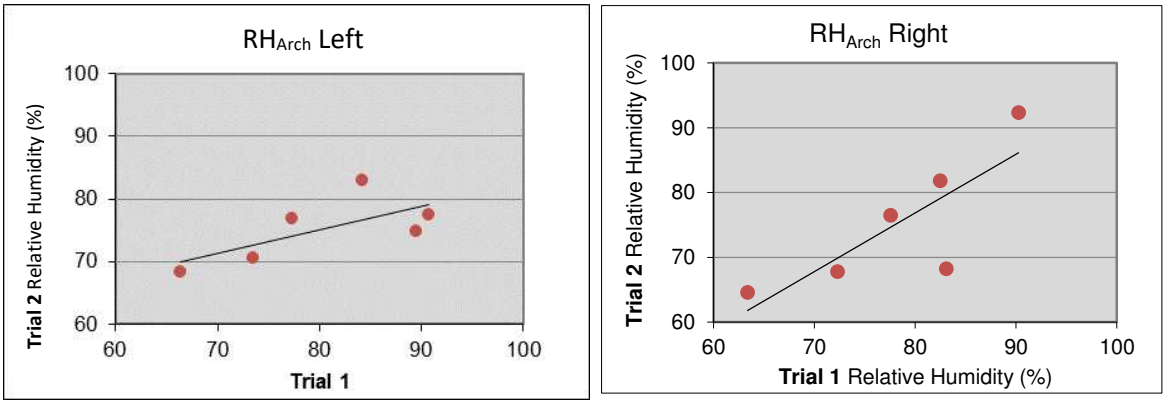
Agreement Between Test and Re-test

Agreement between test and retest (trial 1, 2) for each sensor (RH_{Toe} left and right / RH_{Arch} left and right) are illustrated in a scatterplot below (Figures 5.13 and 5.14). The scatterplot displays trial 1 on the horizontal (x) axis, and trial 2 on the vertical (y) axis. Each participant is identified by a single dot on the graph which is located so that the coordinates of the point (the X and Y values) match the participant's mean RH recorded in trial 1 and trial 2. The straight line represents identical temperature readings on re-test. The mean of the subjects for trial 1 was subtracted from that of trial 2 giving the change in mean. This ranged from -5.11 to 0.4 [±SD 4.37 to 7.1], which incorporates both the systematic and random change.



(a) **(b)**

Figure 5.13: Scatterplot of test retest for RH mean scores, **(a)** RH_{Toe} left RH and **(b)** RH_{Toe} right RH.



(a) **(b)**

Figure 5.14: Scatterplot of test retest for RH mean scores, **(a)** RH_{Arch} left RH and **(b)** RH_{Arch} right RH.

5.6 Discussion

This study was designed to investigate the validity of RH sensors (Honeywell HIH 4000) and thermistors (TAD202A) by comparing each to their respective gold standard equipment. Measurements are almost always prone to various errors, which will cause the measured value to differ from the 'true' value. An acceptable measurement error for this study was set in the design phase a priori to be compared with the bias and precision results obtained after analysis of the data.

The first study was concerned with instrumentation accuracy of measurement of temperature and humidity. Results from the 'experimental' sensors (RH sensors and thermistors) showed that the limits of agreement (LoA) were -0.6% (±0.6 SD) and -1.37 ^{0}C (±0.01 SD) which falls within the a priori acceptable measurement error criteria (±2% and ±0.2^{0}C). This implies that *after adjustment*, the experimental sensors were comparable to the gold standard equipment and can be used to measure temperature and RH with confidence. Further analysis indicated a significant correlation between the experimental sensors and the gold standard equipment *after adjustment* (RH sensor 1_{new} r = 1.0; Thermistor 1_{new} r = 0.91). This highly significant correlation coefficient supports the good agreement between the two measurement methods. The analysis of the mean differences between the two methods showed a slightly higher reading by the RH sensor 1_{new} when compared to RH_{ref} with a mean difference of 0.60%. Similarly, results suggested a higher offset of 1.37^{0}C for the thermistor 1_{new} when compared with the gold standard reading. These differences were consistent across all tested sensors (4 thermistors and 4 RH sensors) with negligible deviations and which can therefore be corrected as necessary when interpreting data.

Both the RH sensors and thermistors demonstrated good to excellent reliability in the test re-test repeatability assessment. While results from the paired t-test (table 5.7)

indicated no significant difference between the two trials ($p > 0.05$), it should be noted that this statistical test assesses whether there is any evidence that two sets of measurements agree on average, with a potential to provide misleading estimates (Rankin, Stokes 1998). For this reason, the ICC was used concurrently.

The mean change in temperature reported by the thermistors was less than 1^0C at all tested sites indicating the experimental thermistors provide a valid tool for temperature measurement. Similarly, RH sensors demonstrated a good to excellent reliability. However, the change in mean and standard deviations were noted to be relatively high between trials (trial 1, trial 2). Previous literature (Gefen 2011) recommended that for optimum skin resilience to pressure, RH level at the interface between the skin and supporting surface should be close to 40%. Findings from the study suggest that in-shoe RH at the interface between the skin and the shoe, ranged between 77 and 93%, suggesting that the change in mean and standard deviation [-5.1%, ±7 SD] observed during the reliability study would not be clinically relevant in these high RH levels recorded, which were significantly above 40% required level.

The sample size for preliminary study 2 n=6, was similar to other relevant studies (van Marken Lichtenbelt, Wouter D, Daanen et al. 2006b, Buono, Jechort et al. 2007, Smith, Crabtree et al. 2009, Gant, Atkinson et al. 2006, Hershler, Conine et al. 1992). Sample size is only likely to effect the LoA and not estimations of typical error which have expected values that are independent of sample size (Hopkins 2000b). Therefore, typical error and 95% CI were calculated in order to address these concerns.

In the context of the current study, knowledge of the measurement error, and whether there was a constant error of the sensors which will be used in the main study was crucial. Findings indicated that the experimental sensors may be used with confidence in

measuring and reporting the true value of RH with acceptable levels of agreement. An assessment of degradation or variation of recordings due to sensor usage and aging (Hubbart, Link et al. 2005) was tested after their application in-shoe in the participant group as this reflected real usage and is presented in section 5.5.9.

Chapter 6

Study 1

The Influence of Mediterranean Seasonal Variation on In-shoe Microclimate in Healthy Adults

6.1 Introduction

The work of this thesis focuses on in-shoe temperature and humidity parameters during ambulation. Although it would seem reasonable to expect different in-shoe parameters for the same shoe under different ambient conditions (summer and winter), the evolutionary pattern of both in-shoe RH and temperature needs to be investigated during ambulation as they are influenced by the body's physiological response to both exercise and the environment.

The core temperature of the human body is normally maintained at 37°C with physiological responses occurring for thermal homeostasis. During exercise several physiological and thermal changes are elicited in the healthy human body, such as metabolic rate variation and increased internal heat (Tansey, Johnson 2015). Changes in temperature at the peripheries can occur as a result of environmental changes, with a healthy human body's response to this being peripheral vasoconstriction instigated by low temperatures or peripheral vasodilatation in response to elevated temperature, together with other physiological mechanisms such as sweating, to inhibit or encourage or prevent heat loss.

Skin temperature and humidity are fundamental variables in human physiology, and their measurement poses various challenges to researchers. The real time measurement of distal extremity temperature and relative humidity may have a variety of uses. Knowledge of in-shoe microclimate would enhance the ability to observe human physiology during ambulatory living, which may in turn help in better understanding tissue response to various in-shoe mechanical forces, which have been implicated to be detrimental factors in the development of diabetic foot ulceration (DFU) in high risk patients, as discussed in Chapter 2. This study therefore aims to determine whether seasonal variation has an influence on the in-shoe microclimate, namely temperature and relative humidity (RH) in a healthy population. The goal of this experiment is to study healthy individuals to minimise potential

confounding influences from any medical conditions, such as diabetes mellitus. Therefore, results obtained from the current study will provide important normative data (study 1) that is essential to determine the influence of seasonal variation on in-shoe microclimate in healthy participants, to be later compared (Chapter 7) to that observed in a diabetic sample (study 2).

6.2 Aims

- To investigate the influence of seasonal variation on the in-shoe temperature and RH levels during ambulation in a healthy population.

- To determine normative in-shoe temperature and RH kinetics in a population of healthy adults.

6.3 Objectives

- To measure in-shoe temperature and relative humidity (RH) during ambulation in a healthy population in summer using previously validated instrumentation.

- To measure in-shoe temperature and relative humidity (RH) during ambulation in a healthy population in winter using previously validated instrumentation.

- To assess and compare in-shoe temperature and RH kinetics during ambulation between summer and winter.

6.4 Ethical Considerations

Ethical approval was granted from the local ethics committee, University of Malta, after a formal proposal was presented (Appendix III). All ethical issues including informed consent and safety have been further discussed in Chapter 4, section 4.6.

6.5 Methods

Two sets of data collection were organised at the Biomechanics Lab, Faculty of Health Sciences, University Malta, twice involving the same human cohort, during two peak periods of the winter and summer seasons, February 2015 and August 2015.

6.5.1 Subject Cohort

Fourteen healthy Maltese adults, 5 males and 9 females, of a mean age of 49 years (±13.3), a mean weight of 75kg (±11.6), and a mean height of 165.7 cm (±12.2) participated in this study. They were informed about the study protocol, verbally and in writing, and signed a consent form, after satisfying the inclusion/exclusion criteria listed in the table below (Table 6.1).

Table 6.1: Inclusion/Exclusion Criteria - Healthy Participant Group	
Inclusion	Exclusion
Males & females Aged over 18 years Healthy (no reported illness or handicap) Intact epidermis No signs of neuropathy/peripheral disease Walked unaided Able to walk on a treadmill comfortably	Any foot deformity Foot pain Diabetes mellitus/RA Current or h/o foot ulceration Participants showing unwillingness to participate.

6.5.2 Experimental Procedure to Investigate Seasonal Influence on In-shoe Microclimate.

A detailed write-up of the protocol used during this study was presented in Chapter 4. This provided an important description of the methods and protocol used for data acquisition during the summer and winter trials in order to minimise measurement errors (random & systematic errors) that may interfere with the interpretation of results. These included; same sample population (n=14), identical conditions for both trials (such as shoes, socks and participant physical exertion), acclimatization protocol, placement of sensors, and walking speed. Since the primary aim of this study was to evaluate in-shoe microclimate during two separate conditions (summer and winter) on the same study cohort, all windows were kept open for a number of hours prior to data collection for the ambient room (experimental room) temperature and humidity levels to stabilise and reflect the outside atmospheric temperature and humidity.

As described previously (Chapter 4), two thermistors and two RH sensors on each foot were placed between the hallux and 2nd toe and below the navicular. For the purpose of ease of understanding, the thermistors will be referred to as T_{Toe} and RH_{Toe} representing readings from thermistor and RH sensor located between hallux and 2nd toe and T_{Arch} and RH_{Arch} representing temperature and RH readings from below the navicular. Temperature and RH were recorded for the whole 39-minutes of treadmill walking at a self-selected speed representing moderate exertion, as per Borg RPE Scale protocol (Borg 1990). For statistical purposes the readings logged every minute were considered for analyses, giving a total of 39 data points (time) per sensor.

6.5.3 Data Treatment

The recorded in-shoe temperature and RH data were treated as per the protocol previously presented and discussed in Chapter 4, section 4.5.

6.5.4 Data Analysis

The statistical analysis was performed using Microsoft Excel and the IBM Statistical Package for Social Science (SPSS) software, version 23. The data were assessed for normal distribution using the Shapiro-Wilk test and depending on the result, a paired sample t-test or Wilcoxon Signed Ranks test was used to compare mean temperature/RH between two seasons (summer & winter). The Paired sample t-test is a parametric test used when the measurements (temperature/RH) have a normal distribution. Conversely, the Wilcoxon Signed Ranks test is a non-parametric test and is used when the measurements have a non-parametric distribution.

Hypothesis:

(H_0) The null hypothesis specifies that there is no significant difference in mean temperature/RH between summer and winter (accepted if the *p* value exceeds the 0.05 level of significance).

(H_1) The alternative hypothesis specifies that there is a significant difference in mean temperature/RH between summer and winter (accepted if the p value is less than the 0.05 level of significance).

Further analysis of data using Generalized Estimating Equation (GEE) models investigating the relationship of foot temperature and RH to time, season, location and orientation was performed. The GEE method focuses on average change response over time and the impact of covariates in these changes. It models the mean response of linear function of covariates of interest via a transformation or 'link' function which can be considered repeated measures analogs of linear regression or logistic regression. Unlike other commonly used tests, such as the Repeated Measures ANOVA, GEE does not require the outcome variable to have a particular distribution. This is an important feature which is beneficial in studies with skewed data or when the distribution of data is difficult to verify due to a small sample size. GEE is a highly recommended statistical test to help estimate the average change per group and measure population-average effects of covariates of interest (Ma, Mazumdar et al. 2012).

6.6 Results

This study explored the influence of seasonal variation on in-shoe microclimate during ambulation by comparing in-shoe temperature and RH kinetics between seasons, summer and winter. As well as the same protocol being applied in data collection in both seasons (as detailed in Chapter 4) the same data analysis was applied. The results indicated that there was a marked difference in in-shoe temperature recordings between summer and winter at both locations (T_{Toe}, T_{Arch}). However, difference in RH levels were not significant.

6.6.1 Summary of Key Findings of the Study on the Influence of Season on In-shoe Microclimate

The main purpose of this section is to present key-points on results achieved from continuous measurement of ambulatory foot temperature and RH with the aim of providing some insight into the data provided by it. These results provide data on the variation of in-shoe foot temperature and RH, during exercise in healthy individuals living in a Mediterranean climate.

In-shoe Temperature Findings

- Healthy participants' foot skin temperature on both feet (left, right) and both locations (toe, arch) was higher in the summer than winter throughout the whole trial

  (summer vs winter $T_{Toe,}$ $p < 0.01$; $T_{Arch,}$ $p < 0.001$) - ***section 6.6.4***.

- Ambient temperature has a significant influence on in-shoe temperature kinetics as in-shoe temperature increases during treadmill walking - ***section 6.6.4***.

- There was a greater rate of increase in in-shoe skin temperature in the winter when compared to summer. Foot skin temperature was colder in the winter at base line, and warmed rapidly to reach similar in-shoe temperature as for summer values by the end of the trial - ***section 6.6.4***.

In-shoe Relative Humidity Findings

- Healthy participants' foot skin RH was similar in the summer and winter throughout the whole trial.

  (summer vs winter $RH_{Toe,}$ p >0.05; $RH_{Arch,}$ p > 0.05) - ***section 6.6.5***.

- Season does not appear to influence in-shoe RH kinetics since difference was not significant between summer and winter, p > 0.05. There was a trend for toe and arch relative humidity measurements recorded in summer to be higher than those recorded in Winter throughout the whole trial but this difference was not significant - ***section 6.6.5.***

- RH in the toes was higher than RH in the arch throughout most of the trial, p < 0.05, and this was more evident in summer - ***section 6.6.5***.

A detailed description of the results is presented below.

6.6.2 Experimental Room Ambient Climate Data

The protocol used for measuring ambient temperature within the experimental room for both trials (summer and winter) has been explained previously in Chapter 4. Table 6.2 below presents room ambient temperature and relative humidity recorded for both seasons. The data was assessed for statistical difference between seasons using the Paired Sample t-test.

Table 6.2: Experimental room ambient temperature and humidity mean recordings, measured using a calibrated and certified humidity and temperature data logger (CEM DT-172)

Ambient Climate	***Mean***	***SD***	***Difference between seasons*** *Paired Sample T-test*
Relative Humidity (%) Summer	70.4	6.2	0.20
Relative Humidity (%) Winter	67.8	3.9	
Temperature (^{0}C) Summer	28.2	0.6	0.01
Temperature (^{0}C) Winter	17.1	0.4	

The Paired Sample t-test results show that there was no significant difference in room ambient RH between summer and winter (p = 0.2), while a significant difference was observed between seasons in room ambient temperature (p = 0.01).

6.6.3 Test for Normalcy of Data

The Shapiro-Wilk test was performed using the individual data measurements to assess the normality assumption of temperature and RH readings obtained from both sensors, at both locations (toe and arch) and orientations (left and right) in summer and winter.

Results obtained from the Shapiro-Wilk Normality Test (Table 1 and 2, Appendix VII) indicated that the distribution varied in normality $p = 0.00$ to $p = 0.990$ across both seasons. While some of the data had a normal distribution, other data sets had a skewed non-normal distribution. Therefore, both parametric (Paired Sample t-test) and non-parametric (Wilcoxon Signed Ranks test) tests were considered for further analysis to compare mean temperature/RH between two seasons - summer and winter. It should be noted that no difference in significance was observed between both tests, therefore, only results for the Paired Sample t-test will be presented.

6.6.4 Influence of Seasonal Variation on In-shoe Temperature in Healthy Individuals.

Measurement recordings of mean in-shoe temperature data at one-minute interval over a 38-minute physical activity trial are illustrated below (Figure 6.1).

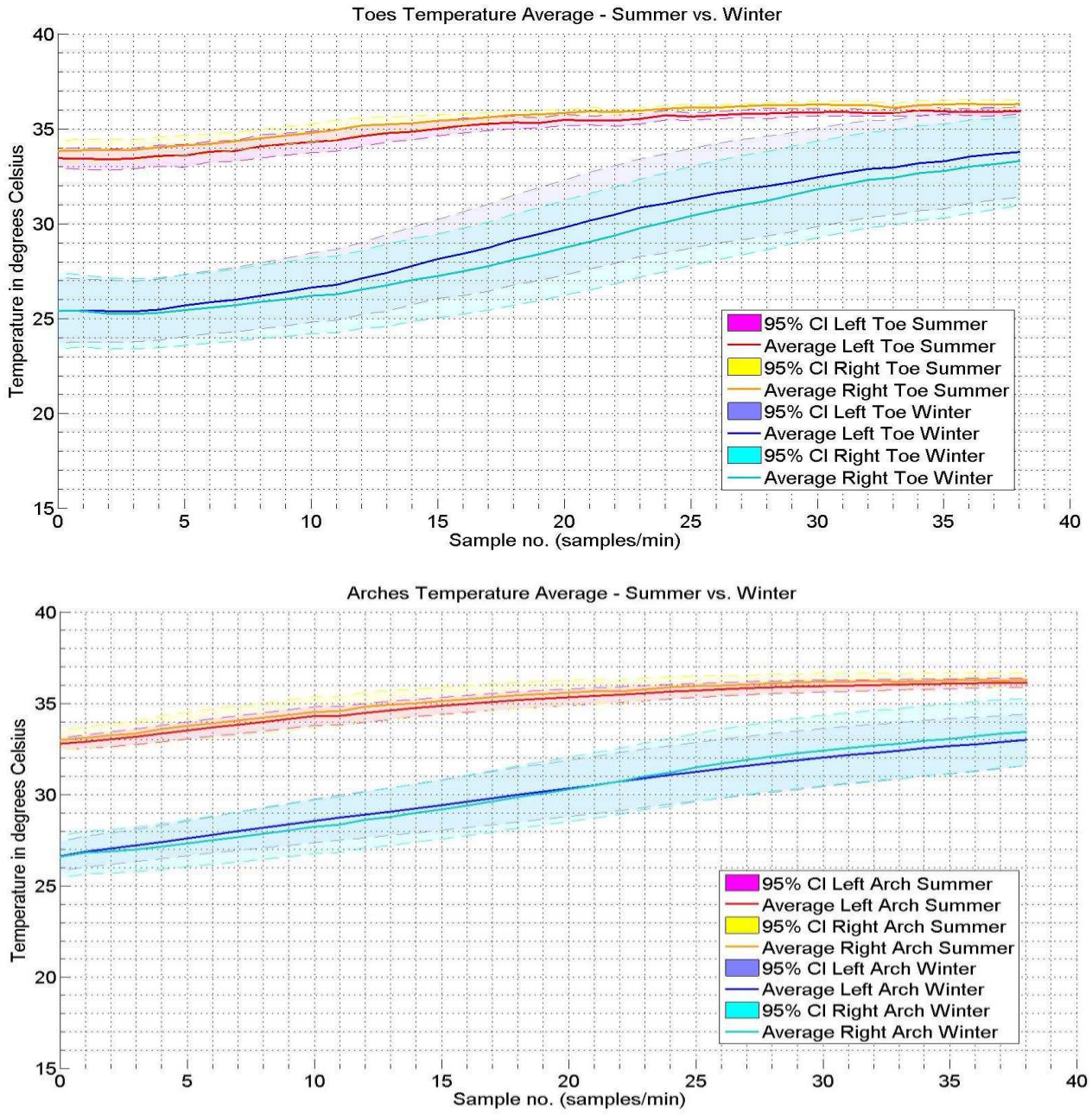


Figure 6.1: Line graph illustrating in-shoe mean temperature (^{0}C) at toes and arches for summer and winter with CI values in healthy participants

The figure above shows a line graph of the mean skin temperature recorded with 95% CI values at the toes and arches of all the healthy participants over time. The red and orange lines represent data recorded at the left and right foot respectively in summer. The dark blue and light blue represent data recorded at the right and left foot respectively in winter. The same line graphs with CI values presented above (Figure 6.1) are also presented separately per each location in Appendix XI. Figure 6.1 demonstrates how foot mean temperatures increase as sampling time increases, with increments being more conspicuous in winter than summer, showing a higher rate of temperature change recorded in the winter trial. The most important rise in temperature is observed in winter between the 15^{th} and 35^{th} minute where an increase of 8.1^0C was observed in the mean toe temperature and an increase of 6.6^0C was observed in the mean arch temperature when compared to an increase of 2.5^0C and 3.3^0C in the toe and arches respectively in summer.

No significant difference ($p > 0.05$) was found between the left and right foot within the same season (Appendix VIII). Since no difference was observed, data for the left and right foot were merged for further analysis.

The Paired Sample t-test (Table 1, Appendix VIII) was used to analyse whether significant differences exist in in-shoe mean temperatures between seasons at the arch and toe. Results demonstrated that there was a significant difference ($p < 0.01$) between seasons in both arches and toes, indicating that seasonal variation had an influence on in-shoe temperature in both studied locations throughout the trial. It was also observed that temperatures recorded in winter were more dispersed than those recorded in summer, with wider standard deviations. Results therefore show that the alternative hypotheses (H_1) can be accepted since the mean in-shoe temperature varies significantly between summer and winter with p values less than the 0.05 criterion. The interaction between season and in-shoe temperature kinetics in both toes and arches was further analysed using GEE models and are presented in section 6.6.4.4.

The plethora of data derived from the current study warranted further in-depth analysis of the temperature kinetics exhibited by both toes and arches. Results are presented as data from the toes and arches separately, which will later be compared in order to elicit in-shoe foot temperature kinetics in healthy adults which may be used as baseline data for further research.

6.6.4.1 In-shoe Toe Temperature (T_{Toes}) Kinetics in Healthy Participants

In order to analyse the temperature kinetics recorded in the toe region between seasons over a period of moderate physical activity (treadmill walking), each data point was plotted on a line graph showing data for each participant (Figure 6.2). In the plot below, each participant (n=14) is represented by a different colour.

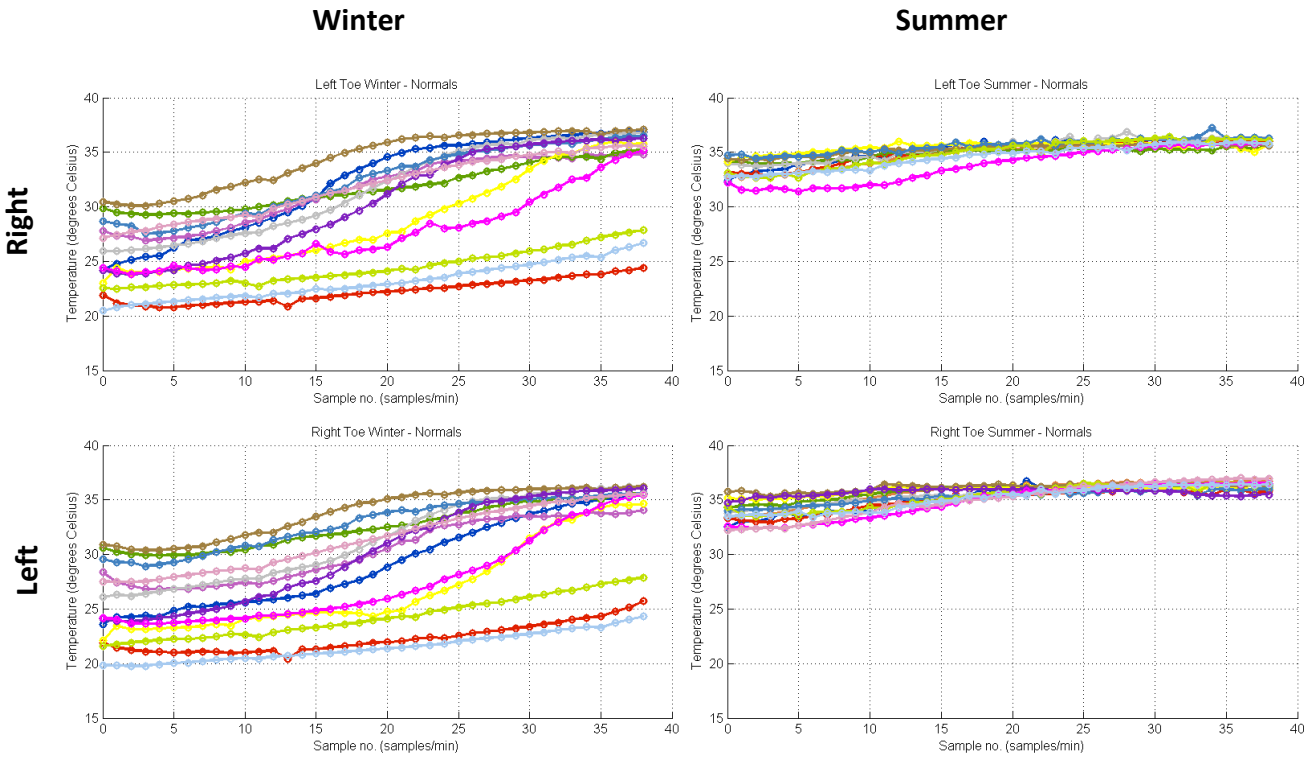


Figure 6.2: Individual healthy participant in-shoe temperature (^{0}C) kinetics at the Toes (T_{Toes}) over a 39-minute trial for the (a) left foot and (b) right foot, both seasons.

The plotted graphs clearly illustrate the influence of seasonal variation on in-shoe temperature kinetics which was evidenced statistically in table 1, Appendix VIII. Immediately, what is visually evident is the difference in variability among participants, both between seasons and within the trials. It appears that in-shoe temperature variation is influenced by both ambient temperature and the individual's physiological (thermoregulatory) response to moderate exercise.

At the start of the trial (T_0) a wider inter-participant in-shoe temperature variation (which decreases towards the end of the trial) is evident in both seasons, while most participants achieved similar in-shoe toe temperatures towards the end of sampling time (T_{38}). After the start of the trial, as sampling time increased (treadmill walking time), variability among most participants decreased, with the line graph of most participants (all except for three participants) converging at the end of the trial. This can be more easily observed in the winter plots, since there was a notable temperature difference from the start to the end of sampling time – figure 6.2. The standard deviations of these plots further support this observation since the summer standard deviations ranged from ±0.37 to ±1.0, while the winter plots have wider standard deviation ranging from ±3.1 to 4.7 (see Table 1, Appendix VIII).

This pattern is observed in all participants, except for 3 participants who had an initial lower temperature at the start of the trial compared to the rest and who maintained lower temperatures until the end of the winter trial. When analysing the winter plots, similar in-shoe temperature kinetics are observed in most participants with an S-shaped line graph. However, it should be noted that the three participants with the lowest initial toe temperatures appeared to have a slower rate of increase in temperature, illustrated by a more linear graph.

An important observation which is evident both in the mean plots (Figure 6.1) and in the individual plots (Figure 6.2), is an initial temperature drop occurring in the initial few minutes of treadmill walking (lasting for approximately 4 minutes). This temperature drop is observed in both summer and winter, but is more evident in the winter plots. This is explored further within the discussion section of this chapter.

Notably, the individual participant plots of the in-shoe temperature kinetics illustrate a distinct similarity between the left and right foot of each participant. The similarity between the contra-lateral limbs is consistent in both seasons. This is further evidenced statistically in the results presented in Appendix IX where no significant difference was observed (Independent Sample t-test $p > 0.05$). The similarity between left and right foot is additionally confirmed using GEE Models (see Section 6.6.4.4). This is also further explored in the discussion section of this chapter.

6.6.4.2 In-shoe Arch Temperature (T_{Arch}) Kinetics in Healthy Participants

In order to illustrate the temperature kinetics recorded in the arch region between seasons, each data point was plotted on a line graph to illustrate the temperature kinetics during 39-minutes of physical activity in summer and winter for each participant (Figure 6.3).

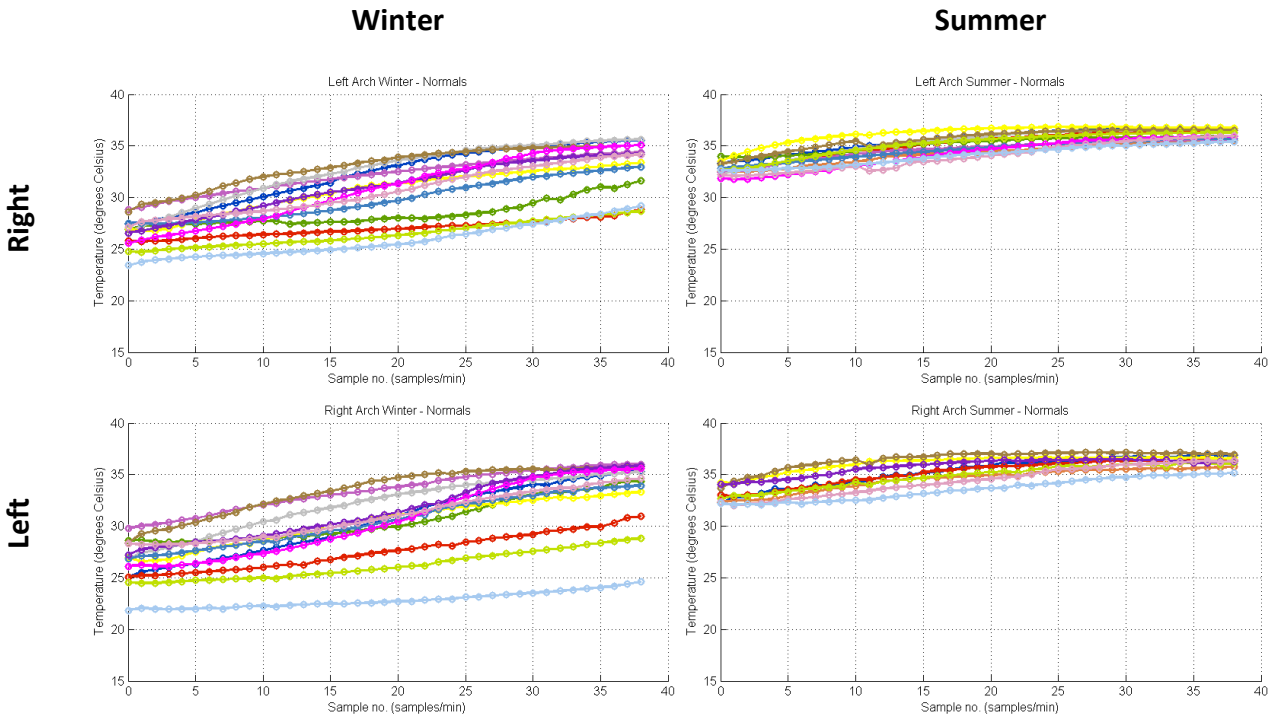


Figure 6.3: Individual healthy participant in-shoe temperature (^{0}C) kinetics at the arch (T_{Arch}) over a 38-minute trial for the (a) left foot and (b) right foot, both seasons.

The plotted results illustrated in figure 6.3, demonstrate that, as observed in the toe region, most of the participants demonstrated an initial in-shoe arch temperature variability which decreased towards the end of the trial. The variability of this distribution at the start of the trial appears to be influenced by ambient temperature, as the variability at the start of sampling time appears to be much smaller in summer when compared to winter. In-shoe

kinetics in the arch appear to have similar trends to those observed at the toe area (the data for which was presented in the previous section) where the same 3 participants who maintained lower toe temperatures exhibited a similar trend in the arch region. These three participants also maintained lower temperatures when compared to the rest. As observed in the toes, the similarity of in-shoe temperature kinetics at the arches between the left and right foot of individual participants is evident when observing the plots illustrated in figure 6.3. As with the toes, a similarity between the contra-lateral limbs is consistent in both seasons.

6.6.4.3 In-shoe Temperature Kinetics in Healthy Participants: Toes Vs Arches

The in-shoe mean temperature data of the toes and arches were compared to analyse for statistical difference between the two locations (Appendix X, table 1) using an independent sample t-test. A significant difference in in-shoe temperature was observed until the 13th minute in winter, after which no further significant difference was observed, as the temperature in the toes increased after that time, reaching that of the arch. No difference is observed in summer ($p > 0.05$). This observation suggests that the toes are more affected by ambient temperature compared to the arch area when exposed to a cold climate, in fact, this difference in temperature was not observed in the summer, when mean ambient temperature was 28.2^{0}C.

A notable observation was the lack of initial drop in in-shoe temperature at the arch area, when compared to the toes, in both seasons. In contrast, the temperature in the arch initially showed an increase at the start of the treadmill walking and continued to increase until the end of sampling time.

6.6.4.4 Generalized Estimating Equations: **In-shoe Temperature**

As detailed earlier in section 6.5.4, Generalized Estimated Equations (GEE) models are appropriate to analyse related longitudinal data and repeated measures designs (Ballinger 2004). These models extend the traditional repeated-measures models because they accommodate dependent variables that follow any distribution within the exponential family (see Section 6.5.4 in Chapter 6).

In-Shoe Temperature Analysis

The first GEE model relates foot temperature (dependent variable) to four categorical predictors, which include time (1 to 38 minutes), season (summer and winter), location (toe and arch) and orientation (left and right). The model also includes two interaction terms time*season and time*location to examine how the temperature increases with exercise time between different seasons and different locations. Since the Shapiro Wilk test showed that for different combinations of time, location and orientation levels, the temperature distributions satisfied the normality assumption, it is possible to assume a normal distribution and an identity link function. To fit the GEE model the participant number is declared as the subject variable.

The correlation matrix represents the within-subject relationships and there are five structures available in SPSS (Independent, Autoregressive, Exchangeable, M-dependent and Unstructured). The QIC information criterion (Quasi Akaike Information Criterion) is used to identify the best correlation structure for the model, where the optimal correlation structure has the lowest QIC value. Table 6.3 shows the QIC values for the five correlation structures.

The QIC information criterion (Table 1, Appendix XII) shows that the **exchangeable** structure is the best correlation structure for this GEE model. This structure has homogenous correlations between elements and is also known as a compound symmetry correlation structure.

Table 6.3: Analysis of individual in-shoe temperature data measurements

Significance of main and interaction effects using GEE Exchangeable structure modelling

Model Term	Wald	df	P-value
Intercept	5126.2	1	0.000
Time	3861322.1	38	0.000
Season	45.8	1	0.000
Location	.800	1	0.371
Orientation	.088	1	0.767
Time * Season	361259850193	38	0.000
Time * Location	559346354	38	0.000

Table 6.3 above shows that Time, Season and their interaction are significant effects. This is clearly shown in Figure 6.4 below where the two skin temperature line graphs are steep, well-separated and are not parallel. While Table 6.3 also shows that Location is not a significant main effect ($p > 0.01$) its interaction with time is significant ($p < 0.01$). This is clearly shown in Figure 6.5 where the two temperature line graphs are quite overlapping but not parallel. Table 6.3 and Figure 6.6 show that Orientation and its interaction with time are not significant effects since the two temperature line graphs are overlapping and parallel.

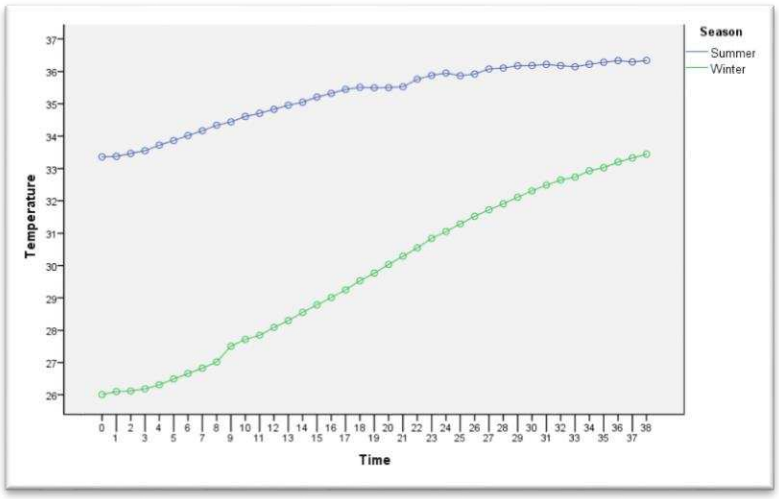


Figure 6.4: GEE Model - Mean in-shoe foot temperature (^{0}C) by Time and Season, Summer & Winter

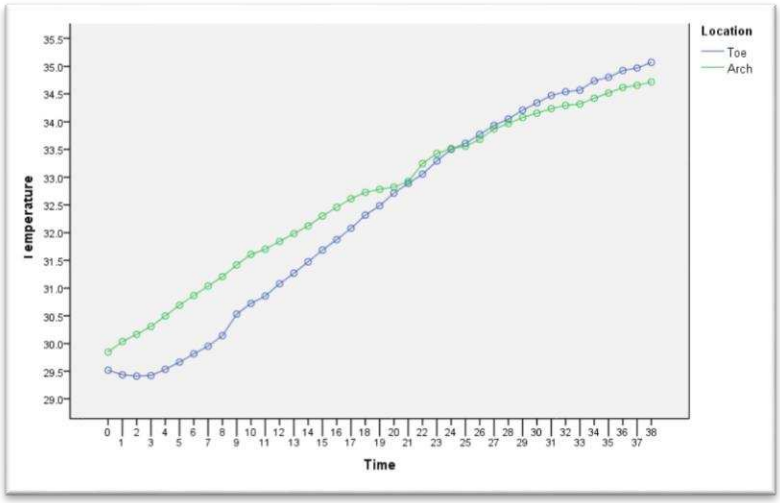


Figure 6.5: GEE Model - Mean in-shoe temperature (^{0}C) by Time and Location, Toe & Arch

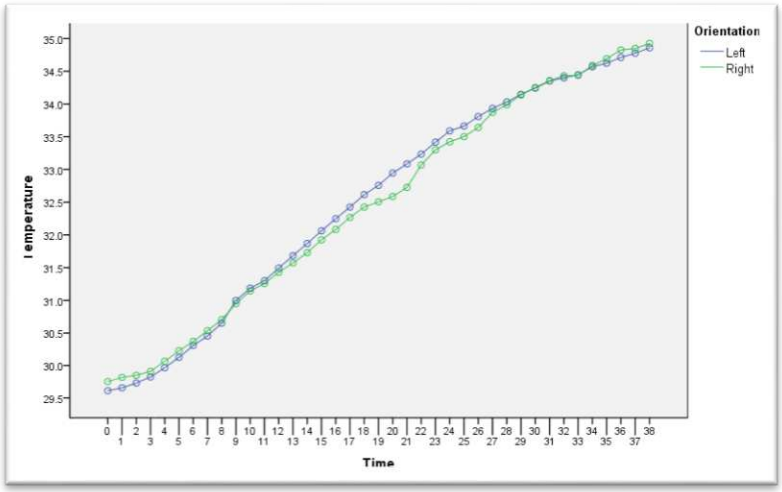


Figure 6.6: GEE Model - Mean in-shoe foot temperature (^{0}C) by Time and Orientation, Left & Right

6.6.5 Influence of Seasonal Variation on In-shoe RH in Healthy Individuals

Measurement recordings for mean in-shoe skin RH data at one-minute intervals over the 38-minute trial of moderate treadmill walking are illustrated below (Figure 6.7).

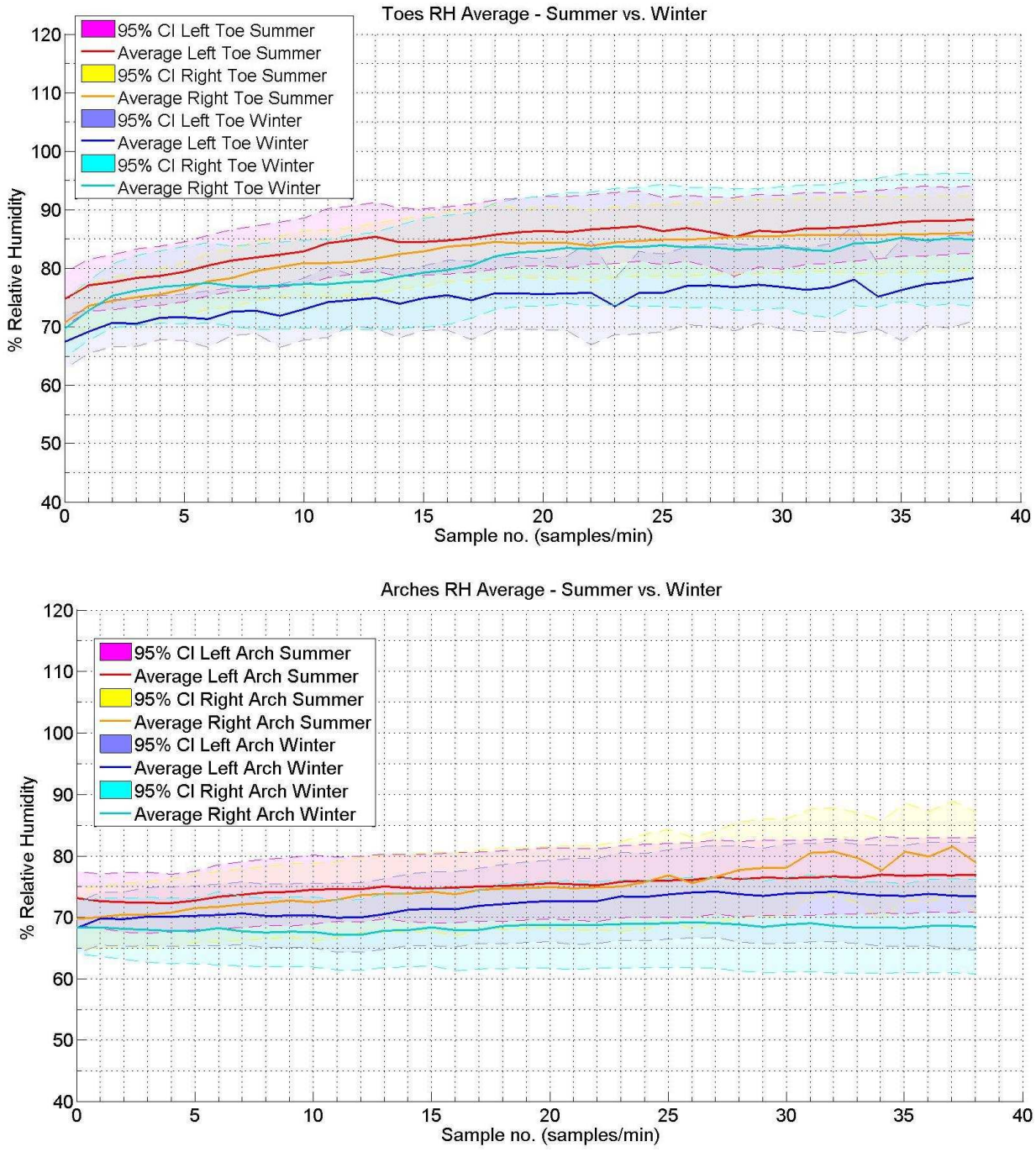


Figure 6.7: Line graph illustrating in-shoe mean RH (%) at toes and arches for Summer & Winter with 95% CI values in healthy participants

The figure above (see Figure 6.7) shows a line graph of the mean in-shoe RH recorded with 95% CI values at the toes and arches of all the healthy participants over time. The red and orange lines represent data recorded at the left and right foot respectively in summer. The dark blue and light blue represent data recorded at the right and left foot respectively in winter. The same line graphs with CI values presented above (Figure 6.7) are also presented separately per each location in Appendix XI.

Results obtained from the study demonstrate that there was no significant difference in in-shoe relative humidity between summer and winter, either at the toes or the arch ($p > 0.05$) throughout the whole trial. The Paired Samples t-test was used to compare in-shoe mean RH between seasons at the arch and toe region (Table 2, Appendix VIII). It is worth noting that the confidence intervals (CI) was observed to be wide in both seasons at both toe and arch, as illustrated in the figure above (see Figure 6.7). No significant difference was found between the left and right foot ($p > 0.05$; Paired Samples t-test; see Table 5, Appendix IX).

Data provided (Table 1, Appendix VIII) also illustrates how mean RH increased as sampling time increased (walking time), with a higher increment observed at the toes (Toes: - summer 15%; winter 13%) when compared to the arches (Arches: summer 6.8%; winter 2.6%). These in-shoe RH patterns, for each studied location, were similar in both seasons.

The interaction between season and in-shoe RH kinetics in both toes and arches was further analysed using GEE models and are presented in section 6.6.5.4.

6.6.5.1 In-shoe Toe RH (RH_{Toe}) Kinetics in Healthy Participants

In order to analyse the RH kinetics recorded in the toe region between seasons over a period of treadmill walking, each data point was plotted on a line graph illustrating data for each individual participant (Figure 6.8).

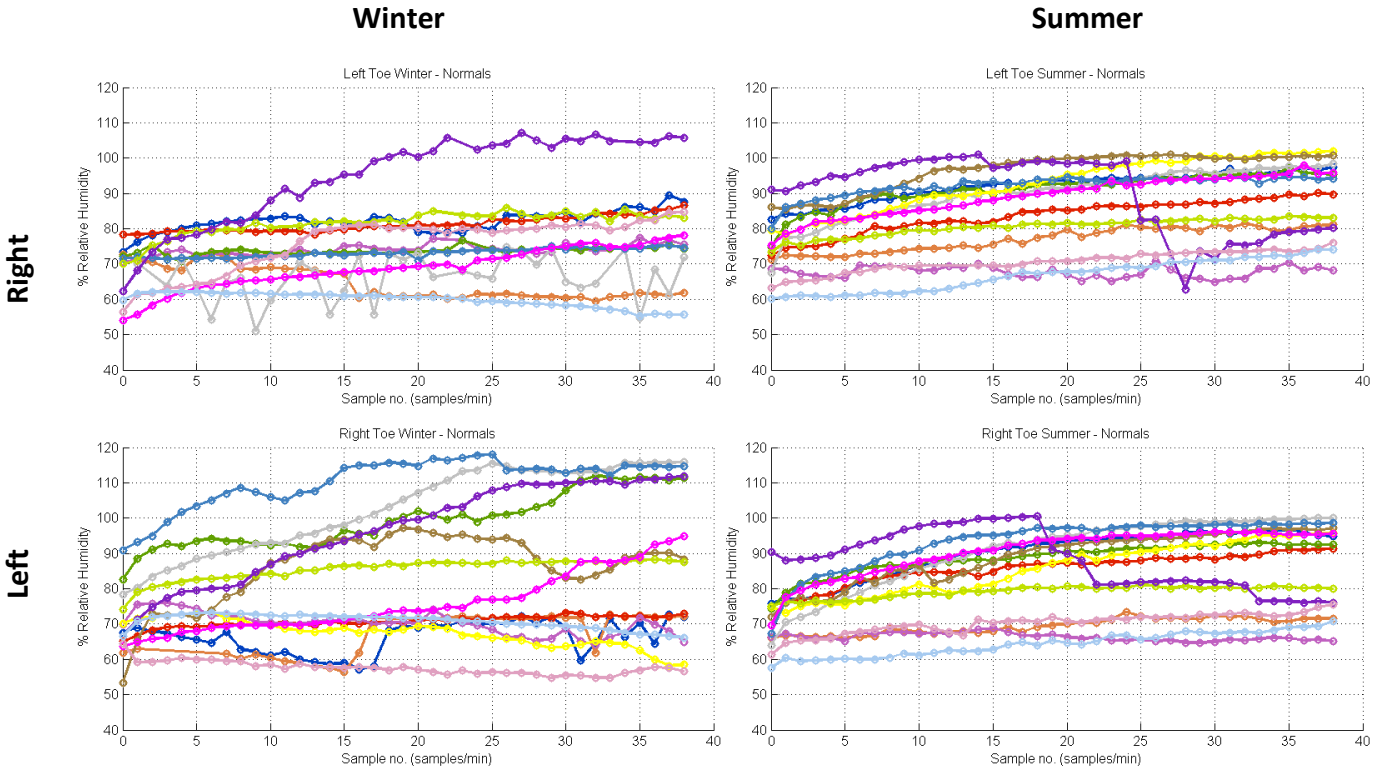


Figure 6.8: In-shoe RH (%) kinetics at the forefoot (RH_{Toe}) over a 38-minute trial for the (a) left foot and (b) right foot, both seasons in healthy participants

The pattern of in-shoe RH changes over time was shown to increase at the toe area in both seasons by approximately 15%. An initial wide RH variability among participants is evident in both summer and winter and remains relatively constant throughout the trial, contrasting with the in-shoe temperature kinetics results, where variability among participants decreased as temperature increased. The variability among participants does not appear to be influenced by ambient temperature as the variability and SD among participants are similar in both seasons. It must be noted that all participants wore identical

socks during both trials which were provided by the researcher, while using their own preferred walking shoes using the same pair for both winter and summer as described in Chapter 4.

6.6.5.2 In-shoe Arch RH (RH_{Arch}) Kinetics in Healthy Participants

In order to analyse the RH kinetics recorded in the arch region between seasons, each data point was plotted on a line graph to illustrate the RH kinetics during 38-minutes of physical activity in summer and winter for every participant (figure 6.9).

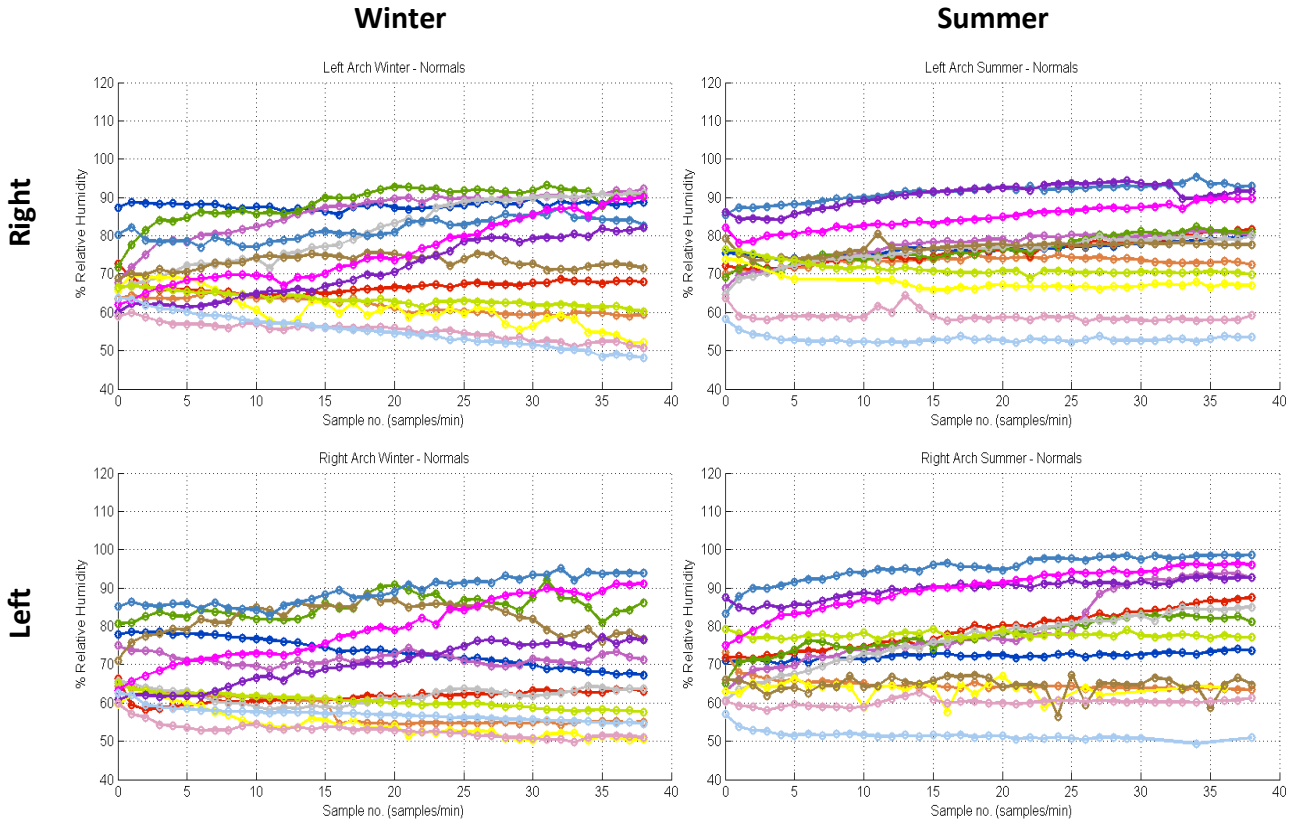


Figure 6.9: In-shoe RH (%) kinetics at the arch (RH_{Arch}) over a 38-minute treadmill walking trial for the (a) left foot and (b) right foot, for both seasons in healthy participants

The pattern of RH changes over time is shown to increase at the arch area in both seasons with a non-significant (p > 0.05) larger increase in summer when compared to winter (6.8%, 2.6% respectively). The variability among the participants is comparable to that observed at the toe area, where initial variability at the start of the trial was maintained throughout the 38-minutes walking. It should be noted that the standard deviation at the end of the trial was > ±13%.

6.6.5.3 In-shoe RH Kinetics in Heathy Participants: Toes vs Arches

In-shoe mean RH data of the toes and arches were compared to analyse for significant difference between the two locations - Appendix X, Paired Sample T-test. At the start of the trial, initial in-shoe RH was similar to that observed at the toe region. However as sampling time (treadmill walking) increased, RH at the toes increased at a higher rate. A significant difference between the two areas was observed (p < 0.05). This difference in RH changes between the 2 areas occurs slightly faster in summer where a significant difference is evidenced after 3 minutes of walking, compared to 9 minutes in winter. In-shoe RH kinetics are further explored using GEE models below.

6.6.5.4 Generalized Estimating Equations (GEE): **In-shoe RH Analysis**

The second GEE model relates foot relative humidity (dependent variable) to the main effects of time, orientation, location, season and the two interaction effects of time*season and time*location, to examine how relative humidity increases with exercise time between different seasons and different locations. Since the Shapiro Wilk test showed that, for different combinations of time, location and orientation levels the temperature distributions satisfied the normality assumption, it is possible to assume a normal distribution and an identity link function. To fit this GEE model, the participant number is

declared as the subject variable. The QIC information criterion (Quasi Akaike Information Criterion; Table 2, Appendix XII) shows that the **independent** structure is the best correlation structure for this GEE model.

Table 6.4: Analysis of individual in-shoe RH data measurements

Significance of main and interaction effects using GEE Exchangeable structure modelling

Model Term	Wald	df	P-value
Intercept	1919.1	1	0.000
Time	5360961622.8	28	0.000
Season	12.9	1	0.000
Location	24.3	1	0.000
Time * Season	171119855541.0	28	0.000
Time * Location	1203405883659.5	29	0.000

Table 6.4 shows that Time, Season and their interaction are significant effects. This is clearly shown in Figure 6.10 overleaf where the two temperature line graphs are steep, well separated and are not parallel. Table 6.4 also shows that Location and its interaction with time are significant effects. This is clearly shown in Figure 6.11 where the two temperature line graphs are well separated and are not parallel.

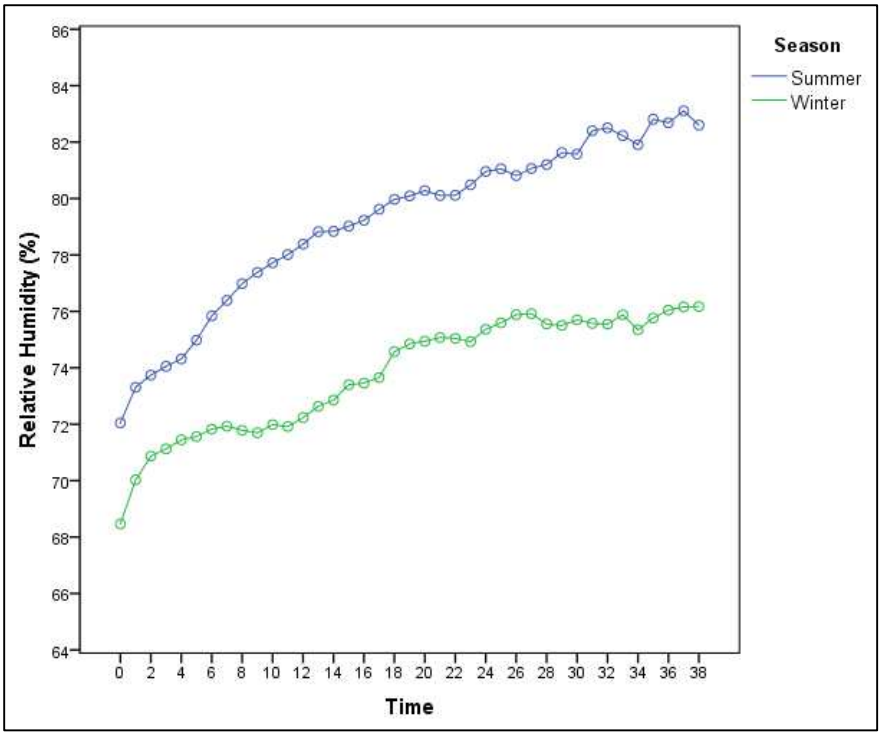


Figure 6.10: GEE Model - Mean in-shoe foot RH (%) by Time and Season, Summer & Winter

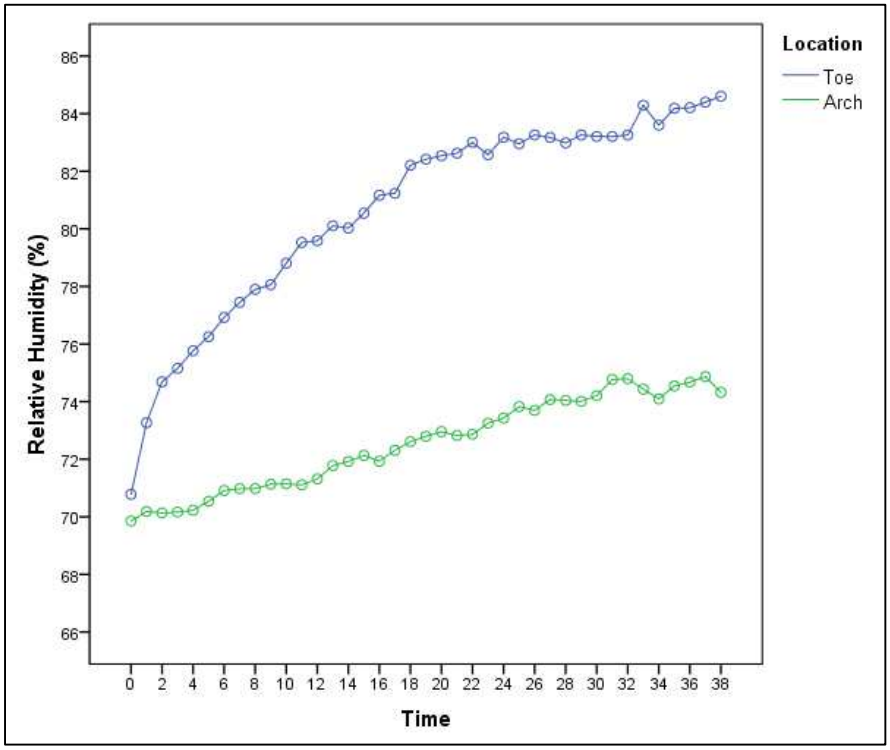


Figure 6.11: GEE Model - Mean in-shoe RH (%) by Time and Location, Toe &

6.7 Synoptic Discussion

The main aim of this part of the work in this thesis was to investigate the influence of seasonal variation on healthy individuals' in-shoe microclimate during treadmill walking. The main objective of this prospective experimental study with healthy individuals was to measure in-shoe temperature and RH kinetics during moderate physical activity and to assess whether these dynamics were different between summer and winter in a Mediterranean climate. From an in depth search of the literature and other resources including opinion pieces (Tulley 2008), it appears the present study is the first to measure and monitor skin temperature and RH in-shoe during ambulation in Mediterranean winter and summer climates, establishing the human response in these parameters as walking time progresses and as they evolve depending on ambient climate. Therefore, results from this study present novel findings pertaining to the influence of season on in-shoe temperature and RH kinetics during moderate physical exercise (walking) in healthy individuals, providing normative data with which other cohorts can be compared, adding to the body of knowledge which was still currently lacking (see Section 2.6)

A significant difference was observed ($p < 0.01$) in in-shoe temperature during 38-minutes of treadmill walking, measured during different seasons. However, a limitation of this analysis should be noted that, when multiple comparisons are made of the same/related data, the chance of rejecting the null hypothesis and accepting a difference increases as the number of comparisons increases. Although the chance of error is 0.05 for each individual comparison, the potential cumulative chance of error for the multiple comparisons that were made, is likely to be greater than 0.05. No difference was exhibited in in-shoe RH kinetics ($p > 0.05$). A discussion of findings of this study is presented below.

Finding 1: *Difference in in-shoe skin temperature at the Toe (T_{Toe}) and Arch area (T_{Arch}) between summer and winter was significant throughout the whole trial ($p < 0.01$; see Section 6.6.4). As may be expected, healthy participants' foot skin temperature on both feet was warmer in the summer than winter.*

Results from the current study demonstrate that foot temperature of healthy individuals can vary considerably across different seasons and that this variation is influenced by ambient climate. It was observed that in an ambient temperature of 28.2 ^{0}C (±0.6^{0}C; typical of summer season in a Mediterranean climate) foot temperature during treadmill walking increased by 3^{0}C (from 33^{0}C to 36^{0}C). A different foot temperature pattern of change was observed in winter, with mean foot temperature increasing by 7^{0}C (from 27^{0}C to 34^{0}C) over 38 minutes of walking, when the ambient temperature was 17.1^{0}C (±0.4^{0}C; typical of a winter season). Consistent with this notion, ambient temperature was found to have significant effects on in-shoe temperature kinetics during exercise as demonstrated in the GEE model graph (Figure 6.4).

While previous studies in the literature differ methodologically, the current results are in agreement with the conclusions by most authors (Foltyński, Mrozikiewicz-Rakowska et al. 2014, Nardin, Fogerson et al. 2010, Martinez Cuervo, Soldevilla Agreda et al. 2007), who acknowledged the influence of ambient temperature on distal skin temperature. A detailed discussion of the current results in the light of previous literature is presented in Chapter 8.

Finding 2: *Ambient temperature has a significant influence on in-shoe temperature kinetics as it increases during treadmill walking in healthy participants.*

In-shoe foot skin temperature was colder in the winter at baseline, and warmed rapidly to reach similar in-shoe temperature as for summer values, by the end of the period of walking. These results therefore demonstrate that, for moderate physical activity, the rate of temperature increase depended on initial in-shoe foot temperature, which in turn

was influenced by ambient climate. As walking progressed, the difference in temperature between seasons decreased, resulting in similar temperatures in summer and winter, by the end of the trials. This indicates that the final in-shoe temperature reached was not influenced by ambient climate but may be more likely influenced by a physiological process imposing a 'thermoregulatory limitation' on the body. It is known that exercise is associated with multiple thermoregulatory processes involving hemodynamic changes (Johnson 2010). Since exercise is linked to hemodynamic changes and to heat generation within the body, marked changes in thermoregulatory processes during exercise are expected. Results therefore demonstrate that the kinetics of in-shoe skin temperature observed in this study may reflect these thermoregulatory processes. This finding will be discussed in further detail in Chapter 8 in the light of previous works.

Finding 3: *A significant temperature difference ($p < 0.05$) between the toes and the arches in both summer and winter at the beginning of the walking (Time 0) was observed. This difference became non-significant within 1 minute of walking in summer and after 13 minutes in winter (see Section 6.6.4.3).* ***Finding 4:*** *Location is not a significant main effect; however, its interaction with time is significant (time*location, section 6.6.4.4).*

Foot skin temperatures at the arches and toes were similar in summer almost throughout the whole trial, while they were significantly different ($p < 0.05$) during the first 13 minutes in winter. However, it should be noted that the difference between the toe and the arch (Appendix x, table 1 & 2) in the first few minutes of the trial is within the measurement error reported in the repeatability study (Preliminary study 2, Chapter 5). Therefore, although significant, these results may be due to measurement variability and not location. Temperature kinetics differed between these two locations as the rate of increase in in-shoe temperature in the arch area was more linear when compared to the toe region in both seasons. Furthermore, in winter the toes exhibited a greater sensitivity to ambient climate than the arch, as the toes were cooler than the arches after acclimatization to ambient climate and throughout the first 13-minutes of moderate exercise. This may also reflect the thermoregulatory processes causing vasoconstriction in

the peripheries in cooler climates. After 13th minutes of moderate treadmill walking (in winter) the *rate of increase* in temperature at the toes accelerated rapidly, reaching that of the arch, reflecting a possible 'threshold of internal temperature', which is possibly due to cutaneous vasodilation initiating a heat loss response (Kenny, Sigal et al. 2016). This threshold is reached earlier in summer since ambient temperature is higher. A further in-depth discussion related to thermoregulation during treadmill walking will be presented in Chapter 8. This discussion will also include comparison of results between healthy and diabetic participant groups.

Finding 5: *No significant difference in temperature was found between the left and right foot within the same season, $p > 0.05$.*

In-shoe temperature readings and kinetics at the arches and toes of the left and right foot were similar in both seasons. Similar results were reported in a previous study (Foltyński, Mrozikiewicz-Rakowska et al. 2014) demonstrating no significant difference in skin temperature between the left and right foot ($p = 0.79$). These results therefore suggest that in a healthy individual no difference in in-shoe temperature should be observed between both feet during ambulation when using identical hosiery.

Finding 6: *Inter-participant temperature variation was larger in winter when compared to summer.*

The variation of in-shoe temperature between healthy participants at the initial phase of the trial was more evident in winter than in summer. During acclimatization, participants' feet were not equipped with any insulation and in winter foot temperature varied significantly between participants, ranging from 20^0C to 31^0C. This inter-participant temperature variation was maintained throughout most of the trial. It is therefore apparent that these results suggest that different individuals may have slightly differing peripheral thermoregulation mechanisms when exposed to colder temperatures, as reported in a similar study by Nardin et al. (2010).

Finding 7: *A small but consistent initial temperature drop was observed at the toe area in both seasons, while it was absent in the arch area*

The current study showed a small but consistent initial drop in in-shoe skin temperature of 0.1 degrees in the first 5 minutes in winter at the toe region (section 6.6.4.1). However, this small initial reduction falls within the measurement errors of the sensor (see Section 4.2.1). Therefore, more-sensitive sensors, with lower measurement error would need to be developed and used, before this observation can be confirmed as clinically meaningful. While a small decrease in temperature of 0.1^0C may not be clinically relevant *per se*, it provides possible evidence of a skin reflex vasoconstriction response, when demand for blood perfusion by working muscles is increased, during the initial stages of exercise. Although no previous studies have documented this response in the foot during ambulation in-shoe, this initial temperature drop (possibly reflecting a haemodynamic thermoregulatory response) during exercise is consistent with previous observations in other parts of the body (Zontak, Sideman et al. 1998, Merla, Mattei et al. 2010, Svaic, Lukenda et al. 2015, Tanda 2015a). Interestingly, the arch area did not demonstrate this pattern, which could be explained by two possible reasons: either due to muscle bulk (Abductor Hallucis) beneath the location of sensor placement. It has been suggested that the skin over exercising muscle mass tends to be warmer than skin over other structures, as exercise progresses (Tanda 2015a), thus obscuring any temperature drop. Or due to greater amount of ventilation occurring because of a bellows action in-shoe which has been reported at an open space such as beneath the arch in a closed shoe (Satsumoto, Takeuchi et al. 2011)

Finding 8: *There was no significant difference in mean in-shoe RH at the toe (RH_{Toe}) and arch area (RH_{Arch}) between both seasons, $p > 0.05$.*

The RH at the toe and arch area was similar between summer and in winter. While the recorded ambient climate for summer and winter seasons differed in ambient temperature, they did not vary in ambient RH. This was also reflected in the initial in-shoe RH readings which were similar between both seasons and in the RH kinetics as they evolved

over the 38 minutes of moderate treadmill walking. While results demonstrate comparable in-shoe RH dynamics between both ambient climates, there was a non-significant trend of approximately 5-10% lower RH in winter than summer (where mean maximum in-shoe RH was shown to reach 82% compared to 76% in winter). In studies investigating footwear comfort, microclimate sensations are largely influenced by relative humidity levels of the air inside the footwear (Irzmańska 2015, Barkley, Bumgarner et al. 2011). Previous research has established that the optimum level inside the shoe is 60-65% (Bergquist, Holmér 1997a). As the presented results show, the optimum humidity levels were exceeded in both locations that were studied and in both ambient climates. The influence of RH levels on footwear comfort and the clinical implications associated with it will be further discussed in the main discussion chapter (Chapter 8).

Finding 9: *RH in the toes was significantly higher ($p < 0.05$) than RH in the arch throughout most of the trial and this was more evident in summer.*

An important finding reported in the current study is the different RH pattern of changes observed between the toes and the arches, where in-shoe RH in the toe region exhibited a larger increase of 10% after 25 minutes of exercise (from 71% to 82%) compared with a 4% increase in the arch area (68.5% to 73%) during the same time. Relative humidity in the toe region at the end of the walking trial was in fact significantly higher in the toes when compared to the arches in both seasons. While significant, it should be noted that the difference in RH between the toes and the arch (Appendix X, table 3 & 4) is within the measurement error reported in the repeatability study (Preliminary study 2, Chapter 5) and therefore, these results may be due to measurement variability and not location.

These high RH values may result in excessively moist skin at the toe area due to poor evaporation of sweat caused by closed footwear, thus foot skin in healthy individuals often feels moist and its RH higher compared to exposed hairy skin. This is likely to affect skin surface co-efficient of friction (Tang, Ge et al. 2009), which in a healthy individual with

normal sensation is managed within the physical characteristics of skin. However, this may have adverse clinical implications in diabetic patients and is further discussed in Chapter 8.

Finding 10: *A significant inter-participant in-shoe RH variability in both seasons was noted, which was more conspicuous in winter. This is also evidenced by wider SDs in winter when compared to summer.*

Inter-participant in-shoe RH variability observed in the current study is in agreement with previous works where a high degree of variation of sweat rates between individuals was reported during exercise (Holmes, Miller et al. 2011). The increased variability in winter may also be associated with increased variability in in-shoe temperature in the same season.

Chapter 7

Study 2

In-shoe Microclimate during Mediterranean winter and summer in Participants living with Diabetes Mellitus.

7.1 Introduction

The basic function of any kind of footwear is to provide protection from any potential hazards to which the foot is exposed, especially when used to protect the insensate foot, such as in diabetes (Cavanagh 2004). Published literature related to footwear in diabetes is usually based on research undertaken in countries having cooler climates, predominantly the UK, USA and northern Europe. Following this, based on their recommendations, clinical guidelines are developed by international organizations, namely the National Institute for Clinical Excellence (NICE), International Diabetes Federation (IDF), American Diabetes Association (ADA) and the World Health Organization (WHO), with the intention of these guidelines being adopted across affiliated countries. Malta is one of these countries with its own Diabetes Association which is in turn part of the IDF. While the evidence, on which footwear guidelines are based, is still being questioned (Maciejewski, Reiber et al. 2004), the transferability of such guidelines to countries with warmer climates has been of clinical concern. However, no robust research has explored this issue to date. Footwear research related to the diabetic foot has mainly concentrated on stability, shock absorption and pressure reduction. Notably, literature concerning patterns of temperature changes in the footwear (Herold et al 2010) and inside the shoe during exercise is scarce in this vulnerable population. The recommended use of appropriate footwear in diabetes typically refers to a closed shoe that is made of soft leather with a rubber sole. Anecdotal evidence (Bergin, Nube et al. 2013) suggests that patients using closed footwear in the hot summer months expose their feet to a hot and humid environment, a condition which may be detrimental to skin resilience, as proposed in Chapter 2.

Skin temperature and humidity are fundamental variables in human physiology, particularly in the diabetic population as they may be influencing factors in the development of foot ulceration, as evidenced in other specialized areas related to tissue viability and decubitus ulceration (Martinez Cuervo, Soldevilla Agreda et al. 2007). Since their measurement poses various challenges to researchers, knowledge of in-shoe micro climate kinetics during ambulation is limited. Therefore, a preliminary study (Chapter 6) was

undertaken in order to establish temperature and humidity kinetics during treadmill walking in healthy participants. Findings demonstrated that in-shoe microclimate kinetics in healthy individuals are influenced by seasonal variation and revealed that thermoregulatory function may be reflected in these measurements. For the work of this chapter, the same protocol was repeated on a population with diabetes to assess the in-shoe temperature and RH kinetics during exercise. The study presented in this chapter therefore aims to investigate whether seasonal variation has an impact on the in-shoe microclimate during exercise, in individuals living with diabetes. Of course, it was necessary to overcome some challenges to the applicability of the measurement of in-shoe microclimate in this small sample group of people (using the sensors). The findings will be compared to those obtained in the healthy population highlighting any existing differences between these two studies.

This research will shed light on the appropriateness of guidelines (based on research carried out in cooler climates) related to footwear that is being worn in warmer Mediterranean climates. It is hoped that results from this research will initiate discussions related to the development of more climate-specific guidelines associated with therapeutic footwear.

7.2 Aims of the Study

- To investigate the influence of seasonal variation on in-shoe temperature and RH levels during ambulation in a small sample cohort of participants living with diabetes.

- To assess whether there is a difference in in-shoe temperature and RH kinetics between diabetic (DM) and previously established data in healthy participants (Chapter 6).

7.3 Objectives

- To measure in-shoe temperature and relative humidity (RH) during ambulation in a DM population in summer using previously validated instrumentation (see Chapter 5).

- To measure in-shoe temperature and relative humidity (RH) during ambulation in a DM population in winter using previously validated instrumentation.

- To assess and compare in-shoe temperature and RH kinetics during ambulation between summer and winter.

- To assess and compare in-shoe temperature and RH kinetics during ambulation between DM and previously established baseline data (healthy individuals, Chapter 6).

7.4 Ethical Considerations

Permissions to conduct this study were obtained from the local ethics committee, University of Malta, after a formal proposal was presented (Appendix III) as detailed in section 4.6. However, in view of the vulnerability of the participants due to diabetes, additional care was given in order to eliminate any possible known risks to injury. These included:

1) A safety inspection was done by the researcher in order to eliminate any potential hazards, such as tripping from loose wiring and other 'obstacles'.
2) A thorough inspection of the footwear was done by a qualified podiatrist to confirm that that the shoes were adequate to be used during the trial without causing injury.

3) Each participant was assessed by a qualified podiatrist for any injuries or abrasions prior to the start of the trial and re-assessed after the trial to ensure that no injuries were sustained.
4) Each participant was assessed for peripheral sensory neuropathy as per exclusion criteria. It was emphasised that if the participant experienced any pain or discomfort during the trial, they were asked to immediately notify the researcher so that the trial will be terminated and feet inspected.
5) Additionally, during the trial, participants were asked every ten minutes whether any discomfort was being experienced due to the sensors.

7.5 Methods

Two sets of data collection were organised at the Biomechanics Lab, Faculty of Health Sciences, University Malta, twice on the same human cohort. Ambient climate was set at similar temperatures and RH levels reflecting summer and winter seasons as recorded during the study in healthy participants (Chapter 6).

7.5.1 Subject Cohort

A convenience sample of six Maltese adults living with diabetes, 4 males and 2 females, of a mean age of 69 years (±4.5), a mean weight of 75.4kg (±13.1), and a mean height of 166.1 cm (±14), were recruited for the study. They were informed about the study protocol, verbally and in writing, and signed a consent form, after satisfying the inclusion/exclusion criteria listed in the table below (see Table 7.1). All participants were assessed for peripheral arterial disease (PAD) and neuropathy by an experienced podiatrist as described in chapter 4. One male participant who felt fatigued after 15 minutes of

treadmill walking was immediately assisted and his participation in the study had to be terminated.

Table 7.1: Inclusion/exclusion Criteria – DM participant group	
Inclusion	**Exclusion**
Males & females Aged over 18 years Diabetes Mellitus Intact epidermis No signs of neuropathy/peripheral disease Walked unaided Able to walk on a treadmill comfortably	Any foot deformity Foot pain RA Current or h/o foot ulceration Participants showing unwillingness to participate. Smokers

7.5.2 Experimental Procedure to Investigate Seasonal Influence on In-shoe Microclimate.

The same protocol was used for data collection as that implemented for study 1 with healthy participants (see Chapter 6). A detailed write-up of the procedure used during this study was presented in Chapter four (see Section 4.4.1). This provided an important description of the methods used for data acquisition, including; acclimatization protocol, placement of sensors, and treadmill walking speed. Data for this study was collected in the same experimental room as that used for the baseline data (healthy participants). For this data collection, all windows and doors were closed and ambient climate was controlled artificially. In order to ensure that the climate in the experimental room reflected the climate recorded for the healthy participant group (baseline data), ambient room

temperature was controlled using air-conditioning and RH was controlled using a humidifier.

7.5.3 Data Treatment & Analysis

The recorded in-shoe temperature and RH data were treated and analysed as described in Chapter 6 (see Section 6.5.3 and 6.5.4). The data were assessed for normal distribution, and depending on the result, a paired sample t-test or Wilcoxon Signed Ranks test was used to compare mean temperature/RH between the two seasons (summer and winter). The paired sample t-test is a parametric test used when the measurements (temperature/RH) have a normal distribution. Conversely, the Wilcoxon Signed Ranks test is a non-parametric test and is used when the measurements have a non-parametric distribution.

(H_0) The **null hypothesis** specifies that the mean in-shoe temperature and relative humidity (RH) is not different between summer and winter and is accepted if the *p* value exceeds the 0.05 level of significance.

(H_1) The **alternative hypothesis** specifies that the mean temperature and RH are significantly different between summer and winter and is accepted if the p value is less than the 0.05 criterion.

Further analysis of data using Generalized Estimating Equation (GEE) models investigating the relationship of foot temperature and RH to exercise time, season, location and group was performed (as described in Section 6.5.4).

7.6 Results

Results from this small cohort of DM participants (n = 5) indicate that there is a marked difference in mean in-shoe temperature recordings between summer and winter at both locations (T_{Toe}, T_{Arch}), while mean RH levels (RH_{Toe}, RH_{Arch}), were not different between seasons. In the remainder of this chapter the key findings of this study will be presented, followed by a brief discussion of the results in light of previously established in-shoe temperature and RH kinetics in a healthy participant group (Chapter 6).

7.6.1 Key Findings of the Study

Finding 1: In-shoe Temperature

- Mean in-shoe skin (foot) temperatures (at the toes and arches) recorded in summer were significantly higher ($p < 0.001$) than those recorded in winter throughout the trial (walking) for this diabetic participant group. Similar to the healthy participant group, the foot was warmer in Summer when compared in Winter in the diabetic group (see ***Section 7.6.4***).
- Mean in-shoe skin temperature for the diabetic participant group was significantly lower than that recorded for the healthy participant group throughout the walking trial, in both winter and summer seasons. This difference was more conspicuous in summer (see ***Section 7.6.4***).
- A delayed increase in temperature was observed in the diabetic participant group, when compared to the healthy participant group, possibly due to impairment in the thermoregulatory process (see ***Section 7.6.4.3***).

Finding 2: In-shoe Relative Humidity

- Season does not appear to influence in-shoe RH kinetics, since there was no significant difference between summer and winter in the diabetic participant group throughout the whole exercise trial (summer vs winter RH_{Toe}; $p > 0.05$; RH_{Arch} $p > 0.05$; see ***Section 7.6.5****)*.

- No significant differences in skin RH were noted between the diabetic participant group and the healthy participant group ($p > 0.05$; see ***Section 7.6.5****)*.

- Foot relative humidity (RH) increases as exercise time progresses; however, this trend is more noticeable for toe rather than the arch (see ***Section 7.6.5****;* Increments at toes – summer, 14.7%; winter 15.2%; Increments at arches - summer 5.6%; winter 5.6%).

7.6.2 Experimental Room Ambient Climate Data

Room ambient temperature and humidity levels were recorded using a previously established protocol described in Chapter 4, for both seasons (table 7.2). This data was analysed using the paired sample t-test to establish whether any difference existed between both seasons. Moreover, ambient climate data was compared with that observed during study 1 (healthy participant group, normative data).

Table 7.2: Experimental room ambient temperature and humidity mean recordings, measured using a calibrated and certified humidity and temperature data logger (CEM DT-172)

Ambient Climate	***Mean***	***SD***	***Difference between seasons*** *Paired Sample T-test*	***Difference between study 1 & 2*** *Paired Sample T-test*
Relative Humidity (%) Summer	71	±2.82	0.31	0.41
Relative Humidity (%) Winter	69.8	±2.01		0.30
Temperature (^{0}C) Summer	24.7	±0.82	0.01	0.23
Temperature (^{0}C) Winter	17.8	±0.57		0.81

Results showed that there was no significant difference (p = > 0.05) in room ambient RH between summer and winter, while a significant difference in ambient temperature was observed between seasons (p < 0.05). When comparing ambient climate between study 1 (healthy participant group) and 2 (DM group), no significant difference was observed, both in summer or in winter (p > 0.05).

7.6.3 Test for Normalcy of Data

The Shapiro-Wilks test was used to assess the normality assumption of the temperature and RH distribution obtained for both sensors, at both locations (toe and arch) and orientations (left and right) in summer and winter.

Results obtained from the Shapiro-Wilks Normality Test (Table 3 and 4, Appendix VII) indicate that the distribution is normal since the *p*-value exceeded the 0.05 level of significance. Therefore, a parametric test (paired sample t-test) was considered for further analysis to compare the mean relative humidity values between different locations (toe and arch), different seasons (summer and winter) and different groups (healthy and diabetic).

7.6.4 Influence of Seasonal Variation on In-shoe Skin Temperature in People Living with Diabetes and Compared with Healthy Individuals

Measurement recordings of mean in-shoe skin temperature data at one-minute interval over a 38-minute treadmill walking in DM participants are illustrated below (see Figure 7.1).

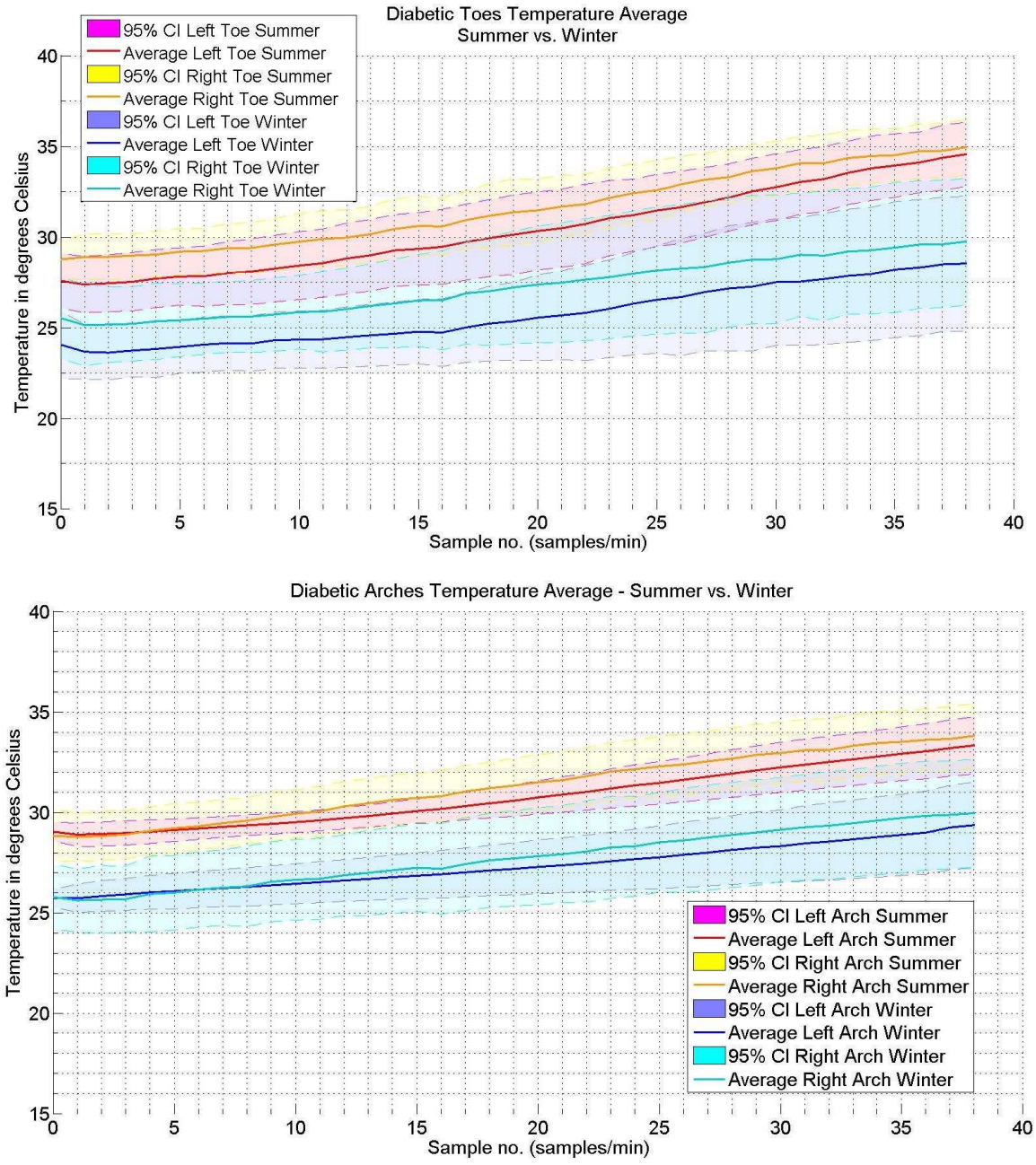


Figure 7.1: Line graph illustrating in-shoe skin mean temperature and CI values at the toes and arches for summer and winter during 38 minutes of moderate walking in a diabetic participant group.

The line graph illustrates mean in-shoe temperature recorded with 95% CI values at the toes and arches of all DM participants according to exercise time. The red and orange lines represent data recorded from the left and right foot respectively in summer. The dark blue and light blue lines represent data recorded in the left and right foot respectively in winter. The graph demonstrates how foot mean temperatures increase as exercise time progresses.

Unlike the in-shoe temperature kinetics observed in the healthy participants (Figure 6.1, where the graph was 's-shaped') the rate of temperature increase observed in the DM participants is relatively linear throughout the exercise. Also, in contrast with the healthy participants, the DM group demonstrated a larger increase in temperature throughout the whole exercise in summer when compared to winter. This pattern was demonstrated in both toes and arches. The increments in temperature (^{0}C) from the start of walking to the end of the exercise period were:

Diabetic Participant Group (Study 2)

Toes = 6.58^{0}C in summer and 4.35^{0}C in winter

Arches = 4.63^{0}C in summer and 3.91^{0}C in winter

Healthy Participant Group (Study 1)

Toes = 2.54^{0}C in summer and 7.78^{0}C in winter

Arches = 3.67^{0}C in summer and 6.93^{0}C in winter

The paired sample t-test (Table 3, Appendix VIII) was used to analyse whether a significant difference existed in the in-shoe mean temperature and RH data between seasons in DM participants at the arch and toe over the 38-minute exercise trial. Data for the left and right foot were merged since no significant difference was found between them

$p < 0.05$ (Appendix VIII). Results (Table 3, Appendix VIII) demonstrated that there was a significant difference ($p < 0.01$) between seasons in both arches and toes, indicating that seasonal variation had an influence on in-shoe temperature in both locations on the foot, throughout the exercise trial. It was also observed that, in a similar way to the healthy participants (Chapter 6), DM participants demonstrated more dispersed temperature data in winter than summer. Results therefore show that the alternative hypotheses (H_1) can be accepted, since the mean temperature is significantly different between summer and winter with p value less than the 0.05 criterion across the exercise trial. The interaction between season and in-shoe temperature kinetics in both toes and arches was further analyzed using GEE models and are presented in section 7.6.4.3.

In order to further analyse the data from the DM participants, individual line graphs of in-shoe temperature and relative humidity were plotted for both seasons and both locations, as given below. This method of analysis was more appropriate due to the small number of participants and allowed the identification of trends of in-shoe kinetics of each individual.

7.6.4.1 In-shoe Toe Temperature (T_{Toe}) Kinetics in People Living with Diabetes

In order to analyse the temperature kinetics recorded in the toe region between seasons over a period of 38 minutes of treadmill walking, each data point for each participant was plotted on a separate line graph. In the plot below (Figure 7.2), each participant (n=5) is represented by a different colour.

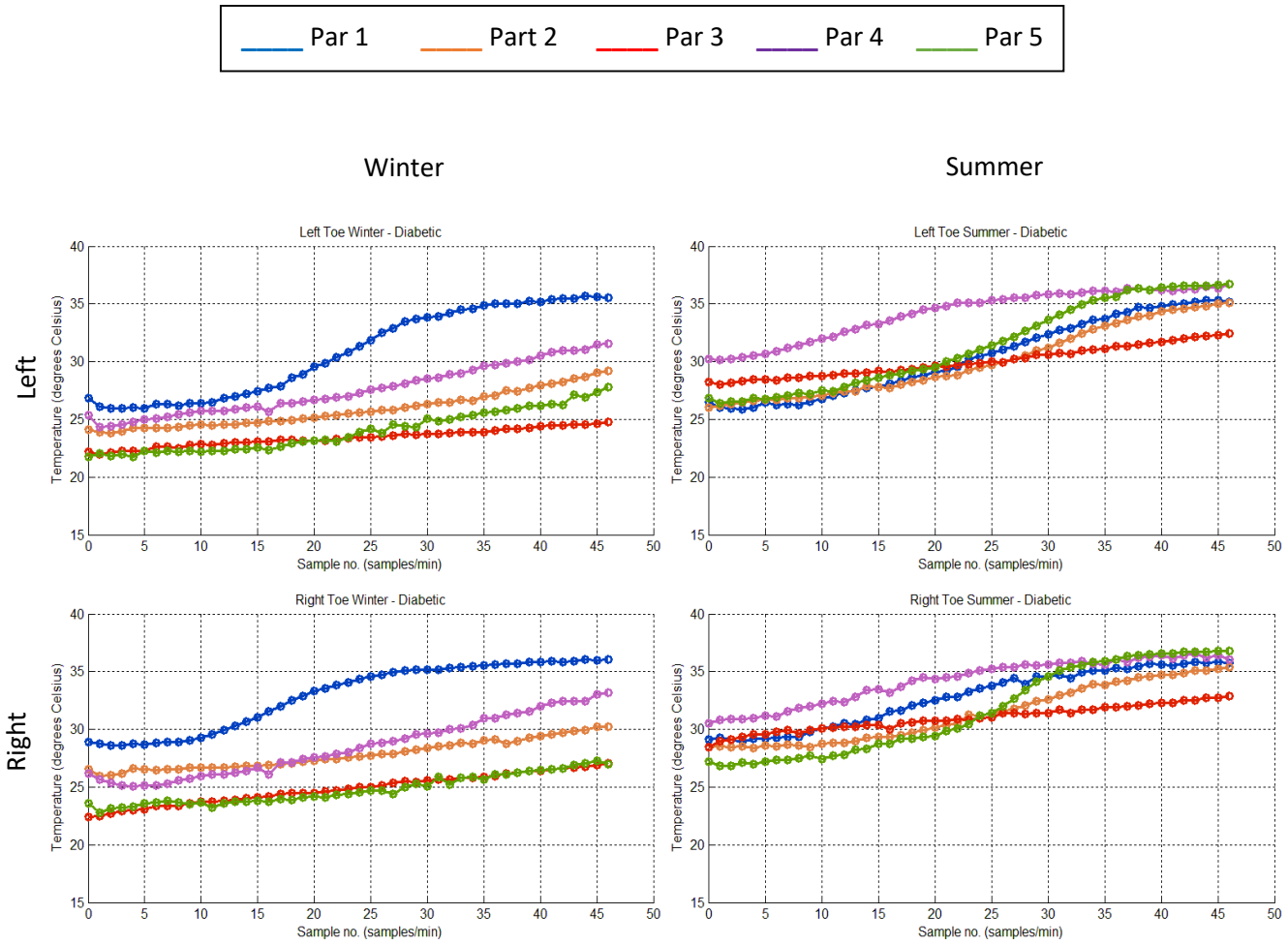


Figure 7.2: Individual participant (DM) in-shoe skin temperature kinetics at the Toes (T_{Toes}) over a 38-minute trial for the left foot and right foot, both seasons.

The plotted graphs illustrate in-shoe temperature at the toes as treadmill walking time progresses over the 38-minute trial, as recorded in the left and right foot in both seasons. While the influence of seasonal variation was evidenced statistically (see Table 3, Appendix VIII), the temperature kinetics were similar for both seasons with similar rates of temperature increase recorded in both summer and winter. This is in contrast to that observed in the healthy participant group (see Figure 6.2) where in-shoe temperature rate of increase was more marked in winter. However, the diabetic group forms a small study sample so findings are not generalizable, and could be a direction for further study.

The pattern of in-shoe temperature changes at the toes among participants living with diabetes, was also different to that of the healthy participant group. It appeared that, unlike the healthy participants, in-shoe toe temperature changes amongst DM participants was not influenced by ambient temperature, since the distribution of recorded temperatures at the initial part of the trial was similar in both seasons. However, it should be noted that, after acclimatization, initial mean in-shoe temperature at the toes was 5^0C lower than that recorded the healthy participant group.

The influence of the physiological response to moderate exercise on in-shoe temperature at the toes, also appears to be different from that observed in the healthy participant group. This is illustrated in the above graph plots where the changes in in-shoe temperature increased as treadmill walking progressed, resulting in a wider distribution of in-shoe temperature at the end of the trial (T_{38}). This pattern was observed in both summer and winter and is the opposite of the pattern observed in the healthy participant group where temperature variation among participants decreased as temperature increased.

In a similar way to the healthy participant group, most DM participants exhibited an initial temperature reduction occurring at the start of treadmill walking and lasting for approximately 4 minutes. This temperature fall was observed in both summer and winter

but was more evident in the winter plots. This will be explored further within the discussion section of this chapter.

As shown statistically (independent-sample t-test $p > 0.05$, in the results presented in Appendix IX), the similarity of in-shoe temperature kinetics at the toes between the left and right foot of individual participants was also evident when observing the plots illustrated in Figure 7.2. The similarity between the contra-lateral limbs was consistent in both seasons. This is further explored in the discussion section of this chapter.

An independent sample t-test was performed to analyse whether there was a statistical difference in in-shoe temperatures at the toes between the healthy participants and the DM participants. Results presented in Table 1 (Appendix XIII) indicate a significant difference ($p < 0.05$) in temperatures recorded in summer throughout of the whole of the treadmill walking trial. However, when analysing the Winter results, no difference was observed in the first 19 minutes, after which a statistically significant difference ($p < 0.05$) was achieved until the end of the trial. This difference is probably due to the enhanced rate of temperature increase in the healthy participant group during the 15^{th} and 35^{th} minute, which was not manifested in the DM group.

7.6.4.2 In-shoe Arch Temperature (T_{Arch}) Kinetics in People Living with Diabetes

In order to analyse the temperature kinetics recorded in the arch region between seasons, each data point was plotted on a line graph to illustrate the in-shoe temperature kinetics during 38-minutes of physical activity in summer and winter for each individual participant in the DM group (see Figure 7.3). Each participant is represented by a different colour.

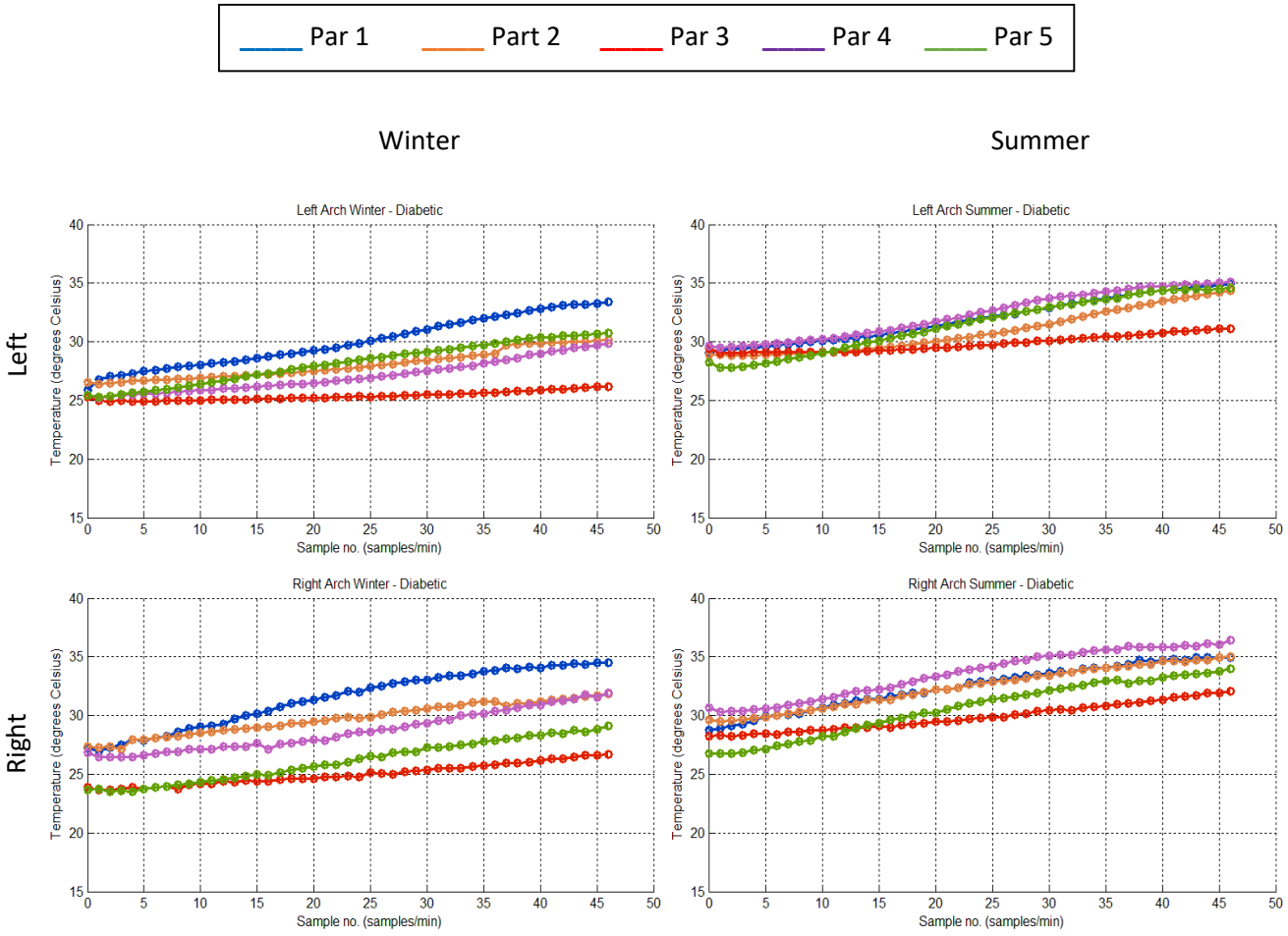


Figure 7.3: Individual DM participant in-shoe skin temperature kinetics at the Arch (T_{Arch}) over a 38-minute trial for the (a) left foot and (b) right foot, both seasons.

The line graphs above (Figure 7.3) illustrate in-shoe temperature at the arch as it evolves over the 38-minute treadmill walking trial, recorded in the left and right foot in both seasons. While the influence of seasonal variation was statistically evidenced (Table 3, Appendix VIII) the temperature kinetics were similar for both seasons, with similar rates of temperature increase recorded in both summer and winter. This was also in contrast to that observed in the healthy participant group (Figure 6.2) where in-shoe temperature rates of increase were more marked in winter.

The in-shoe temperature changes amongst the DM participants observed in the arch area exhibits similar patterns to those observed in the same participant group in the toe region during both walking trials (summer and winter). A non-significant difference was also observed ($p > 0.05$) in in-shoe temperature kinetics between toes and arches (Appendix X), meaning that both temperature kinetics and temperature recordings were comparable between these two regions in the DM group. The key observations are:

1) Ambient temperature did not influence initial in-shoe temperature recordings.
2) Physiological thermoregulatory responses to moderate treadmill walking appeared to result in an increase in variability between participants as the walking progressed.
3) An initial temperature decrease, at the start of exercise, was observed in the arch area in some participants, similar to the toe area.
4) No significant difference ($p > 0.05$) in in-shoe temperature kinetics was observed at the arch area between the left and right foot of individual DM participants (Appendix IX).

Statistical tests (independent sample t-test and Mann-Whitney tests) were performed to analyse whether there was a statistical difference in in-shoe temperatures at the arches between the healthy participants and the DM participants. Due to the small sample size in the DM group, results of normality testing suggested that it would be more conservative to

use no-parametric tests. Results presented in Table 3 (Appendix VIII) indicated a significant difference in temperatures ($p < 0.05$) recorded in summer throughout the treadmill walking. However, when analysing the winter results, no difference was observed between the 1st and 7th minute, after which a statistically significant difference was observed ($p < 0.05$) until the end of the walking trial.

7.6.4.3 Generalized Estimating Equations – In-shoe Temperature

As detailed in Chapter 6 (Section 6.5.4) Generalized Estimating Equation (GEE) models are appropriate for analysis of correlated longitudinal data. These models extend the traditional repeated-measures models because they accommodate dependent variables that follow any distribution within the exponential family.

In-Shoe Temperature Analysis

The GEE model relates foot temperature (dependent variable) to four categorical predictors, which include time (1 to 38 minutes), season (summer and winter), location (toe and arch) and group (healthy and diabetic). The model also includes all four main effects and three interaction terms (time*season, time*location and time*group) to examine how the temperature increases with exercise time, between different seasons according to different locations on the foot and between different groups. Since the Shapiro-Wilks test showed that for the majority of the different combinations of time, location and orientation levels, the temperature distributions satisfied the normality assumption, it appeared appropriate to assume a normal distribution and an identity link function. To fit the GEE model the participant number is declared as the subject variable.

The correlation matrix represents the within-subject relationships and there are five structures available in SPSS (Independent, Autoregressive, Exchangeable, M-dependent and Unstructured). The QIC information criterion is used to identify the best correlation structure for the model where the optimal correlation structure has the lowest QIC value (Table 3, Appendix XII). The QIC information criterion shows that the **exchangeable** structure is the best correlation structure for this GEE model. This structure has homogenous correlations between elements and is also known as a compound symmetry correlation structure.

Table 7.3: Analysis of individual in-shoe temperature data measurements

Significance of main and interaction effects for model fit

Model Term	Wald	df	P-value
Intercept	10916.8	1	0.000
Time	2106379208.9	38	0.000
Season	124.017	1	0.000
Location	5.006	1	0.025
Group	31.680	1	0.000
Time * Season	57699343233.6	38	0.000
Time * Location	5776.903	38	0.000
Time*Group	13645.478	38	0.000

Table 7.3 shows that Season, Location, Group and Time are significant main effects of Temperature. Moreover, the interaction of Season, Location and Group with Time are also significant interaction effects. This is clearly shown in Figures 7.4, 7.5 and 7.6 where the temperature line graphs are steep, well separated and are not parallel.

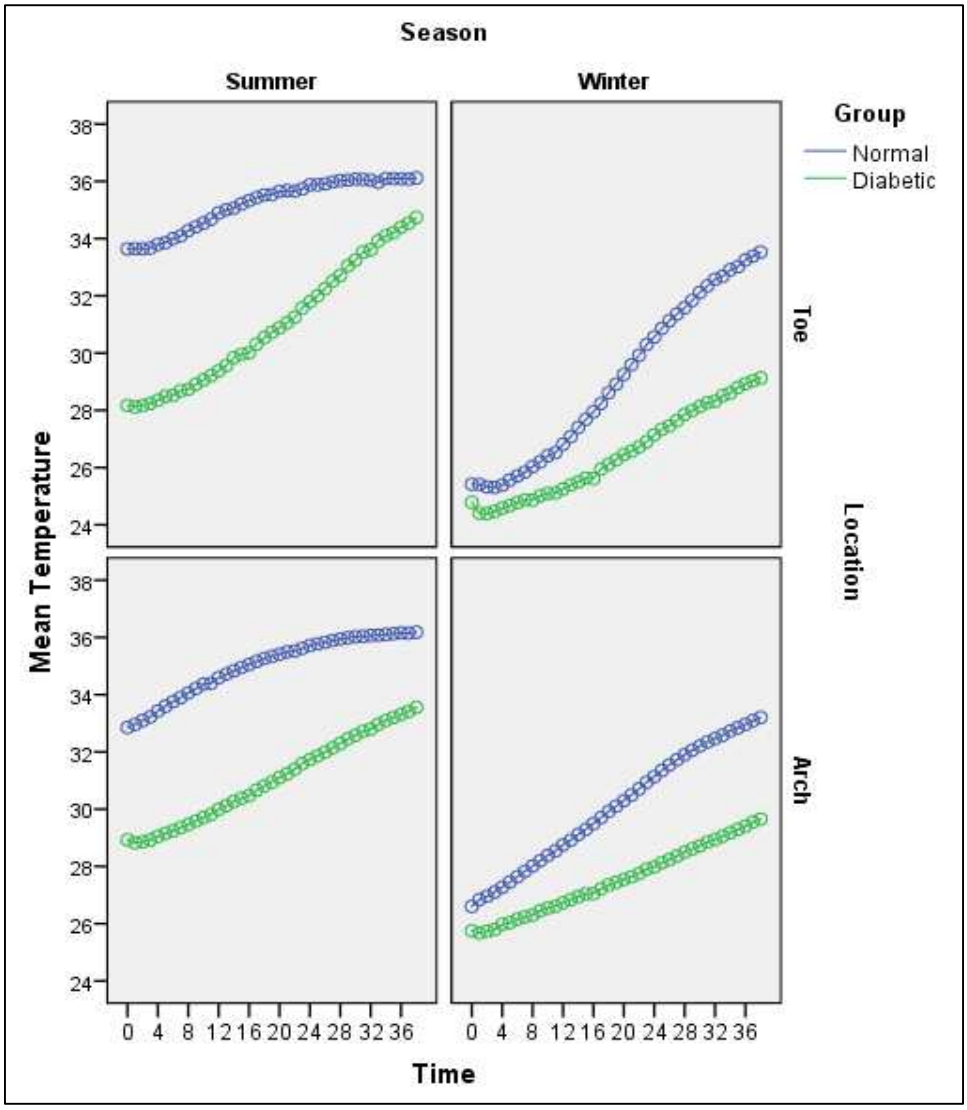


Figure 7.4: GEE Model - Mean temperature (^{0}C) clustered by Group, Season, Location and Time in healthy individuals and people with diabetes

Figure 7.4 clearly shows that temperature increases with an increase in treadmill walking time. Moreover, the temperatures of the healthy group are much higher than the diabetic group in both summer and winter. Group is a significant main effect since the temperature line graphs for the normal and diabetic groups are well separated and the interaction of Group with Time is significant since the line graphs are not parallel. It is interesting to note that while the temperature line graphs for the normal and diabetic groups tend to converge with an increase in treadmill walking time in summer, the temperature line graphs tend to diverge in winter.

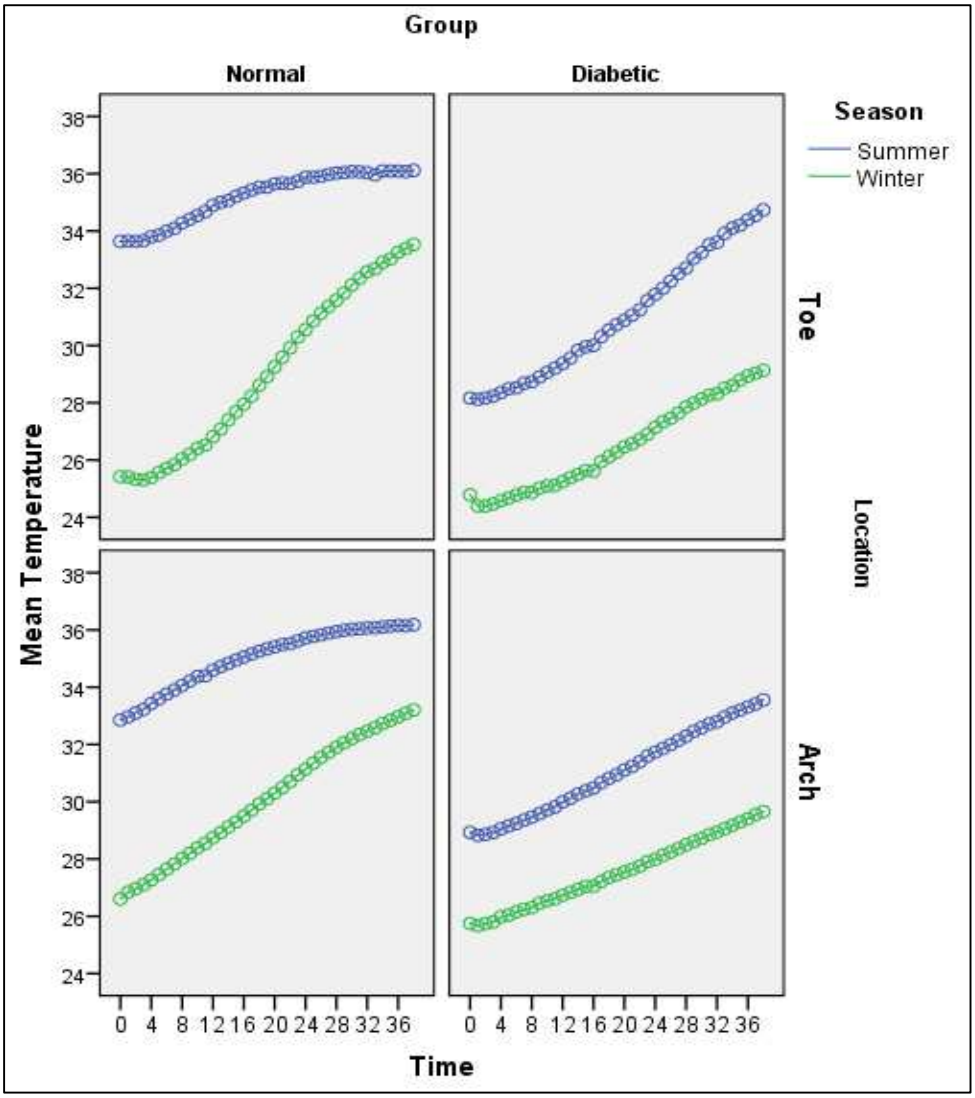


Figure 7.5: GEE Model - Mean temperature (^{0}C) clustered by Season, Group, Location and Time in healthy individuals and people with diabetes

Figure 7.5 clearly shows that temperature increases with an increase in treadmill walking time. Moreover, the summer temperatures are much higher than the winter temperatures, for both the healthy and diabetic groups. Season is a significant main effect since the temperature line graphs for the winter and summer seasons are well separated and the interaction of Season with Time is significant, since the two line graphs are not parallel, particularly for the healthy group.

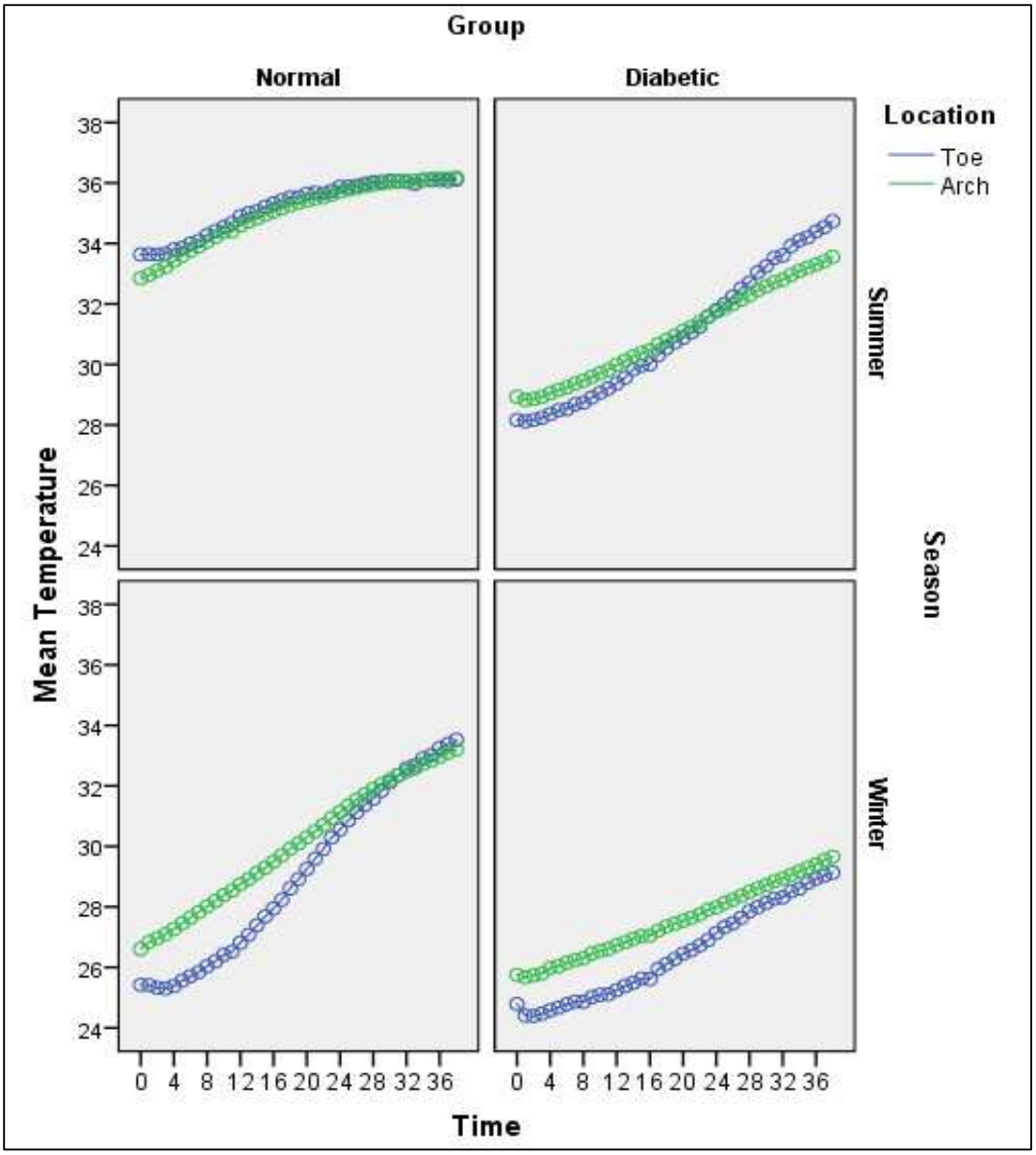


Figure 7.6: GEE Model - Mean temperature clustered by Location, Group, Season, and Time

Figure 7.6 clearly shows that temperature increases with an increase in treadmill walking time. The arch temperature is higher than the toe temperature in winter for both the healthy and diabetic groups; while the temperature of the toe and arches are quite similar in summer. Location of the sensor on the foot was a significant main effect since the temperature line graphs are separated, particularly in winter. Moreover, the interaction of Location with Time was significant since the temperature line graphs are not parallel, particularly for the normal group.

7.6.5 Influence of Seasonal Variation on In-shoe Skin RH in People Living with Diabetes, Compared with Healthy Individuals

Measurement recordings for in-shoe RH data at one-minute intervals over the 38-minute treadmill walking trial are illustrated below (Figure 7.7). Data for left and right foot were merged since no significant difference was found between them (independent sample t-test, $p > 0.05$; see Appendix X). The paired samples t-test (see Table 3, Appendix VIII) was used to compare in-shoe RH between seasons at the arch and toe region.

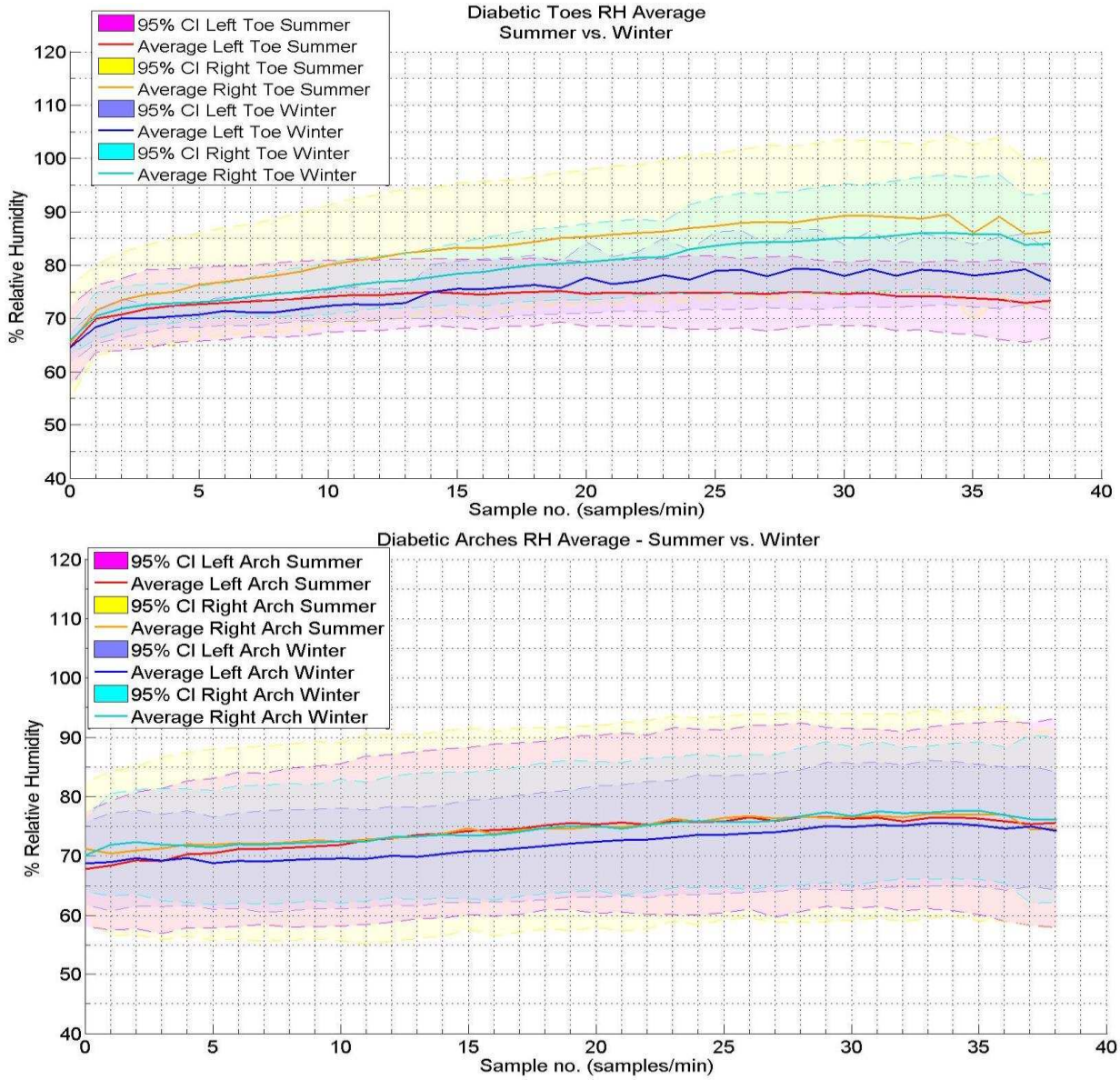


Figure 7.7 - Line graph illustrating in-shoe mean skin RH (%) with CI values at toes and arches for Summer & Winter during 38 minutes of moderate exercise (DM group, n = 5).

Figure 7.7 shows a line graph of mean RH recorded at the toes and arches of all DM participants (n=5) according to treadmill walking time. The red and orange lines represent data recorded from the left and right foot respectively in summer. The dark blue and light blue lines represent data recorded in the left and right foot respectively in winter. The graph demonstrates how mean skin RH increases as treadmill walking time progresses, with a higher increment observed at the toes when compared to the arches. These in-shoe RH patterns, from the start to the end of the walking trail, were similar for both seasons with a similar RH increase occurring in summer and in winter:

Toes: Summer RH = 14.7%; Winter RH = 15.2%.

Arches: Summer RH = 5.6%; Winter RH = 5.6%.

The in-shoe RH kinetics at both toes and arches occurring in both seasons were comparable to those observed in the healthy population (see Section 6.6.5.1 and 6.6.5.2).

Results obtained from this study demonstrate that there was no significant difference in in-shoe relative humidity between summer and winter in DM participants both at the toes and arches, when analysed using paired sample t-tests and Wilcoxon Signed-Rank tests ($p > 0.05$) throughout the treadmill trial (as shown in Table 4, Appendix VIII). It is worth noting that the standard deviation (SD) at the end of the trial was greater than at the start in both seasons at both toes and arches. The interaction between season and in-shoe RH kinetics in both toes and arches was further analysed using GEE models and are presented in section 7.6.5.3.

Although statistical tests using means have been conducted, to give an indication of trends in this participant group, interpretation of data derived from individual participants maybe more useful if analysed separately.

7.6.5.1 In-shoe Toe RH (RH_{Toe}) Kinetics in People Living with Diabetes

In order to analyse the RH kinetics recorded in the toe region between seasons over a period of 38-minutes of moderate treadmill walking, each data point for each participant was plotted on a line graph. In the plot below (Figure 7.8), each participant (n=5) is represented by a different colour.

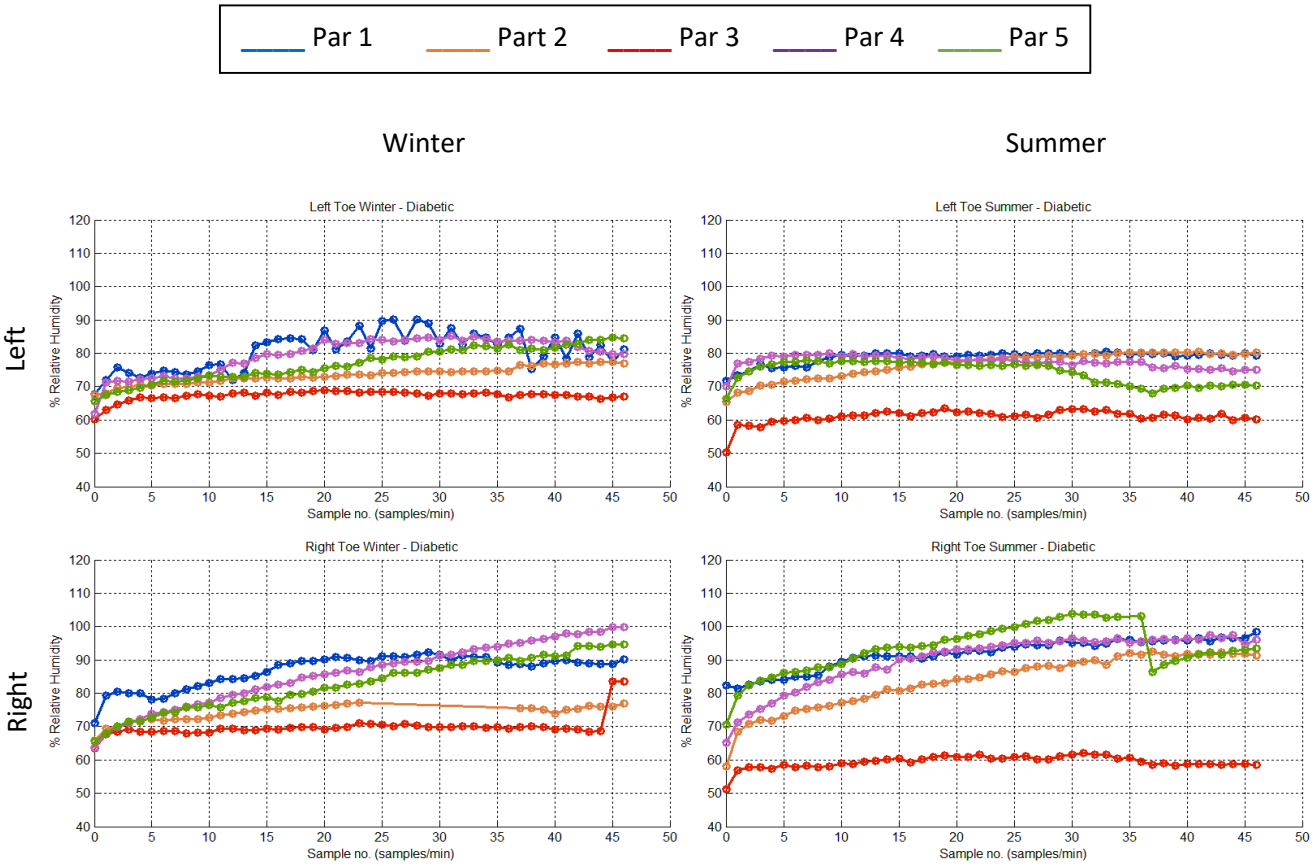


Figure 7.8: Individual participant in-shoe RH kinetics at the Toes (RH_{Toes}) over a 38-minute trial for the left and right foot, both seasons.

The pattern of changes in in-shoe RH over walking time is shown to increase at the toe area in both seasons by approximately 15% and this is similar to the healthy participant group. An initial wide RH variability among DM participants is evident (mostly in summer) and this increases as walking time progresses, resulting in a greater dispersion in summer (±13.5 SD) when compared to winter (±9.1 SD; see Table 4, Appendix VIII). This pattern of participant variability which increases as walking progresses, was also evident in the healthy participant group. However, the latter group resulted in a greater dispersion in winter, at the end of the walking trial (summer ±11.5 SD; winter ±18.1 SD; see Table 2, Appendix VIII). The initial variability among DM participants at time 0 does not appear to be influenced by ambient climate as the variability and SD among participants is similar in both seasons (Table 4, Appendix VIII).

It must be noted that all participants wore identical socks during both trials which were provided by the researcher, while using their own preferred walking shoes (identical in both trials) as described in Chapter 4. While efforts were made to maintain consistency between trials, inherent difficulties, discussed in detail in Chapter 4, were encountered during data collection, some of which have been evidenced in the graph above. For example:

1) The line graph representing RH data for participant 2 (winter for the right toe; Figure 7.8) illustrates missing data points between 25 to 37 minutes. This reflects the removal of 'abnormal spikes' and 'dips' (of more than 5% RH per minute) possibly due to sensor movement during exercise, which probably placed strain on the wire connections.

2) Replacement of sensors due to connection breakdown is evidenced in the data line graph representing participant 5 (summer for the right toe; Figure 7.8). A sudden drop in RH (of approximately 18%) at 37 minutes reflects the removal of hosiery causing a drop in RH until the sensor at the toe was replaced. Since hosiery had to

be removed, this also affected the RH readings at the arch area during the same period (Figure 7.9).

The independent sample t-test and Mann-Witney U test were performed to analyse whether there was a statistical difference in in-shoe RH at the toes between the healthy participants and the DM participants, giving an indication of possible trends in this population (DM). Results presented in Table 4 (Appendix VIII) indicate that there is no significant difference in RH ($p > 0.05$) as recorded in summer and in winter, throughout the whole treadmill walking trial.

7.6.5.2 In-shoe Arch RH (RH_{Arch}) Kinetics in People living with Diabetes

In order to analyse in-shoe RH kinetics, recorded in the arch region between seasons over a period of 38-minutes of moderate treadmill walking, each data point for each participant was plotted on a line graph. In the plot below (Figure 7.9), each participant (n=5) is represented by a different colour.

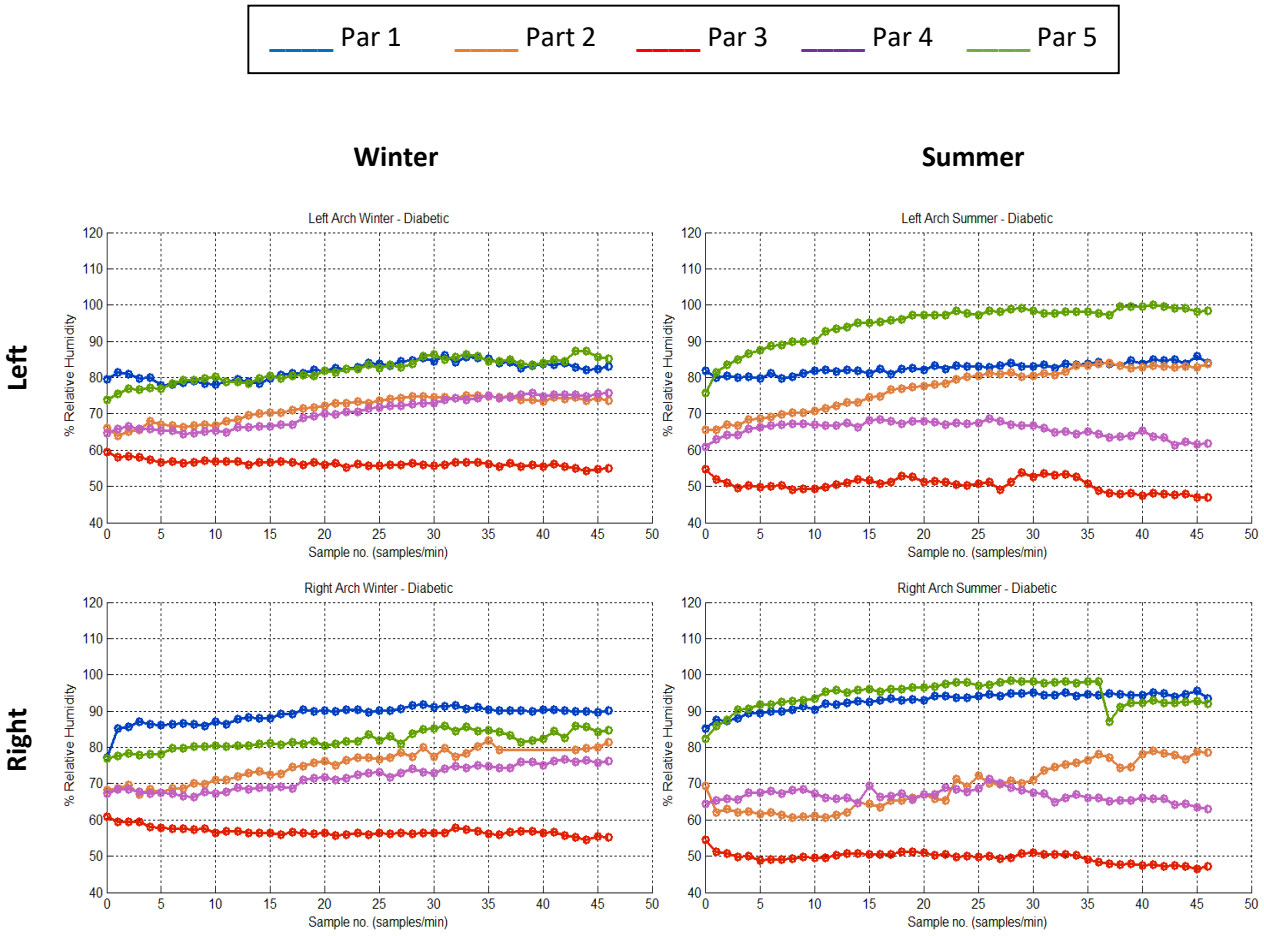


Figure 7.9: Individual DM participant in-shoe RH skin kinetics at the Arch (RH_{Arch}) over a 38-minute trial for the left and right foot, both seasons.

The line graph above (Figure 7.9) illustrates in-shoe RH at the arch as it evolves over the 38-minute exercise trial, recorded in the left and right foot in both seasons. While the lack of influence of seasonal variation was evidenced statistically (see Table 4, Appendix VIII) indicating no difference in RH between seasons ($p > 0.05$), similar in-shoe RH kinetics are illustrated in the graphs above.

The in-shoe RH variation amongst participants observed in the arch area exhibited similar patterns to those observed in the same participant group in the toe region. This is also evidenced statistically since no significant difference ($p > 0.05$) in in-shoe temperature kinetics between toes and arches was observed (Appendix IIX), meaning that both temperature kinetics and actual temperature recordings were comparable between these two regions in the DM group.

The independent sample t-test and Mann-Witney U test were performed to analyse whether there was a statistical difference in in-shoe RH at the arches between the healthy participants and the DM participants giving an indication of possible trends in this population (DM). Results presented in Table 2 (Appendix XIV) indicate that there was no significant difference in RH ($p > 0.05$) recorded both in summer and in winter throughout walking, in a similar way to those observed in the toe readings (Table 1, Appendix XIV).

7.6.5.3 Generalized Estimating Equations - In-Shoe RH Analysis

The second GEE model relates foot relative humidity (dependent variable) to the main effects time, orientation, location, season, group and the three interaction effects (time*season, time*group and time*location) to examine how relative humidity increases with exercise time between different seasons, different locations and between both groups. The correlation matrix represents the within-subject relationships and there are five structures available in SPSS (Independent, Autoregressive, Exchangeable, M-dependent and Unstructured). The QIC information criterion is used to identify the best correlation structure for the model where the optimal correlation structure has the lowest QIC value. The QIC information criterion shows that the **exchangeable** structure is the best correlation structure for this GEE model (Table 4, Appendix XII). This structure has homogenous correlations between elements and is also known as a compound symmetry correlation structure.

Table 7.4: Analysis of individual in-shoe RH data measurements

Significance of main and interaction effects for model fit

Model Term	Wald	df	P-value
Intercept	1838.842	1	0.000
Time	509941.545	37	0.000
Season	10.986	1	0.001
Location	24.438	1	0.000
Group	0.051	1	0.821
Time * Season	2228.225	37	0.000
Time * Location	175419770389.4	38	0.000
Time*Group	108026938627.6	38	0.000

Table 7.4 shows that Season, Location and Time are significant main effects of Relative humidity. However, Group is not a significant predictor. Moreover, the interactions of Season, Location and Group with Time are all significant interaction effects. This is clearly

shown in Figures 7.10, 7.11 and 7.12 where most of the relative humidity line graphs are steep, well separated and are not parallel.

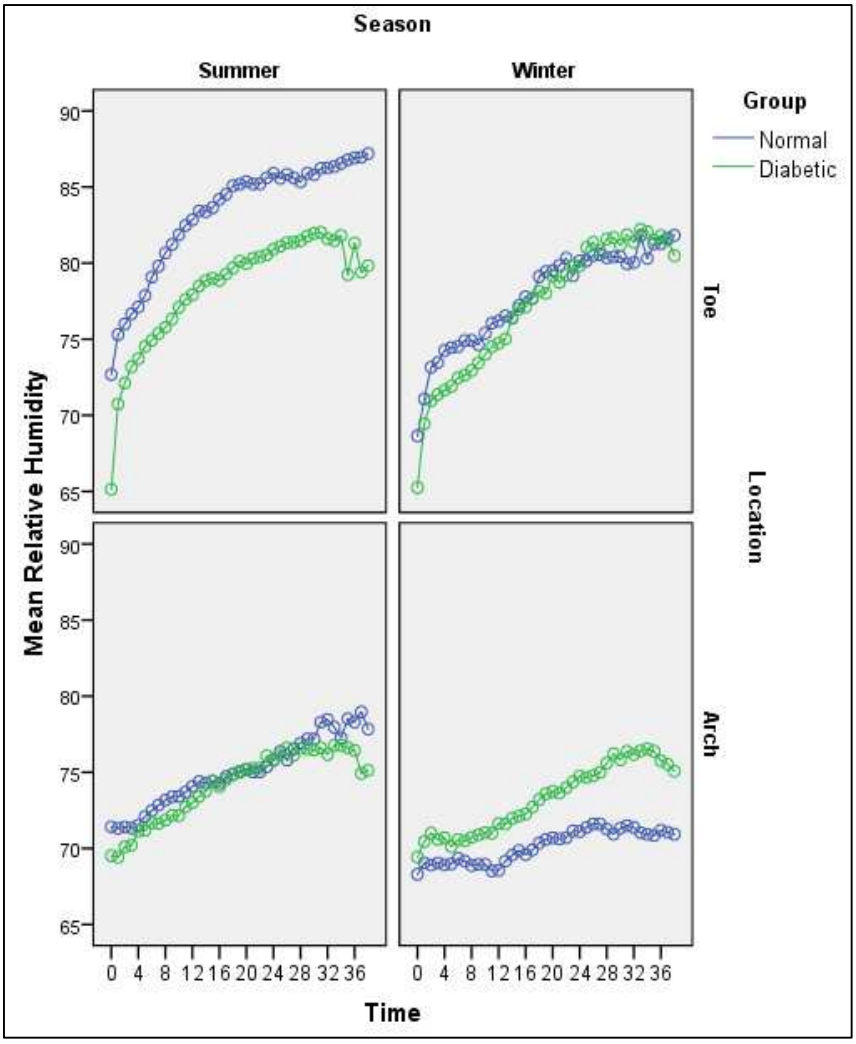


Figure 7.10: GEE Model - Mean skin relative humidity (%) clustered by Group, Season, Location and Time

Figure 7.10 clearly shows that relative humidity increases with an increase in training Time. Although Group is not a significant main effect, it is evident that the toe relative humidity in summer is larger for the healthy group compared to the diabetic participant group, while the arch relative humidity in winter is larger for the diabetic group compared to the healthy group. The interaction of Group with Time is a significant effect since the line graphs are not parallel.

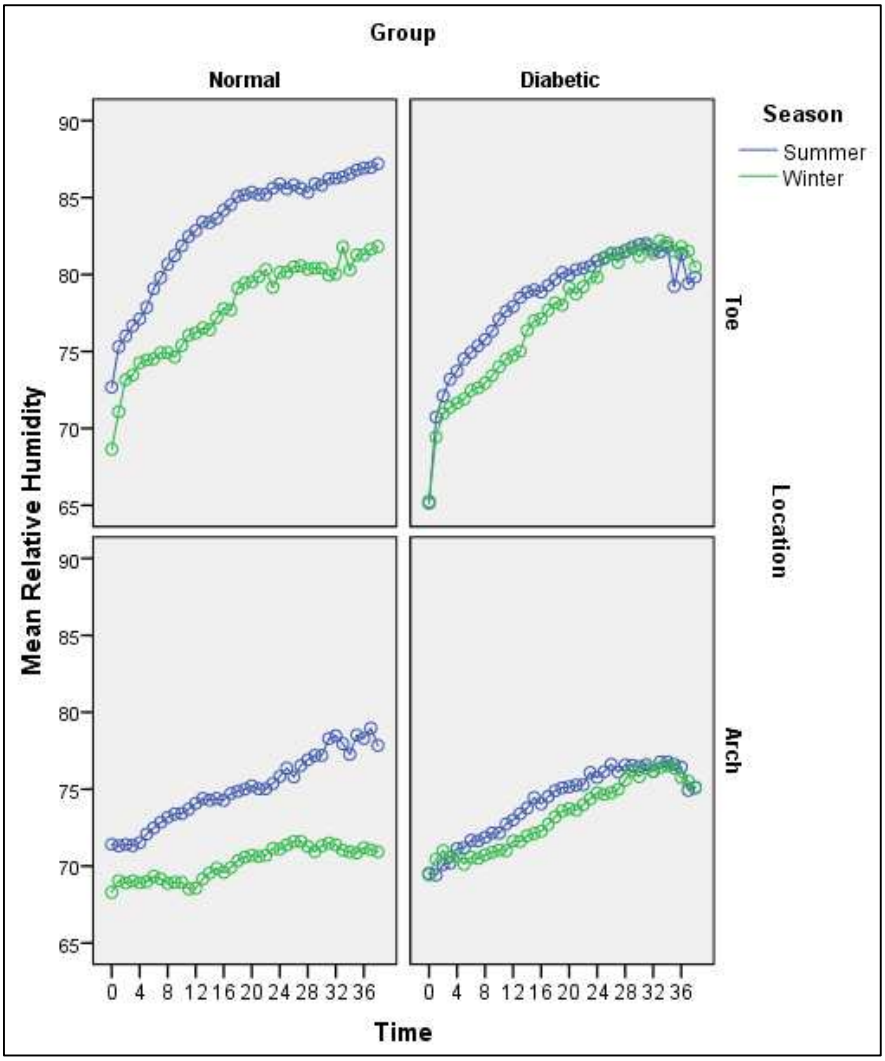


Figure 7.11: GEE Model - Mean skin relative humidity (%) clustered by Season, Group, Location and Time

Figure 7.11 clearly shows that relative humidity increases with an increase in walking time. The summer relative humidity readings are much higher than the winter relative humidity for the normal group but marginally higher for the diabetic group. Season showed a significant main effect since the relative humidity line graphs for the winter and summer seasons are well separated, particularly for the healthy group. Moreover, the interaction of Season with Time was significant since the two line graphs are not parallel, particularly for the healthy group.

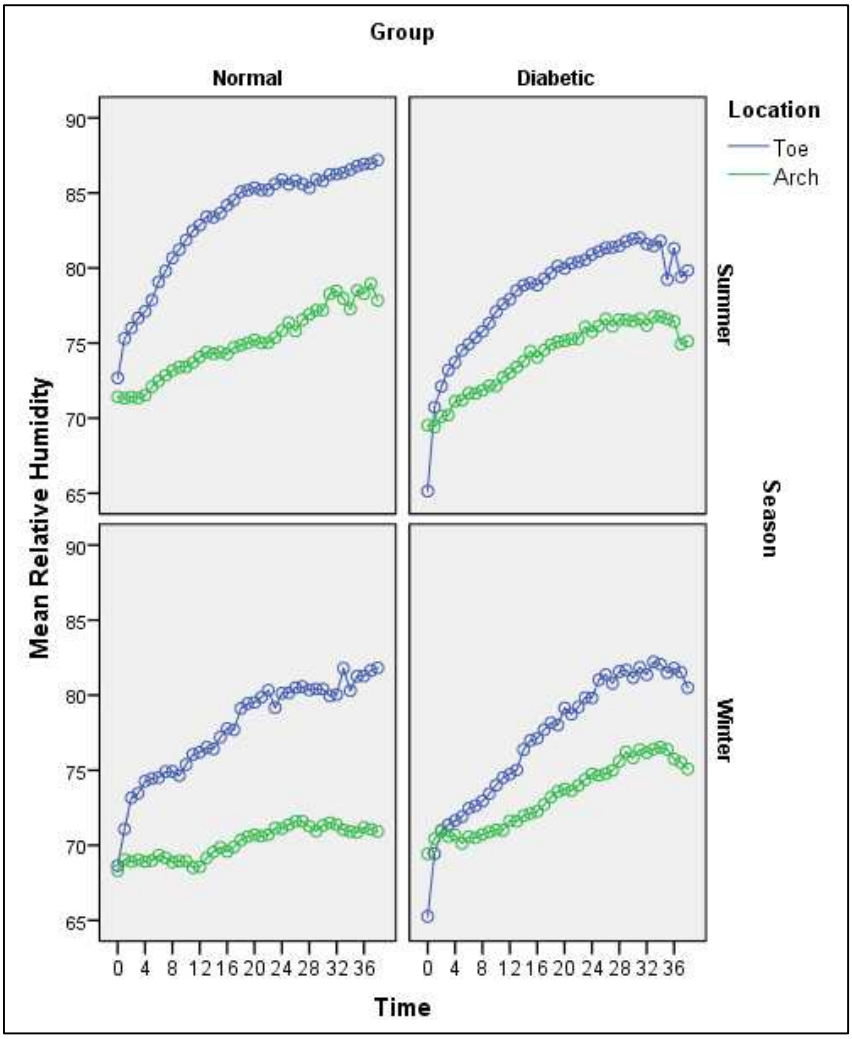


Figure 7.12: GEE Model - Mean relative humidity clustered by Location, Group, Season, and Time

Figure 7.12 clearly shows that relative humidity increases with an increase in walking time. The toe relative humidity is considerably higher than the arch relative humidity in both summer and winter for both the normal and diabetic groups. Location of the sensor on the foot displayed a significant main effect since the relative humidity line graphs are well separated, particularly in winter. Moreover, the interaction of Location of the sensor on the foot with Time was significant since the relative humidity line graphs are not parallel, particularly for the healthy group.

7.7 Synoptic Discussion

Finding 1: *Mean in-shoe temperatures (at the toes and arches) recorded in summer were significantly higher ($p < 0.001$) than those recorded in winter throughout the treadmill walking trial for the diabetic participant group.*

In the diabetic participant group, the foot was significantly warmer in summer when compared to winter which was also demonstrated when analysing the data using the GEE model approach. Season was identified to have significant effects on in-shoe temperature. These results are congruent with those observed in previous work of this thesis, in chapter 6, for the healthy participant group. This indicates that foot temperature can vary considerably across different seasons and that this variation is influenced by ambient climate. Similar results were observed in another study (Foltyński, Mrozikiewicz-Rakowska et al. 2014) investigating the influence of ambient temperature on foot temperature in healthy participants and in patients with diabetic foot ulceration. The authors demonstrated a significant correlation between foot temperature and ambient temperature in both healthy and diabetic individuals, with a greater correlation coefficient observed in healthy participants (healthy; r=0.69 & diabetic r=0.61). Further in-depth discussion of this work will be provided in Chapter 8.

Finding 2: *The arch and toe in-shoe mean temperatures are lower in the diabetic group than in the healthy participant group in both winter and summer. This difference is more pronounced in summer.*

In summer a statistical difference ($p < 0.001$) for in-shoe foot temperature kinetics is observed throughout the walking trial. The data show that foot temperatures in participants with diabetes were significantly lower than those in healthy participants both at the start (after 15 minutes of acclimatization at rest) and throughout the 38-minutes of moderate treadmill walking. Although no significant difference ($p > 0.05$, table 7.2) was observed in ambient summer temperature between study 1 (healthy participant group) and study 2 (DM participant group), it is worth noting that ambient summer temperature for

study 1 (24.0^0C) was slightly higher when compared to study 2 (28.2^0C), possibly influencing skin temperature at the start of the trial after acclimatization. Additionally, although significant the temperature difference at the toes between the healthy participant group and the diabetic group during the last five minutes of treadmill walking is within the measurement error reported in the repeatability study (Preliminary study 2, Chapter 5). Therefore, although significant, these results may be due to measurement variability and not group.

In winter, similar in-shoe mean temperature kinetics between diabetic and healthy participants were observed in the first 20 minutes at the toes. After that, a statistically significant difference was demonstrated ($p < 0.05$). It is worth noting that during this period the healthy participant group demonstrated an increased rate of temperature change (15^{th} - 35^{th} minute, Chapter 6, Figure 6.1) probably reflecting the thermoregulatory skin response to exercise (vasodilatation). The same concept is also reflected in the arch in-shoe kinetics where similar in-shoe mean temperature patterns between diabetic and healthy participants are observed in the first 6 minutes, after which a statistically significant difference is demonstrated ($p < 0.05$). This possibly indicates a different thermoregulatory response. This is discussed in further detail in Chapter 8.

Finding 3: *In-shoe mean temperatures increased as treadmill walking time progressed for both participant groups.*

Findings demonstrated that in-shoe mean temperatures at both toes and arches increased over time. Notably, in the healthy participant group, these increments were more conspicuous in winter than summer. In the diabetic group increments were similar for both seasons. It therefore follows that, while for the healthy participants the rate of temperature increase depended on initial in-shoe foot temperature (as discussed Chapter 6), this was not evident in the diabetic participant group. In the diabetic participant group there were similar increments in both seasons, even though initial temperatures between seasons

were significantly different. This may also be attributed to likely thermoregulatory dysfunction which may be present in the diabetic participant group.

Finding 4: *An initial temperature fall was observed during the first three minutes of walking in some participants, both at the toe and the arch.*

An initial decrease in temperature of approximately 0.1^0C was observed in the current study (as also evidenced in the healthy participant group). As discussed in Chapter 6, this small initial reduction falls within the measurement errors of the sensor (see Section 6.7, page 162). Therefore, more-sensitive sensors, with lower measurement error would need to be developed and used, before this observation can be confirmed as clinically meaningful. This initial decrease can probably be attributed to a skin reflex vasoconstriction response when the demand for blood perfusion by working muscles increases at the onset of exercise (Zontak, Sideman et al. 1998, Svaic, Lukenda et al. 2015, Merla, Mattei et al. 2010). Interestingly, this observation was demonstrated also in the arch area in the diabetic participant group, but it was absent in the same area in the healthy participant group. This is further explored in the discussion chapter (Chapter 8).

Finding 5: *There was no significant difference in in-shoe RH at the toes and arches between summer and winter, throughout the treadmill walking trial for the diabetic group ($p > 0.05$).*

The results demonstrated that in-shoe RH at the toes and arches was similar for both seasons, as also observed in the healthy participant group. In-shoe RH kinetics were comparable for both seasons in the diabetic participant group indicating that ambient climate did not influence in-shoe RH dynamics. While a non-significant trend towards lower in-shoe RH values (5-10%) in winter is observed in the healthy group, in-shoe RH in the diabetic group was practically identical for both seasons. However, the wide standard deviation (SD) of in-shoe RH measurements should be noted.

While it is acknowledged that the diabetic participant group was a small sample size, and thus these findings are not likely to be generalizable, nonetheless they suggest the useful applicability of in-shoe temperature and RH measurement in diabetic participants. It provides the foundation for further research in this field, particularly in a Mediterranean climate. Although mean temperatures and RH values were analysed in this sample group, the findings are meant to provide some insight into the trends in in-shoe microclimate kinetics during exercise rather than proposing generalised inferences in this population. This information is novel and cannot be compared to previous research. However, comparisons can be made with the earlier work of this thesis, which established 'normative' in-shoe microclimate kinetics (Chapter 6). It is suggested that individual participant analysis in the light of normative trends (in healthy participants) may be more useful in diabetes, as it may highlight important discrepancies. Further insight into the findings of this study and suggestions of important clinical implications are provided in the next chapter (Chapter 8).

Chapter 8

General Discussion

8.1 Introduction

The work of this thesis sought to investigate the influence of seasonal variation (Mediterranean Climate) on in-shoe microclimate in the context of diabetic foot ulceration. It was hypothesised that a warmer climate may result in higher temperature and relative humidity levels within the shoe, when compared to a cooler climate, thereby exposing the foot to an unfavourable environment especially during the Mediterranean summer months. As elicited earlier in this thesis, previous clinical studies (Wu, Ahn et al. 2009, Donovan, Dinh et al. 1993, Maklebust, Sieggreen 1996, Knox 1999), predominantly related to the study of human 'supporting surfaces' in the context of bedding material and pressure ulceration have shown that temperature and humidity have an important role in the development of such pressure ulcers in bed-ridden or wheelchair-bound patients. However, the knowledge gained from such trials has not been transferred to the diabetic foot, despite the similarity between both clinical scenarios exhibiting common characteristics with the skin being subjected to a combination of mechanical stress, high levels of temperature and relative humidity, and also subjected to limited air flow. The similarity of the ambient environment surrounding the skin in bed-ridden patients and the skin in the shoe of diabetic patients, has not been linked, in the literature. This lack of knowledge transfer may be attributed to the lack of technology to measure in-shoe microclimate especially during exercise. This has prevented studies to explore the effects of ambient climate on in-shoe microclimate. Therefore, the results of the work of this thesis, hopefully will go some way towards filling this gap in knowledge.

The work of this thesis demonstrated, for the first time, that seasonal variation has a significant influence on in-shoe microclimate. In-shoe temperature kinetics appear to be significantly different in the (Mediterranean) summer season compared to winter during walking. The findings of the work of this thesis on a small group of participants living with diabetes, also suggest that measured in-shoe temperature kinetics potentially revealed impaired thermoregulatory patterns. This is similar to what has been demonstrated in other parts of the body (Eg: hands). Moreover, these findings provide evidence that the measured

in-shoe temperature and RH, reach levels which may be detrimental to skin resilience. In view of this new knowledge, special consideration of in-shoe RH and temperature, in the context of diabetic foot ulceration (DFU) is emphasised. Therefore, the influence that ambient climate might have on in-shoe microclimate, within the DFU pathway, is proposed (see Figure 8.1). In this chapter, this proposed concept is explored in the light of the current findings and previous literature, highlighting novel information emerging from the work of this thesis, with proposals for new considerations in the theoretical mechanism underpinning the development of DFU.

8.2 The Influence of Seasonal Variation on In-shoe Microclimate

Results from the current study demonstrate that foot temperature is influenced by seasonal variation in both healthy individuals and individuals with diabetes mellitus. In-shoe RH reflected ambient RH (which was similar in both seasons) and was not influenced by the different ambient temperatures observed between summer and winter. Previous medical research investigating RH in-shoe is limited, with reports focusing mainly on system applicability rather than measured levels of in-shoe relative humidity (Maluf, Morley et al. 2001, Sandoval-Palomares, Yáñez-Mendiola et al. 2016), while other research has focused solely on foot temperature (Nardin, Fogerson et al. 2010, Foltyński, Mrozikiewicz-Rakowska et al. 2014, Shimazaki, Murata 2015, Kang, Hoffman et al. 2003, Reddy, Cooper et al. 2016). In view of this, the following section will therefore discuss the findings of the present study in the light of similar previous literature, and will focus on temperature and humidity separately.

8.2.1 Influence of Ambient Climate on Foot Skin Temperature

As previously discussed in Chapters 6 and 7, the results observed in the work of this thesis are consistent with previous literature investigating the influence of ambient temperature on foot skin temperature in healthy and diabetic participants (Foltyński, Mrozikiewicz-Rakowska et al. 2014, Nardin, Fogerson et al. 2010). In their study, Nardin et al. (2010) investigated the influence of seasonal variation on foot skin temperature patterns as they varied over a 32-hour period, indoors and outdoors. Although the study design is different from that employed in the current work, their observations are congruent with those reported in this thesis where a higher ambient temperature was associated with higher foot skin temperature. In their study, in winter, when temperature was -4.5^{0}C, the lowest foot temperature recorded during activity was 15.9^{0}C and in summer at a temperature of 33.9^{0}C, the highest mean foot skin temperature was 35.5^{0}C. However, it is important to note that in their study, Nardin et al. (2010) investigated the influence of seasonal variation in a North-eastern American region which has a continental climate, notably different from the Mediterranean winter and summer seasons studied in the work of this thesis. Similarly, Foltyński et al. (2014) also reported a correlation between ambient temperature and foot skin temperature, although in their study different ambient temperatures were derived from incidences of lower or higher temperatures due to varying locations such as indoors or outdoors during their data collection, making direct comparison of in-shoe temperature kinetics with the results from the current work difficult. Additionally, their ambient climate reflected a continental climate since their study was conducted in Warsaw, with cold winters and mild summers, differing distinctly from the Mediterranean climate studied in the current work of this thesis.

However, differing results were reported by Kang et al (2003) who stated that foot temperature in healthy subjects was not correlated with ambient temperature. As stated in the literature review (Section 2.6), this disagreement could be attributed to the method employed to measure ambient temperature. In the work of this thesis ambient temperature

was recorded within 50cm of the participants to avoid body heat interference and improve reliability of results over previous research.

Therefore, both the current work and also most of the previously published research discussed above, suggest that ambient climate has a significant influence on in-shoe microclimate, possibly surmising that in cooler climates, in-shoe temperatures are lower than those experienced in warmer climates. It should be noted that studies evaluating footwear efficacy in diabetes have predominantly emerged from countries with a cooler climate, namely Washington DC (Reiber, Smith et al. 2002), United Kingdom (Edmonds, Blundell et al. 1986), Netherlands (Bus, Valk et al. 2008) and Germany (Busch, Chantelau 2003), hence it could be that in-shoe temperature was not as relevant as it may be in warmer climates, although further research is required in this regard.

While most of the previously published studies reported a significant correlation between foot temperature and ambient temperature, in both healthy and individuals with diabetes, the work of this thesis is the first to report the influence of the Mediterranean climate on in-shoe skin temperature. None of the studies discussed above measured in-shoe RH. Moreover, the studies discussed above did not investigate in-shoe foot skin temperature kinetics as they change during physical activities such as walking. Some recent works have been published (Shimazaki, Matsutani et al. 2016, Reddy, Cooper et al. 2016, Sandoval-Palomares, Yáñez-Mendiola et al. 2016) where in-shoe temperature kinetics during walking were reported but only in one controlled climate without comparing the influence of different climates. The pattern of in-shoe temperature kinetics reported in these studies are discussed in the light of the current work in the following sections.

8.2.2 In-shoe temperature kinetics

Four research studies have investigated in-shoe temperature kinetics in relation to different walking speeds (Shimazaki, Murata 2015, Shimazaki, Matsutani et al. 2016) and to the applicability of a portable in-shoe measuring system with implications to the diabetic foot (Reddy, Cooper et al. 2016, Sandoval-Palomares, Yáñez-Mendiola et al. 2016), which are further discussed below in the light of the current work of this thesis.

Sandoval-Palomares et al. (2016), evaluated in-shoe microclimate in 2 healthy participants in the context of assessing the applicability of a portable system for monitoring in-shoe microclimate in diabetes. The authors reported an average increase of in-shoe temperature of approximately 6^0C after 40 minutes of treadmill walking, reaching a maximum of 29.4^0C and 29.3^0C in their 2 studied participants. The relatively large increase in temperature and failure to reach similar levels as those observed in the current work could be due to the placement of sensors which were in the insole rather than attached to the skin, thus not measuring actual skin temperature. This could also be the reason behind the different shape of graph presented in their study which was not 'S-shaped' like the that observed in the current work, which as discussed briefly in Chapter 6 probably reflects thermoregulatory processes.

Similarly, Reddy et al. (2016) evaluated an in-shoe temperature measurement system, by measuring in-shoe temperature at different walking speeds. Although the authors describe several limitations mostly attributed to sensor displacement, the in-shoe temperature kinetics illustrated in their study demonstrate a similar pattern to that illustrated in the current work of this thesis. However, further comparisons could not be discussed since the authors fail to report data on ambient temperature studied.

In another study Shimazaki and Murata, (2015) investigated in-shoe temperature kinetics in a controlled ambient climate (28.6^{0}C and 72% RH) comparable to that observed in the summer Mediterranean climate in the current work of this thesis. The increase in temperature at the arch reported by the authors was of 3.7^{0}C, similar to that observed in the current work (3^{0}C). Additionally, the in-shoe temperature kinetics illustrated demonstrate a similar pattern to those observed in the current thesis, although with a slightly earlier sharp increase in temperature occurring after 10 minutes of walking, compared to 15 minutes observed in the present work. This earlier increase in temperature could be attributed to the faster walking speed (6Km/h vs 3.5Km/h in the current study) illustrated in their results, where in fact authors stated that walking speed had a significant influence on in-shoe temperature kinetics.

In another recent study, Shimazaki et al. (2016) evaluated in-shoe temperature kinetics during four different treadmill walking speeds in an ambient climate controlled at 25^{0}C and 50% RH. While the reports on actual in-shoe skin temperature measurements are not very clear, they state that at the reported ambient climate, arch temperature increased by 1.7^{0}C after 30 minutes of walking, which is less than the 3^{0}C reported in a Mediterranean summer in this thesis. However, reaching similar temperatures of 36^{0}C after 38 minutes. The difference in temperature could be attributed to the slightly warmer ambient climate studied in current work (28.2^{0}C and 70.4% RH), possibly suggesting that in warmer ambient temperatures, foot skin temperature in-shoe increases earlier than in cooler ambient temperatures. It has been stated that in warm ambient temperature, it is more difficult for heat to transfer from the surface of the footwear to the surroundings, resulting in more heat retention in the skin of the foot and faster increase in temperature (Shimazaki, Murata 2015). This may imply that in-shoe foot temperatures may increase at a faster rate in warm climates when compared to cooler climates.

8.2.3 Skin Temperature Kinetics and Thermoregulatory Response

As discussed briefly in Chapter 6, the in-shoe temperature kinetics during treadmill walking that were observed in this study, probably reflect the thermoregulatory processes that have been demonstrated in other areas of the body (Kenny, Sigal et al. 2016). However, the current study is the first to demonstrate how seasonal variability influences in-shoe foot temperature as it evolves in healthy participants in different climates during activity and provides a reference for comparison with the diabetic patients. Knowledge of in-shoe foot temperature kinetics informed by the work of this thesis may help to better understand the mechanisms associated with DFU development, occurring within the shoe during ambulation, when the foot is most vulnerable to tissue breakdown.

The findings from the comparison of in-shoe foot temperature in diabetic patients with that observed in healthy individuals (presented in Chapter 7) revealed that in summer, foot temperature was significantly cooler in the diabetic patients, while in winter this difference is less pronounced. In summer the temperature difference is evidenced after acclimatization, where in-shoe skin temperature was approximately 4^0C lower than that observed in healthy participants.

This variation may be primarily due to an impaired thermoregulatory mechanism associated with diabetes, which is manifested as a reduced vasodilatory response by the active vasodilator system, when the body is exposed to exercise in warm and humid ambient conditions (Kenny, Sigal et al. 2016) similar to those studied in this thesis in the summer. In the current work, possible impaired vasodilation in the diabetic patients is further evidenced as exercise progresses via differing in-shoe temperature kinetics that were seen between the participant groups. The dynamics of skin temperature changes, in response to physical exercise in healthy individuals, has been well described by Johnson and Proppe (1996) and Johnson (2010). They described the thermoregulatory response and physiological processes that affect skin temperature.

During dynamic exercise, production of heat increases substantially, and induces an increase in core temperature. Given that heat is the most abundant by-product of cellular metabolism, dynamic exercise, in which a significant percentage of muscle is engaged, causes skin blood flow changes when a body core temperature threshold is reached. This triggers vasodilatation and a subsequent linear increase in skin blood flow, as core temperature continues to rise, as a means of heat loss (Johnson, Proppe 1996). In order to achieve temperature homeostasis, additional heat is eliminated by thermoregulatory reflexes which induce further adjustments of skin blood flow and sweating rate. These adjustments are known to be influenced by type and duration of exercise as well as environmental temperature (Cinar, Senyol et al. 2001, De Lorenzo, Kadziola et al. 1999). Therefore, as evidenced in the work of this thesis and also in previous works (Merla, Mattei et al. 2010, Svaic, Lukenda et al. 2015, Tanda 2015b, Zontak, Sideman et al. 1998) the measured skin temperature kinetics during exercise reflect thermoregulatory responses induced by the exercising heat in the body and is intended to maintain thermal homeostasis. The variations in responses probably reflect local differences in cutaneous blood flow, convective heat delivery (mass flow) and thermal exchanges with the external environment, all related to thermoregulatory responses to moderate exercise (Kenny, Sigal et al. 2016).

The in-shoe temperature kinetics in the healthy individuals therefore probably reflect normal thermoregulatory response during exercise, characterized by an initial slow increase in skin temperature, which increases in rate between the 15th and 35th minute of exercise (Figure 6.1) and is then followed by a slowing in rate of increase of the in-shoe temperature. The small group of DM participants in this study demonstrated a different pattern characterized by a slow linear increase throughout the treadmill walking trial. These results are characteristic of impaired thermoregulatory processes, as previously described by Kenny et al. (2016) who attributed the altered temperature kinetics to delayed achievement of the temperature threshold required to trigger vasodilation in diabetes, indicating impaired vasodilator responsiveness. Therefore, the results from the current work demonstrates that in-shoe temperature kinetics would probably support this idea of

impaired vasodilatory responses. This is consistent with previous literature which report decreased axon-reflex mediated vasodilator responses in patients with type 2 DM, without clinically detectible peripheral neuropathy (Caballero, Arora et al. 1999). Diabetes has been linked to impairment in temperature regulation during exposure to thermal stress (Jan, Shen et al. 2013b, Stirban 2014) due to several microvascular alterations, discussed previously in Chapter 2.

Thermal stress can occur on skin tissues either by exposure to hot and/or humid environmental conditions or by an increase in body temperature following physical activity (Kenny, Sigal et al. 2016). During thermal stress, sensory information both from the skin and muscle and from central thermoreceptors, is transmitted to the pre-optic anterior hypothalamus which is believed to co-ordinate thermoregulatory responses to maintain heat balance (Kenny, Jay 2013). It is the hypothalamus (via sympathetic nerves) that signals the dilatation of peripheral blood vessels in the skin, following thermal stress, with an increase of sweat production to encourage evaporative heat exchange, which is important in preventing body heat retention (Werner 1980).

In a study investigating responses to thermal stress in diabetic patients by local heating of skin temperature over the first metatarsal head, the impaired response was mainly attributed to neurogenic and myogenic controls (Jan, Shen et al. 2013b). The mechanisms underpinning these impairments remain largely unresolved, possibly due to physiological variations in the skin (Jan, Shen et al. 2013b, Jan, Brienza et al. 2005, Liao, Burns et al. 2013, Parthimos, Schmiedel et al. 2011, Kenny, Sigal et al. 2016, Jan, Shen et al. 2013a). Nevertheless, evidence from studies which have investigated microvascular function in diabetes, suggests that such alterations are manifested in a decrease in thermo-sensitivity, causing a higher threshold of thermoregulatory response (Wick, Roberts et al. 2006) and a decrease in maximal capacity of heat loss response through skin vasodilation (Katz, Ekberg et al. 2001, Khan, Elhadd et al. 2000). In addition, impaired distal thermoregulation has also been thought to be suggestive of early signs of diabetic

polyneuropathy (Rutkove, Veves et al. 2009) and may possibly be used for early detection of diabetic patients at risk of foot ulcers (Geyer, Jan et al. 2004, Rossi, Carpi et al. 2006). It is worth noting that the 10-gram Semmes Weinstein monofilament test used in the current work assessed large fibre neuropathy, possibly overlooking participants with early small fibre neuropathy.

The findings of the current work probably reflect the thermoregulatory differences in healthy and DM participants, in agreement with previous literature. Additionally, this altered thermoregulatory function in DM has been previously associated with a deficit in cutaneous capillary blood flow (Colberg, Parson et al. 2003) which has been linked to an increased risk of tissue breakdown through a multifactorial process (to be discussed later in this chapter; (Najafi, Wrobel et al. 2012).

8.2.4 In-shoe Skin RH Kinetics

The work of this thesis has demonstrated that in-shoe Relative Humidity kinetics were comparable for both seasons in the healthy and the diabetic participant group, indicating that ambient temperature did not influence in-shoe RH dynamics and that the increment in skin RH observed in summer was similar to that observed in winter. The inherent difficulties of measuring in-shoe RH during ambulation, mostly due to lack of specific technology and sensor design, has resulted in a paucity of literature related to this subject. Research investigating the influence of ambient temperature on in-shoe RH, is predominantly aimed at assessing comfort in military footwear (Uedelhoven, Kurz et al. 2002), sports footwear (Rebay, Arfaoui et al. 2008) or in extremes of temperature such as in ski boots (Hofer, Hasler et al. 2014) or in fire fighter protective footwear (Irzmańska 2015), with only 2 studies (Maluf, Morley et al. 2001, Sandoval-Palomares, Yáñez-Mendiola et al. 2016) suggesting measuring in-shoe RH with implications to the diabetic foot.

This is the first study to investigate the influence of ambient climate on in-shoe skin RH in healthy individuals and individuals living with diabetes. Hence, current results cannot be directly compared to previous research. While different RH measuring techniques, cohort characteristics and protocols have been utilised in the published literature, common trends in RH kinetics can be gleaned from these studies. Observations related to in-shoe RH dynamics in published literature are congruent with the trends observed in the current work, where initial in-shoe RH was reported to be 65% and increased gradually with walking time to reach 79-80% after 30 minutes (Irzmańska 2015).

In their study, Maluf et al (2001) demonstrated the validity of an in-shoe system to measure in-shoe microclimate and pressure. However, their report focuses on pressure and no information related to in-shoe RH is provided. In a more recent study, Sandoval-Palomares et al (2016) also demonstrated the applicability of a portable system to measure in-shoe microclimate demonstrating in-shoe RH kinetics in two healthy participants. In agreement with the current work, their results illustrate that initial in-shoe RH was similar to ambient RH (50.13% and 50% respectively). However, in-shoe RH increased to an average of 67% after 38 minutes of treadmill walking, which is considerably lower than that observed in the current work where in-shoe RH in healthy participants reached a mean of 87.2% RH at the toes and 77.8% RH at the arch in summer when ambient temperature was 28.2^0C. The reason for this difference could be due to the difference in ambient climate studied (current thesis 28.2^0C, 70.4% RH; Sandoval et al 23^0C, 50% RH), possibly suggesting that warmer and more humid climates may result in increased in-shoe RH, although further studies are needed to sufficiently substantiate this implication. Nevertheless, these results provide some indication of the high RH levels reached in-shoe, particularly in the toe area.

The current findings therefore demonstrate the levels of in-shoe RH as they occur in summer and winter and how they change during physical activities such as walking, providing new knowledge which was not previously available in the literature. This study

also presents a viable protocol that can be applied when measuring in-shoe RH and which can be adopted for future research in this area of study.

The work of this thesis has demonstrated that in-shoe microclimate experienced in a warmer ambient climate (Mediterranean summer climate) differs from that observed in a cooler climate (Mediterranean winter climate). Additionally, the clinical implications associated with this finding pertain to footwear advice and footwear prescription and how ambient climate should be considered within this context. The clinical implications are presented in a separate section later in this thesis. However, the work of this thesis has also provided new data related to in-shoe RH and temperature levels during walking. The relevance of this novel knowledge can be discussed in the light of previous literature related to microclimate and ulceration in other parts of the body and attempts to shed new light on microclimate factors as they may be implicated in the pathway of diabetic foot ulceration.

8.3 In-shoe Microclimate and Risk of Diabetic Foot Ulceration

As elicited earlier in this thesis (Chapter 2, Section 2.5) clinical studies investigating the relationship between microclimate and pressure ulceration in bed-ridden patients, has provided some evidence that thermodynamic and humidity conditions within and around skin tissue significantly increases the susceptibility of tissue breakdown and pressure ulcer development. The quantitative analysis of the effects of skin surface temperature and moisture is sparse, with most studies being purely experimental (using animal models and mathematical models (Clark, Romanelli et al. 2010, Kemuriyama, Niitsuma et al. 2000, Kokate, Leland et al. 1995, Sae-Sia, Wipke-Tevis et al. 2005, Sae-Sia, Wipke-Tevis et al. 2007, Sprigle, Linden et al. 2001, Gefen 2011). Common concluding findings from these studies

state that susceptibility to tissue breakdown increases with increasing RH and increasing temperature.

In a mathematical modelling study (Gefen 2011), a marked decrease in skin tolerance of 19% was observed when ambient temperature increased from 35^0C to 36^0C. At this point, skin temperature was measured at 30^0C. In another study, as skin temperature increased to 35^0C and above, deep tissue damage was observed (Iaizzo 2004) indicating that there is increasing susceptibility to skin breakdown between 30^0C to 35^0C. Additionally, Gefen (2011), showed that when RH increased from 50% to 75% (which encompasses the in-shoe skin RH ranges observed in the current study) skin resilience decreased by 10% when skin temperature was 30^0C. In this model, skin resilience continued to decrease with increasing temperature.

It can therefore be stipulated that skin temperature and RH levels exceeding 30^0C and 50% RH are indicative of unfavourable parameters for skin resilience. In the current study these temperature and RH levels were evident in diabetic individuals during shod treadmill walking in summer, where in-shoe skin parameters exceeded 30^0C and 70% RH after 20 minutes of walking. This indicates that the detrimental risks of RH and temperature on the skin integrity are probably initiated at a relatively early stage of ambulation. However, as detailed in Chapter 2 (see Section 2.4.1), the skin biomechanics of glabrous skin differs from hairy skin in being more robust to withstand mechanical stresses of normal gait, although these characteristics may be altered in diabetes (see Section 2.4.4). These findings therefore demonstrate, for the first time, that the in-shoe temperature and RH levels reached during walking in the work of this thesis, may greatly predispose the skin to ulceration, particularly during summer ambient conditions. They provide evidence that ambient climate may have an important role in DFU and support the proposal that it should be included within the previously accepted pathway to DFU. A new theoretical conceptual framework for the pathway to DFU development, which includes the influence of ambient

climate on in-shoe microclimate, is therefore proposed and illustrated in the diagram below (Figure 8.1).

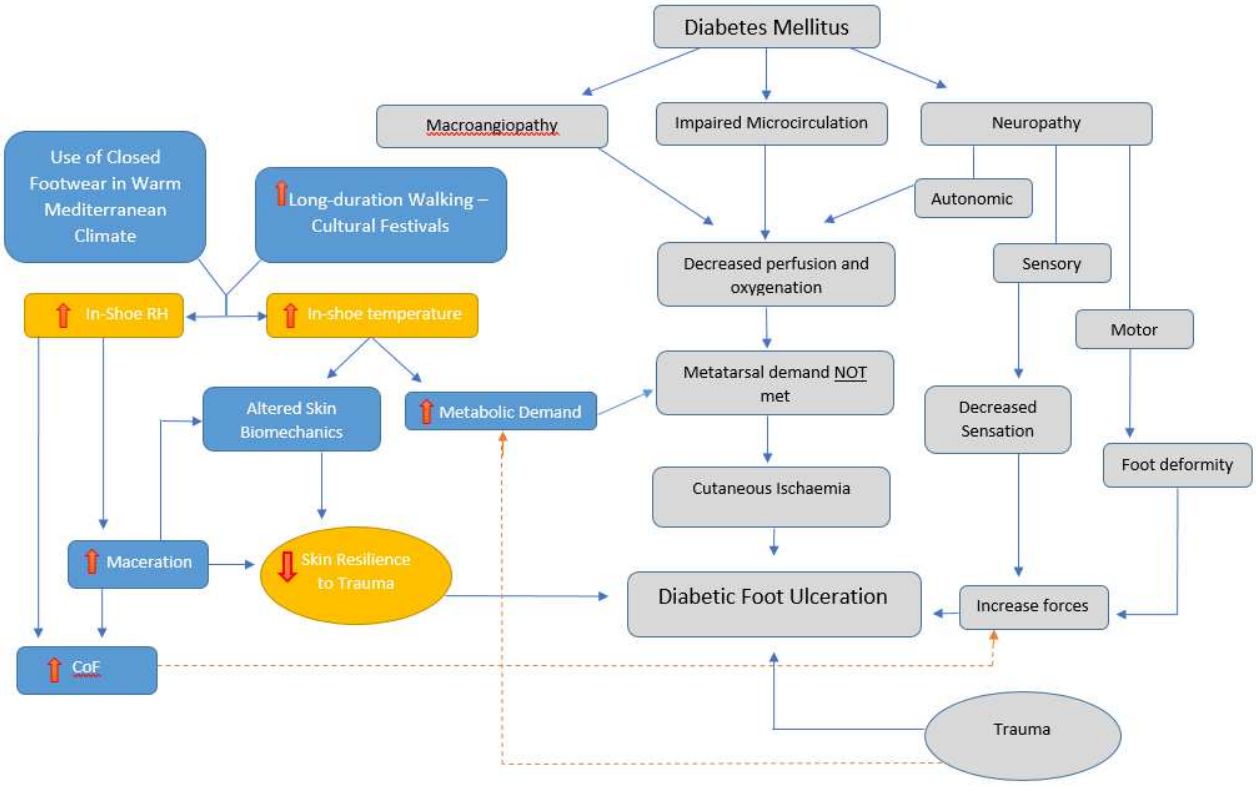


Figure 8.1: Diagrammatic representation suggesting the integration of a new theoretical concept including the implication of ambient climate and in-shoe Temperature and RH (as derived from current work; see Chapter 6 and 7) with the previously accepted pathway of DFU (as adapted from(Frykberg, Zgonis et al. 2006).

The mechanisms by which high RH on the skin surface and increased skin temperature, as individual or related risk factors, contribute to a decrease in skin resilience to mechanical forces (which supports the application of this new theoretical conceptual framework) are discussed further below.

8.3.1 Increased Coefficient of Friction and Over-Hydration of Skin

Primarily, humidity or moisture at the skin surface is known to increase the coefficient of friction between the skin and the contacting surface (Bertaux, Derler et al. 2010, Gerhardt, Strassle et al. 2008, Clark, Romanelli et al. 2010). Ambient RH exceeding 70% has been associated with accumulation of water in the stratum corneum (Bouwstra, Groenink et al. 2008) which weakens the crosslinks between the collagen in the dermis and softens the stratum corneum (Mayrovitz, Sims 2001) reducing the strength of skin tolerance to pressure or shear forces (Maklebust, Sieggreen 1996). Over-hydration is thought to cause swelling of the corneocytes, altering epidermal lipids, impairs the upper horny layer, macerating the stratum corneum (Luebberding, Krueger et al. 2013), increasing the coefficient of friction (CoF) contributing to the adherence of the outer layer of the skin to the supporting surface. Over bony prominences, this increased skin adhesion together with weakened collagen cross-links within the dermal layer creates areas of shear, promoting ulceration (Zhong, Xing et al. 2006, Gerhardt, Strassle et al. 2008).

Additionally, moisture on the skin surface has been shown to render the skin limp and weak, making it less resistant to the increased frictional forces sustained as a consequence to the increased RH (Sopher, Gefen 2011). The detrimental effect of an increase in moisture adjacent to the skin has been demonstrated by tensile tests on excised skin strips in a controlled humidity environment (Wildnauer, Bothwell et al. 1971). In Wildnauer et al. (1971), the tensile strength of the strips decreased by 75% with an increase in relative humidity from 10% to 98%. Skin with such reduced strength may be more prone to mechanical damage from shear stress or abrasion as evidenced in pressure ulcer studies, discussed previously in Chapter 2, Section 2.5.1, where the risk of pressure ulceration increased as friction forces increased in the presence of moisture (Smolander, Holmér 1991, Brown, Pearcy 1986). Frictional forces can mechanically separate epidermal cells at the level of the stratum spinosum, resulting in the formation of blisters (Xing, Pan et al. 2007), which can deteriorate into foot ulcers in the diabetic foot (Figure 2.3, page 26). While these levels of RH have been demonstrated in the current study, clinical experience has also shown the

tendency of foot skin to macerate when it remains in the warm and humid environment of a closed shoe in summer, for long periods of time.

The work of this thesis demonstrated that in-shoe RH before the start of treadmill walking, reflected ambient RH in both groups (healthy and diabetic participant group) and both seasons (summer and winter). Similar observations were recently reported by Sandoval-Palomares et al. (2016) where in-shoe RH at the beginning was 50.13% and 53.01% in their two healthy participants when the ambient climate was controlled at 50%. Therefore, in countries with high humidity levels, high in-shoe RH may possibly be experienced even in individuals with a decreased rate of perspiration (such as neuropathic patients). In Mediterranean countries, like Malta, where RH levels can reach 90%, in-shoe RH levels may therefore need to be considered for further study in patients with diabetic peripheral neuropathy, who are generally assumed to have low levels of perspiration (Fealey, Low et al. 1989).

8.3.2 Altered Tissue Biomechanics and Increased Metabolic Demand

The work of Chapter 6 and Chapter 7 demonstrated how in-shoe temperature kinetics evolve as temperature increases during treadmill walking. Heat within the shoe is generated by radiation from the ambient environment, friction between the foot and the inside of the shoe (Shariatmadari, English et al. 2010) and also due to the increase in body heat as a by-product of increased metabolism during exercise (Shimazaki and Murata, 2015). Having an impaired thermoregulatory mechanism, due to diabetes, which caused a slower rate of temperature increase in the current work, the skin of the foot exceeded 30^0C after 20 minutes of walking, with the feet of some participants exceeding 33^0C after 35 minutes. Clinical studies (Kokate, Leland et al. 1995, Posada-Moreno, Elena Losa Iglesias et al. 2011, Rapp, Bergstrom et al. 2009, Sae-Sia, Wipke-Tevis et al. 2005) as previously discussed in Chapter 2, (Section 2.5.2), have established that elevated skin temperature is

associated with an increased risk of pressure ulcer development. The detrimental effects of an increase in skin temperature have been attributed to two mechanisms in the literature – decreased stress-strain relationship and increased metabolic demand.

In a study investigating the influence of temperature on the stress-strain relationship of pig's skin (Zhou, Xu et al. 2010), it was demonstrated that as temperature increased, skin became softer and the stress needed to achieve the same strain, decreased. The authors attributed the thermal effect on skin's resilience to either denaturatiaion of collagen which leads to remarkable changes in its mechanical properties (Chen, Wright et al. 1998) and also to the possible hydration of collagen occurring during denaturation, a mechanism involving the initial liberation and subsequent absorption of water via water bridges (Chimich, Shrive et al. 1992, Humphrey 2003). A change in water content changes the interactions between collagen, proteoglycans and water molecules (Humphries, Wildnauer 1971, Miller, Wildnauer 1977), influencing the viscoelastic behaviour of the skin (Xu, Seffen et al. 2008). These detrimental effects, associated with an increase in temperature were evidenced in a highly cited study by Kokate et al. (1995) who demonstrated that with same amount of induced pressure, there was no skin damage reported at 25^0C, while considerable damage was reported at 35^0C. The influence of temperature on the mechanical properties of the skin reported in the literature occurred at temperatures similar to those observed in the current work during 38 minutes of walking in summer, but were not reached in winter in the diabetic individuals observed. This highlights the fact that risk of tissue damage is mostly increased when wearing closed footwear in the summer season.

Additionally, an increase in temperature poses further risk to the foot in-shoe, as an increase of 1^0C in skin temperature is associated with an increase of approximately 10% in tissue metabolic requirements (Iaizzo, Kveen et al. 1995). Based on this knowledge, other authors (Bader 1995, Boyko, Ahroni et al. 2001) investigated the relationship between skin temperature and oxygen levels by measuring transcutaneous oxygen pressure ($TcPO_2$),

demonstrating reasonably consistent results of a reduction in nutrient demand of 10% for a 1^0C temperature drop at a given level of blood supply.

While it may be postulated that an increase in temperature may cause vasodilation and hence an increase in nutrient supply to meet the corresponding increase in metabolic demand (Patel, Knapp et al. 1999), some evidence (Lachenbruch 2005b) has demonstrated that in areas under significant stress, vasodilation may be impaired. In this study, Lachenbruch (2005b), showed that the vasodilatory response to increased temperature occurred at low to moderate interface pressure but could not occur at the higher pressure, most likely due to mechanical compression of the vessels. In the healthy individual, reactive hyperaemia protects the tissues from ischaemia after such mechanical stress, which causes mechanical occlusion of the microcirculation in the area. In diabetes, this mechanism is impaired due to impaired vasodilation as explained previously in Chapter 2.

This impairment was evidenced in the foot by Jan et al. (2013b) who demonstrated a smaller myogenic response after the application of mechanical stress on the first metatarsal head, thus leading to a smaller reactive hyperaemia in patients with diabetic peripheral neuropathy, when compared to healthy controls. Lachenbruch (2005b) concluded that ischaemia or tissue breakdown occurs when the heightened need for nutrients in the skin, following temperature increase, cannot be met due to excessive mechanical tissue compression. In the foot, this mechanism may take place when mechanical forces occur over bony prominences, particularly in tight footwear or in the presence of foot deformity, causing tissue injury. While in healthy individuals, protective sensory perception is key for the prevention of such injuries, in the presence of neuropathy they may often result in tissue breakdown (Edmonds, Foster 2006).

While this mechanism of injury which implicates increased temperature as a key factor in the ulceration process, with and without neuropathy (Clark, Romanelli et al. 2010,

Sae-Sia, Wipke-Tevis et al. 2005), it is widely accepted in the literature relating to pressure ulceration, that temperature has been distinctly overlooked as a risk factor in the development of DFU. This is even though the biomechanical, physiological and physical characteristics are comparable in both clinical scenarios. Perhaps of greater relevance to the diabetic foot is the additional possible impairment of cutaneous perfusion which results in the inability of small wounds to heal, which may eventually result in ulceration (Ngo, Hayes et al. 2005). This inadequate small vessel blood supply to the skin causes a deficiency in nutritive flow so that the increased metabolic demand in the tissues due to the increase in skin temperature (occurring in-shoe) is not met. There is ample evidence indicating microvascular dysfunction in diabetes which is in turn associated with impaired vasodilation and abnormal thermoregulation (Kenny, Sigal et al. 2016, Stirban 2014, Wick, Roberts et al. 2006, Ngo, Hayes et al. 2005). The delayed increase in temperature observed in the diabetic patient group in the current work may be suggestive of a delayed vasodilatory response which is consistent with reports of a higher threshold trigger point for active vasodilation in patients with Type 2 DM (Wick, Roberts et al. 2006). Although the physiological and pathophysiological mechanisms of impaired thermoregulation in diabetes are not well understood, existing evidence associated with this impairment suggests decreased axon-reflex mediated cutaneous vasodilator responses (Benarroch, Low 1991) and endothelial dysfunction (Caballero, Arora et al. 1999).

This impairment has been associated with endothelium-dependent and endothelium-independent vasodilatory dysfunction due to diminished nitric oxide synthase activity which causes reduced nitric oxide bioavailability, required for vasodilatation (Kenny, Sigal et al. 2016) and has been shown to exist even in patients with type 2 diabetes without vascular complications (Caballero, Arora et al. 1999). These observations were reflected, to some extent, in the current work where temperature kinetics were suggestive of a delayed threshold for vasodilation and impaired vasodilation response in DM patients without clinical evidence of PAD. Microvascular abnormalities, which can be manifested as abnormal thermoregulation in diabetic patients, is associated with failure of microvascular

perfusion to meet the requirements of increased skin metabolism (Ngo, Hayes et al. 2005, Stirban 2014, Kenny, Sigal et al. 2016, Wick, Roberts et al. 2006) which may occur due to increased temperature, such as that manifested in-shoe in the current work. This may lead to tissue injury which further increases metabolic demand and increases the risk of tissue breakdown, thus possibly playing an important role in the development of diabetic foot ulceration in conditions of increasing in-shoe temperatures (such as those of the work of this thesis). This mechanism is further illustrated in the diagrammatic representation given in Figure 8.1.

8.4 The Mediterranean Climate and DFU

The present work demonstrates that in a Mediterranean summer, in-shoe skin temperature and humidity, when walking using closed footwear, reach levels which may increase the risk of the development of foot ulceration. It highlights the need for these parameters (in-shoe temperature and humidity) to be included as key risk factors in the causal pathway to DFU.

This knowledge is particularly relevant to countries with a warm Mediterranean climate, such as Malta, where patients are advised to wear closed footwear even in summer. Furthermore, the increase in outdoor activities occurring in summer further places diabetic patients at an increased risk due to exposure to the warm ambient environment. For example, in summer it is customary for Maltese people to spend more time outdoors, mostly for recreational reasons since the local social life is considerably different from that lived in the winter months, namely with an increase in the number of village feasts and extended day light time. These feasts are central to the Maltese culture and are often important annual events for families to meet. They are also synonymous with long walks, behind a procession, as the main element of these religious festivities. Wearing closed

footwear in such occasions and in typical Mediterranean summer climates may result in the warm and humid foot conditions, as described above.

This study highlights the fact that potentially detrimental levels of temperature and humidity in-shoe are reached after only 20 minutes of walking, in such ambient climates. During ambulation, the foot is constantly subjected to mechanical forces and elevated in–shoe relative humidity increases the coefficient of friction, causing an increase in shear forces while simultaneously decreasing skin resilience to those forces (due to increased hydration), thus enhancing the risk of tissue injury. Additionally, elevated in-shoe skin temperature increases metabolic demand, which may also be further increased due to the sustained injury. Altered cutaneous perfusion and impaired vasodilatory responses may not allow enough nutrients to meet the increase in metabolic demand, which may result in tissue ischaemia, further increasing the risk of ulceration at vulnerable sites on the foot.

The in-shoe temperature and RH kinetics shown in the work of this thesis are not only suggestive of negative implications for tissue viability, but also to footwear practices. In studies investigating footwear comfort, microclimate sensations are largely influenced by relative humidity levels inside the footwear (Irzmańska 2015). Previous research has established that the optimum level inside the shoe is 60-65% (Bergquist, Holmér 1997b). As the present results show, these suggested optimum humidity levels were exceeded at an early time during walking.

8.5 Clinical Implications and Recommendations

This thesis has provided new knowledge related to the influence that ambient climate has on in-shoe microclimate, and in a Mediterranean climate. While standardisation of care and recommendations for practice are often advised in diabetes care (Bus, Haspels et al. 2011), these findings emphasize that footwear guidelines need to be more climate-specific and global standardised footwear guidelines may be questioned. Footwear guidelines emerging from cooler climates are not transferrable to countries with warmer climates. Therefore, there may be a need for new guidelines related to footwear in diabetes which not only consider biomechanical and mechanical issues but also in-shoe microclimate parameters, and the impact of different climates across the world.

Special care should be applied when recommending physical activity to patients with diabetes. While it is common practice for clinicians to advise patients to walk regularly, the current study has provided some insight into the in-shoe microclimate during ambulation and how it may possibly become unfavourable for tissue resilience and also comfort perception as exercise proceeds. The work in this thesis provides possible indications that physical exercise in summer should not exceed 20 minutes, so that a steep rate of increase in temperature is avoided and in-shoe microclimate is maintained at a more favourable temperature. When providing footwear advice, practitioners should not only consider mechanical issues but also microclimate parameters and patients' characteristics such as lifestyle and ambient climate in which they live, both when indoors and outdoors as it is known to influence in-shoe temperature and RH.

Caution should be practiced when using previously established plantar pressure threshold values, as screening tools for risk of DFU. While it has not yet been demonstrated in DFU, pressure ulcer studies have shown that increased skin temperature and RH, lower the threshold for tissue breakdown, possibly implying that when temperature and RH increase during ambulation, the threshold for ulceration is lowered, hence putting the

patient at risk unknowingly, although further evidence is required. This notion should also be applied by researchers who aim to investigate this possible pressure threshold, emphasizing that microclimate should be considered within this context.

In view of the proposed modified theoretical conceptual framework, related to DFU development implicating in-shoe temperature and humidity as key risk factors, practitioners involved in the care of patients living with diabetes should consider these parameters when providing footwear advice or footwear prescription. Although further research is required in this field, alternative footwear, which allows air movement, (thereby limiting in-shoe RH) should be considered. Medical consultants and the multidisciplinary teams should be provided with this information, particularly those working in Malta and in countries with similar hot ambient summer climates. Advice related to exercise or walking long distances should also take the information gained from this thesis into account, since time of walking has an impact on in-shoe microclimate kinetics which may in turn influence risk of DFU.

8.6 Study Limitations

Although this thesis has demonstrated that the protocol employed and sensors utilised were effective for measuring in-shoe microclimate during treadmill walking, some limitations were apparent in the testing procedures, mostly encountered with the RH sensor and its connectivity. The sensor utilised for measuring in-shoe RH was not specifically manufactured for this purpose and proved to be fragile when subjected to the mechanical forces experienced in-shoe during ambulation. Since the researcher was limited by specific characteristics related to the choice of sensor for use of the study, namely the need for it to be small and compact, it is recognised that the humidity sensor connections of the utilised device were not primarily designed to be used inside shoes during ambulation and to withstand repetitive stress. The 'weakness' of the RH sensors identified during the initial phase of development were later addressed by the researcher and a biomedical engineer in an attempt to improve the device's durability. Modifications included re-wiring, application of casing or protection in order to reduce stress on the sensor's connection.

While it is acknowledged that the diabetic participant group had a small sample size, nonetheless, data retrieved from this work is likely sufficient to adequately suggest the useful applicability of in-shoe temperature and RH measurement in diabetic participants and provides the foundation for further research in this field. Although mean temperatures and RH were analysed in this sample group, the findings are meant to provide some insight into the trends on in-shoe microclimate kinetics during exercise rather than relating generalized inferences in this population.

Another limitation which should be noted in this study is that foot temperature and RH were measured between the hallux and second digit and just below the navicular since these locations were believed to be least likely to cause discomfort or injury during walking. However, measurement of in-shoe microclimate at the plantar surface may also be important, particularly in relation to the diabetic foot, because of the increased risk of tissue

breakdown in this region due to increased stresses sustained during walking. Although this idea was considered at the preliminary phase of this thesis, the available sensors were not robust enough to withstand the stresses sustained on the plantar aspect. Additionally, if the sensors were applied on the plantar surface they could lead to injury.

Despite these limitations, the approach to measurement used in the work of this thesis may be helpful in future research related to the study of in-shoe microclimate possibly with dedicated and specifically-designed sensors, that can withstand physical stress when used in-shoe during ambulation.

8.7 Direction for Future Research

To date there are few published studies that have investigated the influence of ambient climate on in-shoe microclimate, with most of these focusing on footwear comfort and sports footwear. The results of this thesis provide evidence that ambient climate has an influence on in-shoe microclimate and therefore further research is warranted. As the primary exercise protocol employed in the present work was of moderate exertion, future research should utilise **different exercise intensities/speeds** in order to investigate the influence of speed on in-shoe microclimate kinetics for greater application to real world activities. From a practical stand point research investigating such activities outdoor during normal living are also necessary to provide a better understanding of in-shoe microclimate levels during every day ambulatory activities, where radiant heat and direct sunlight may have an influence on in-shoe microclimate. The application of a **portable device** to measure in-shoe parameters in such a study might be better suited. Improved sensor technology, especially for measuring RH that could be used specifically in-shoe and be able to withstand shearing forces typically experienced during ambulation, is warranted.

Since the focus of the current body of work was to investigate the influence of season on in-shoe microclimate, the protocol that was utilised entailed participants using a self-selected walking intensity in both trials. For this reason, of primary concern was the use of the same footwear for both trials and no attention was necessary to footwear material. Following the results from the work of this thesis, further research into the impact of different **footwear materials** and possibly shoe styles (eg: sandals, trainers etc) on in-shoe microclimate is warranted. Future research should focus on the use of alternative materials which promote air flow and compare them with traditional materials used in therapeutic footwear, such as leather. This information would be particularly relevant in hot ambient climates where (to date) leather therapeutic footwear is provided to patients at high risk of ulceration as routine practice.

Of primary concern, the risk of foot ulceration is mostly focused on patients with **peripheral sensory neuropathy,** due to their inability to perceive trauma. Published literature has demonstrated impaired thermoregulation and cutaneous perfusion in diabetic patients, even without neuropathy. Additionally, diabetic patients with neuropathy exhibit reduced perspiration and may therefore exhibit different in-shoe microclimate kinetics from those observed in the current body of work. Future research should therefore investigate in-shoe microclimate parameters as they evolve during ambulation in patients with peripheral sensory neuropathy, to provide a better understanding of the risk associated with these parameters in this diabetes sub-group.

8.8 Conclusion

The work of this thesis sought to elucidate in-shoe microclimate (temperature and relative humidity) in people with diabetes who live in a Mediterranean climate with relevance to diabetic foot ulceration. The main findings, new contributions to the literature, were that ambient climate has an influence on in-shoe microclimate and a seasonal variation in in-shoe temperature kinetics was evident. Additionally, in-shoe temperature and RH kinetics, exhibited during treadmill walking in warm Mediterranean ambient temperatures, reach levels which may create an unfavourable microclimate for skin resilience to in-shoe stresses, possibly due to altered tissue mechanics and increased metabolic demand, associated with the increase in temperature and RH. Finally, in-shoe temperature kinetics reflected the probable thermoregulatory process induced during moderate exercise, in both healthy and diabetic participants, revealing evidence of likely impaired microcirculation and increased risk of tissue injury in diabetic individuals. Consideration of in-shoe microclimate within the causal pathway of DFU is therefore emphasized and a new concept based on early research evidence for incorporating the influence of ambient climate and in-shoe RH and temperature together with other key known physiologic and biomechanical components, is proposed. Future research is warranted to determine the extent of the influence of in-shoe microclimate on diabetic foot ulceration to provide a better understanding of diabetic foot ulcer development, with the aim of reducing rates of ulceration and amputation in this population.

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Appendix I

Literature Review Matrix

Author Name of Journal	Year of Publication	Purpose	Study Design	Sample Size	Includes information on			Weaknesses	Conclusions
					Ambient Climate	In-Shoe Temperature	In-shoe Humidity		
Maluf et al. *Archives of Physical Medicine and Rehabilitation*	2001	To investigate the reliability and validity of a portable device used to monitor changes in plantar pressure, temperature, and humidity	Descriptive	4 Healthy Participants	n/a	n/a	n/a	No information related to in-shoe RH is provided	Authors claim the possibility of long-term, continuous monitoring of in-shoe plantar pressures, temperature, and humidity
Kang et al. *Muscle Nerve*	2003	Foot temperature to identify thermo-regulatory disturbances in poly-neuropathy	Observational	4 Healthy Participants 12 Neuropathic Participants	Varied over 24 hrs	yes	n/a	Ambient temperature measurement was made by placing the sensor in a box inside the sock; Various technical difficulties	Ambient temperature fluctuations were mirrored in changes in foot temperature in neuropathy, not in healthy

Author Name of Journal	Year of Publication	Purpose	Study Design	Sample Size	Includes information on			Weaknesses	Conclusions
					Ambient Climate	In-Shoe Temperature	In-shoe Humidity		
Nardin et al. *JAPMA*	2010	To investigate the influence of ambient climate on foot skin temperature with implications for DFU	Quasi Experimental	39 Healthy Participants	Four seasons	yes	n/a	The participants were different for each season and inter-participant activity during data acquisition also differed, hence making it difficult to directly compare in-shoe temperature values with other studies	Ambient temperature influences foot temperature
Foltyński, et al. *Biocybernetics and Biomedical Engineering*	2014	To assess influence of ambient temperature in foot temperature in ulcerated patients	Quasi Experimental	10 Healthy Participants 20 Neuropathic Participants	Varied over 24 hrs	yes	n/a	n/a	Ambient temperature influences foot temperature

Author Name of Journal	Year of Publication	Purpose	Study Design	Sample Size	Includes information on			Weaknesses	Conclusions
					Ambient Climate	In-Shoe Temperature	In-shoe Humidity		
Shimazaki & Murata *Applied Ergonomics*	2015	To investigate in-shoe temperature kinetics at different walking speeds	Quasi Experimental	17 Healthy Participants	Constant at 28.6 ^{0}C 72% RH	Yes	n/a	n/a	Metabolic heat generation has an impact on temperature profile in-shoe; High temperature associated with high contact
Reddy et al. *Procedia CIRP*	2016	To evaluate an in-shoe temperature measurement system	Quasi Experimental	5 Healthy Participants	n/a	Yes	n/a	Issues with sensor displacement and occasional non-contact	Although authors claim that such systems are feasible, measurement issues should be addressed
Sandoval-Palomares et al. *Sensors*	2016	To assess the applicability of a portable system for monitoring microclimate in diabetes	Observational	2 Healthy Participants	Constant at 23 ^{0}C 50% RH	yes	n/a	Placement of sensors were in the insole rather than attached to the skin, thus not measuring actual skin temperature	Authors claim the possibility in monitoring the temp and RH at the foot-footwear interface

Author Name of Journal	Year of Publication	Purpose	Study Design	Sample Size	Includes information on			Weaknesses	Conclusions
					Ambient Climate	In-Shoe Temperature	In-shoe Humidity		
Shimazaki et al. *Applied Ergonimics*	2016	To evaluate energy balance & in-shoe temperature kinetics during four different treadmill walking speeds	Quasi Experimental	7 Healthy Participants	Constant at 25^0C 50% RH	yes	n/a	n/a	Factors influencing the energy balance for temperature formation were determined

Appendix II

Diabetic Foot Ulceration in Malta:

A Scoping Study

1.0 Background to Study

Diabetes Mellitus is a major health concern around the world and in Malta because of the elevated prevalence of diabetes in this population (DECODE, 2003). Malta has a high prevalence of Type 2 diabetes (10%) when compared to European counterparts (2-3%) (WHO, 2012). In 2007, 246 million people worldwide had diabetes and is expected to rise up to 366 million by the year 2030 (Wild et al., 2004). A large part of the burden in diabetes does not only reflect in health care costs (Levin, 2002) but is also related to the development of chronic complications that usually accompany this condition. One of the most feared complications of diabetes is foot ulceration (Reiber & Ledoux, 2002; Boulton et al, 2004; Boulton et al 2005). Diabetic foot ulceration is usually the result of several factors acting together, with polyneuropathy, altered biomechanics, peripheral vascular disease and inadequate footwear as major factors, often complicated by the presence of infection (Dinh & Veves, 2005; Frykberg, 2003). Each of these components is usually not sufficient to cause ulceration, but it is the combination of two or more factors working together that typically results in tissue breakdown (Reiber et al., 1999). Prescribed therapeutic footwear is commonly used for the treatment or prevention of foot ulceration in diabetes (International Diabetes Federation, 2009).

Despite the Maltese Government's investment in free health care and the provision of free prescribed footwear for the treatment and prevention of foot ulceration for patients suffering from diabetes mellitus, the number of minor amputations has been on the increase in recent years in Malta (Statistics Department Mater Dei, 2011). Management of diabetic foot ulceration continues to be a major concern within the field of diabetes, owing to the cost to individuals and society (Boulton, 2005). Moreover, the role of therapeutic footwear in the prevention of foot ulceration has been questioned for several years and only limited scientific evidence is available to date (Maciejewski et al, 2004; Bus et al, 2008; Reiber et al, 2002).

A retrospective study was undertaken with the aim to establish the nature and magnitude of diabetic foot ulceration in a Maltese diabetic population. It will provide the base to the research and map key concepts required for the main study.

1.1 Research Question

What is the effect of prescribed therapeutic footwear on lower limb morbidity in the Maltese diabetic population?

1.2 Research Aims and Objectives

The aim of this study is to investigate the effect of prescribed footwear on lower limb morbidity in order to obtain a clearer picture on the success rate of therapeutic footwear as a preventive measure in reducing foot ulceration in the Maltese diabetic population. A further aim is to demonstrate the nature and extent of the problem associated with the use of prescribed therapeutic footwear in Malta. These aims will be reached with the following objectives:

- Obtain an overview of the location of foot ulceration in patients with diabetes

- Obtain an overview of lower limb morbidity in patients with diabetic foot ulcerations to demonstrate magnitude of the problem in Malta

- To analyse retrospectively the success rate of the use of therapeutic footwear in patients with a history of foot ulceration

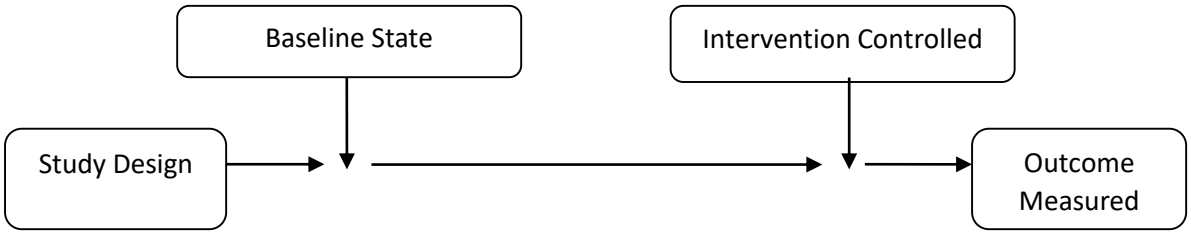
- To form a basis of evidence as a preliminary study and first phase of the PhD project

2.0 Research Design & Method

2.1 Study Design

For the scoping study a retrospective study design was implemented. A retrospective study uses existing patient-focused data which have been recorded for purposes other than research (Hess, 2004; Jansen et al., 2005). The type of retrospective study employed is a case series study. A case series study is a report of multiple similar unusual or instructive cases, where the medical records are the primary source of information to answer a research question (Worster & Haines, 2004). The retrospective study design can help focus the research question, clarify the hypothesis, determine appropriate sample size and identify feasibility issues for a prospective study. More specifically a retrospective case series study can be used to generate a hypothesis that can be investigated more rigorously in a prospective study (Hess, 2004).

Prospective Study



Retrospective Study

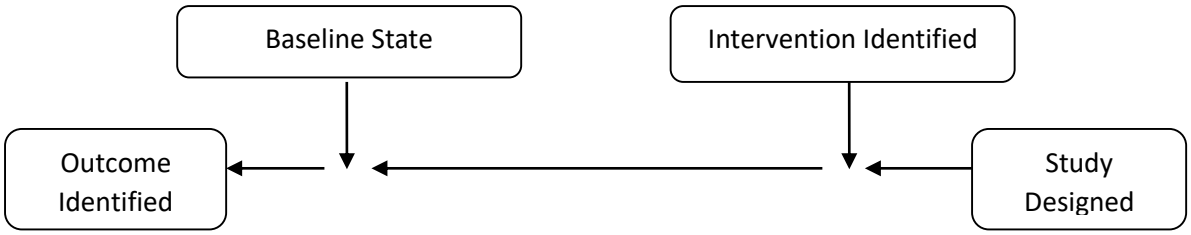


Figure 2.1: Prospective vs. Retrospective study design (Hess, 2004)

The illustration above (figure 2.1) depicts the main differences between the two study designs. In a prospective study, the base line state of the subjects is determined followed by a controlled intervention after which the outcome is measured. In a retrospective study, the baseline state, intervention and outcome measure are obtained from existing data recorded for other purposes (Hess, 2004).

It is important to acknowledge the disadvantages (Boyd et al., 1979; Hess, 2004) of a case series study which are:

- It is uncontrolled
- The investigator depends on the availability of a medical record
- Medical records may be lacking in quality and quantity
- Case serious is subject to selection bias because the investigators select the cases.

Medical records from two main hospitals in Malta were used to identify patients with diabetes suffering from foot ulceration/amputation that have been prescribed therapeutic footwear.

2.1.1 Data Abstraction from Medical Records

When conducting a retrospective case serious study involving review of medical records, it is important to demonstrate that the data was abstracted reliably and in an unbiased manner (Gilbert et al., 1996). The use of explicit inclusion and exclusion criteria define a priori for abstracting variables results in higher inter and intra observer reliability because it reduces subjectivity in interpretation (Horrouitz & Yu, 1984).

2.2 Methods

The data collection for this study was retrieved from two separate hospital settings – main general hospital and rehabilitation hospital, which are however linked by patient referrals as they are both state hospitals. These hospitals do not share a common database, therefore data collection had to be retrieved from two different medical records used by each respective clinical setting. For this reason this study was split into two phases: Phase 1, included an initial search through a database of leading vascular surgeon who leads an outpatient clinic treating the majority of foot ulcerations and runs as a joint service with the diabetic foot clinic in the same hospital. This hospital is the only local state general hospital in Malta where the largest majority of diabetic patients attend for assessment and treatment. Encoded patients' records identified from phase 1 of the study of who have been prescribed therapeutic footwear were then searched through the second database from the rehabilitation hospital which formed phase 2 of this study.

2.2.1 Phase 1

An initial search was done through a leading vascular surgeon's database, within a local general hospital. The data was provided to the researcher by the vascular surgeon who de-identified the data by removing all non-essentials identifiable variables, such as identity card (ID) numbers/hospital numbers, patient's names and addresses. After removing all identifiable non-essential identifiable variables, a unique random number was assigned to each patient in the data base. The key which linked the ID number with the random number assigned was only known by the consultant vascular surgeon. The study population included Type 2 diabetic patients who were referred to the outpatient clinic for treatment of foot ulcerations over a period of 2 years (2009-2011). It was deemed acceptable that this period was long enough to include seasonal variations or other changes over time that are relevant to the research question (Hulley et al., 2001).

The researcher received the data as a Microsoft Access file in a USB flash drive. Data retrieved from these records included medical status, number and location of ulceration and number of referrals for amputations.

Two hundred fifty nine medical histories were randomly selected from a database of 872 medical records.

2.2.2 Sampling Method

The sampling method selected for this study was systematic random sampling. This is a type of random sampling where sample participants are selected from a larger population according to a random starting point and a fixed, periodic interval which should be determined beforehand (table 2.1). Systematic random sampling ensures that the results are representative of the population unless certain characteristics of the population are repeated every 'nth' individual, which is highly unlikely. This sampling method is preferred

over simple random sampling due to its simplicity and because the researcher has the assurance that the population will be evenly sampled without the risk of clustered selection as in simple random sampling. It provides an equal opportunity for each eligible case to be selected without bias (Worster & Haines, 2002)

The sample size chosen for this study is 300 (n) as it is based on the average number of ulcer patients seen in the outpatient clinic by the vascular surgeon in one year (personal communication with Professor Cassar, Consultant Vascular Surgeon, 2011). This number will allow the researcher to make allowances in the event of missing information during data abstraction as missing information in a medical record will be managed by case deletion (Worster & Haines, 2004). Hence, in order to calculate the integer which will serve as the constant difference between any two consecutive participants the following calculation was employed (Table 2.1):

Table 2.1: Systematic Sampling Method

N/n = k

872 / 300 = 2.9

N = Sample population

n = sample size chosen

k = interval

the first sample item selected is the 2nd (random number between 1 & k)

Therefore, every third medical record was scanned for eligibility of the study. If the inclusion criteria (Table 2.2) were not met than the following record was considered. This pattern was repeated until the whole database was scanned. The data base given to the researcher

did not include patients' personal details but were coded prior to use as to safe guard patients' identity.

Data retrieved included:

- ✓ location of ulceration/amputation
- ✓ medical status (neuropathy, neuroischaemia, hypertension, PVD and h/o smoking)
- ✓ referral for amputation/s
- ✓ referral to the orthotics and prosthetics department
- ✓ use of prescribed therapeutic footwear

Data collected was recorded in a specifically designed spreadsheet for data processing and analysis.

2.2.3 Inclusion and Exclusion Criteria

Medical records eligible for the study satisfied all inclusion and exclusion criteria listed in Table 2.2 below:

Table 2.2: Inclusion and Exclusion Criteria

Inclusion Criteria

- ✓ diabetes mellitus
- ✓ neuropathy
- ✓ neuropathy and ischemia
- ✓ foot ulcerations

Exclusion Criteria

- × leg ulceration
- × non-diabetic patients
- × decubitus ulcerations

2.2.3.1 Justification for Inclusion and Exclusion Criteria

The criteria are set to ensure a subject population that will enable the investigation of the set objectives stipulated in this study. For this reason only patients with diabetic foot ulcers who are usually prescribed therapeutic footwear as part of the treatment plan were included. Decubitus ulcerations were excluded as therapeutic footwear is not usually provided to treat these patients. The criteria will also provide equal opportunity for inclusion and not to exclude diabetic subjects that may be using the service provided.

2.2.4 Ethical and Regulatory Consideration

The study was conducted with the intention of protecting human rights of every participant. The University Research Ethics Committee reviewed and approved the research application (Appendix 2). Permissions were also provided from the following cooperating institutions:

- ✓ Data Protection Officer, Mater Dei Hospital (Appendix 3)
- ✓ Leading Consultant Vascular Surgeon, Mater Dei Hospital (Appendix 4)
- ✓ Chief orthotics/prosthetics, Rehabilitation Hospital (Appendix 5)

The identities of all participants were encoded in order to keep confidentiality and anonymity.

3.0 Statistical Analysis and Results

This section presents and organises all the data gathered retrospectively in a more accessible way (Hicks, 2009). Descriptive statistical illustrations have been generated using Microsoft Office Excel 2007.

The systematic sampling method employed in this research yielded a total of 300 records eligible for inclusion to the study. Forty one medical records were omitted due to missing information in the data base. Therefore 259 records were finally used for data analysis (Figure 3.1) which included 273 ulcerations in total.

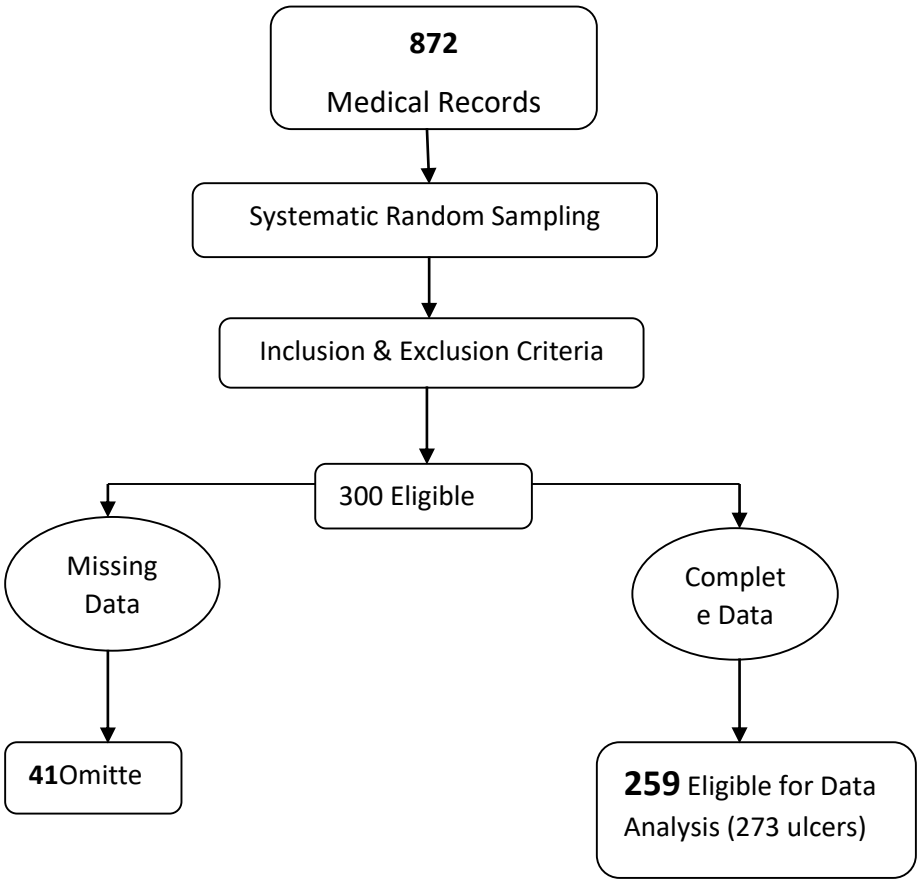


Figure 3.1: Summary of Data Collection Process

3.1 Types of Ulcerations

The pie chart below (figure 3.2) demonstrates the percentage distribution of neuropathic, neuro-ischemic and ischemic ulcerations obtained from the study group (n=273). More than half of the population were documented to have a neuropathic ulcer (66%) followed by neuro-ischaemic (28%) and ischaemic (6%) ulcerations.

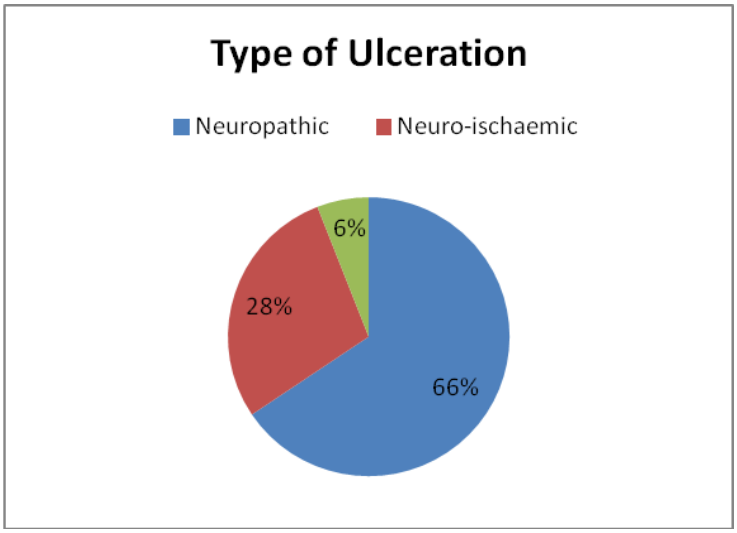


Figure 3.2: Distribution of type of ulceration in the study group

3.2 Location of Ulceration

The results related to the location of ulceration of the medical records analysed are illustrated in the table 3.1 below. Results include number (n), percentage (%) and loication of all ulcerations documented in all eligible medical records.

Table 3.1: Location and Number of Ulcerations		
Location	**Number of Ulcers** (n)	**Percentage** (%)
Hallux	98	37
2nd digit	34	12
3rd digit	24	9
4th digit	26	10
5th digit	20	7
1st MTPJ	38	14
2nd MTPJ	4	1
3rd MTPJ	13	5
4th MTPJ	4	1
5th MTPJ	12	4

The graph below (figure 3.3) illustrates the distribution of the location of foot ulcerations found in the study group (n=273). The majority of plantar forefoot ulceration where present under the hallux (37%) followed by the 1st metatarsal head (14%) and 2nd digit (12%), with the smallest number of ulcerations present under the 2nd MTP joint and the 4th MTP joint (1%).

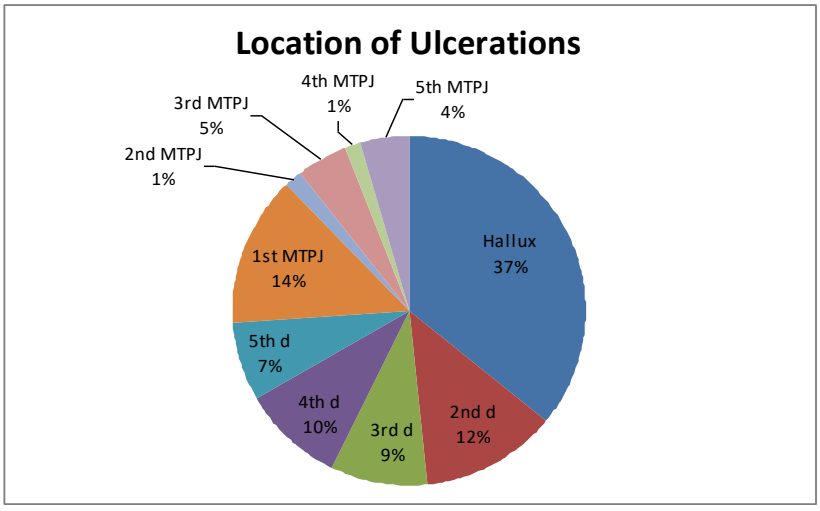


Figure 3.3: Distribution of forefoot ulcerations in the study Group

3.3 Distribution of Amputations

The results related to the distribution of referred amputations as documented in the medical records analysed are illustrated in the table 3.2 below. Results include number (n), percentage (%) and location of all referred amputations documented in all eligible medical records.

Table 3.2: Amputations according to location

Location	**Number of Amputations** (n)	**Percentage out of total amputations %** (n=104)
Hallux	28	26
2nd digit	17	15
3rd digit	8	8
4th digit	12	12
5th digit	10	10
1st MTPJ	12	12
2nd MTPJ	3	3
3rd MTPJ	5	5
4th MTPJ	4	4
5th MTPJ	5	5

The pie chart below (figure 3.4) illustrates the distribution of referred amputations according to location as a percentage of total amputations (n=104) recorded in the medical records of the sample population. The highest percentage of amputations occurred in the hallux (26%) followed by the 2nd digit (15%) and the 4th digit and 1st Ray (both 12%).

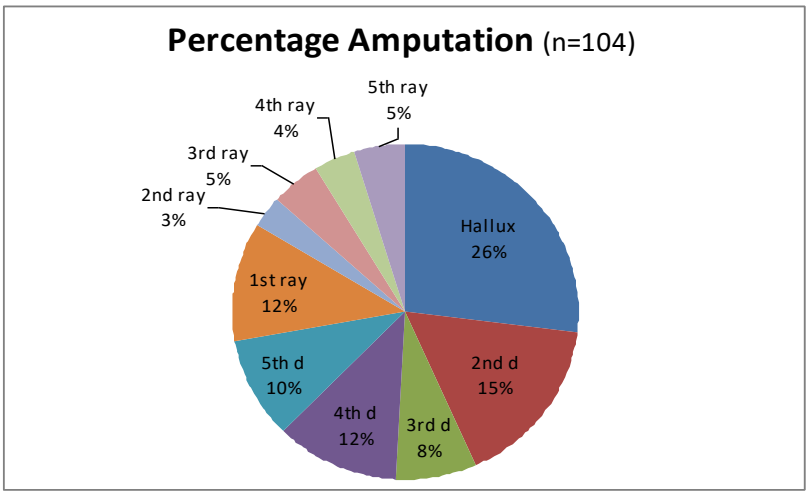


Figure 3.4: Distribution of amputations

3.4 Ulcerations and Amputations Rates

The number and percentage of documented ulcerations which resulted in an amputation are provided in the table 3.3 below, showing that 38% of all foot ulcerations were referred for amputation. Results demonstrate that all (100%) of 4th MTP joint ulcerations were referred for amputation of the 4th ray, followed by the 2nd, 4th and 5th digital ulcerations of which approximately 50% were amputated. It is also noted that although the hallux and 1st MTP joint have the highest ulceration rates over all, 36% and 14% respectively (figure 3.3), they are the locations with the lowest referrals rate for amputation, 28.6% and 31.5% in this sample population as shown in table 3.3.

Table 3.3: Ulceration vs Amputations According to Location			
Location	**Number of Ulcerations** (n)	**Number resulting in Amputations** (N)	**Percentage of Ulcerations resulting in Amputations** (%)
Hallux	98	28	28.6
2nd digit	34	17	50
3rd digit	24	8	33.3
4th digit	26	12	46
5th digit	20	10	50
1st MTPJ	38	12	31.5
2nd MTPJ	4	3	75
3rd MTPJ	13	5	38.5
4th MTPJ	4	4	100
5th MTPJ	12	5	41.7
TOTAL	**273**	**104**	**38**

The graph below (figure 3.5) is an illustration of the percentage of ulcerations resulting in amputation:

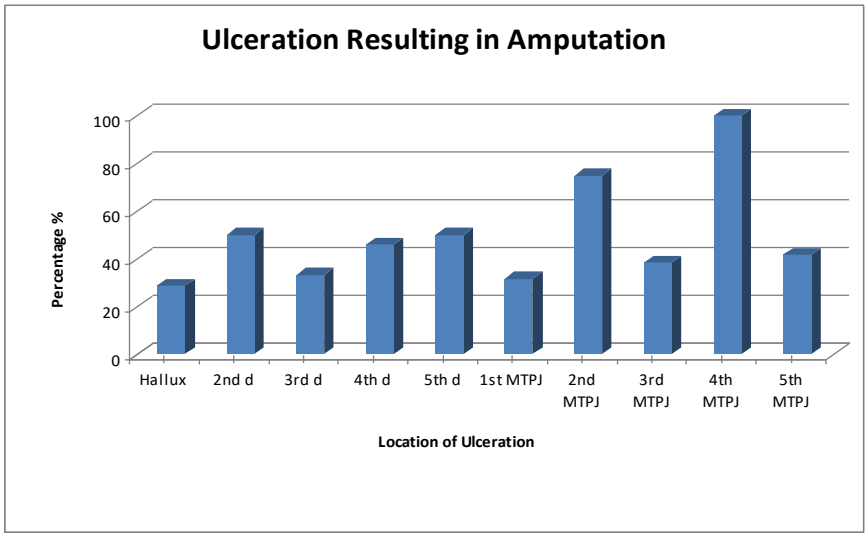


Figure 3.5: Percentage distribution of ulceration resulting in amputation

The graph below (figure 3.6) illustrates the number of ulcerations and number of amputations according to the respective location. Results indicate that the largest number of ulcerations occurred under the hallux and 1st MTP joint, reflecting in the higher number of amputations in the same location.

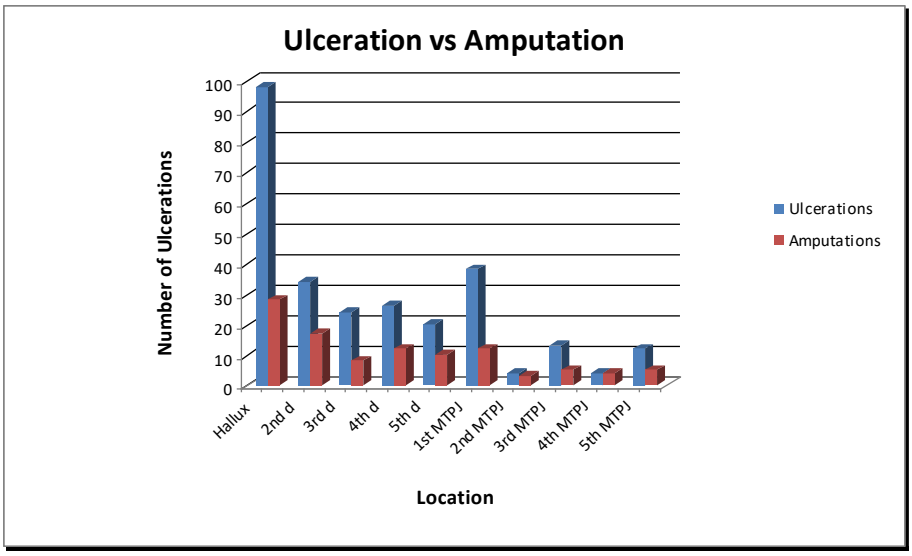


Figure 3.6: Distribution of ulceration and amputation according to location

Appendix III

Research Ethics Approval

UNIVERSITY OF MALTA

Request for Approval of Human Subjects Research
Please type, or print legibly with black pen. You may follow this format on separate sheets or use additional pages if necessary.

FROM: (name, address for correspondence) Stephen Mizzi Room 56, Level 1 Faculty of Health Sciences University of Malta TELEPHONE: 2340 1154 E-MAIL: stephen.mizzi@um.edu.mt COURSE AND YEAR: Phase 2, Phd Project	PROJECT TITLE: The influence of in-shoe mechanical variables and micro-climate in prescribed therapeutic footwear in a Maltese population living with diabetes mellitus.
ANTICIPATED FUNDING SOURCE: (include grant or contract number if known)	SUPERVISOR'S NAME: Professor Kate Springett Head of Department of Allied Health Professions Canterbury Christ Church University kate.springett@canterbury.ac.uk
DURATION OF ENTIRE PROJECT: from March 2014 to June 2014.	

1. Please give a brief summary of the purpose of the research, in non-technical language.

Malta has a high prevalence of type 2 diabetes (10%) when compared to European counterparts (2-3%). Despite the Maltese Government's investment in free health care and the provision of free prescribed footwear for the treatment and prevention of foot ulceration in diabetes, the number of minor amputations has been on the increase in recent years in Malta. Management of diabetic foot ulceration continues to be a major concern within the field of diabetes, owing to the cost to individuals and society. Moreover, the role of therapeutic footwear in the prevention of foot ulceration has been questioned for several years and only limited scientific evidence is available to date.

Since the main aim of this research study is to measure in-shoe variables at the interface between the shoe and the skin in the diabetic foot, a series of preliminary and pilot studies on healthy individuals will be undertaken to enable the researcher to establish a sound methodological approach. This will form phase two of the PhD as it will provide the base to the research which will establish the multifactorial, complex variables which influence the effectiveness of prescribed footwear used for the prevention of diabetic ulceration.

Therefore, the aim of this study is to investigate in-shoe measurement changes in plantar pressure, humidity and temperature that occurs within the shoe of participants during activity.

2. Give details of procedures that relate to subjects' participation
(a) How are subjects recruited? What inducement is offered? (Append copy of letter or advertisement or poster, if any.)

A convenience sample of 15 healthy participants and 10 diabetic participants (with no neurological or vascular problems) will be recruited from the Faculty of Health Sciences, University of Malta. The researcher will distribute an Invitation and Information letter (Appendix 1) by email to all staff through the Administrative Officer (Faculty of Health Sciences), asking them whether they wish to participate. The first to reply and who satisfy the inclusion criteria will be recruited for this study.

The researcher will ensure that no identifiable information will appear in the public domain

No inducement will be offered to participants.

(b) Salient characteristics of subjects—number who will participate, age range, sex, institutional affiliation, other special criteria:

15 healthy participants of any gender will be selected for the study, age ranging from 18-75years not suffering from any medical conditions.

10 diabetic participants will be selected from the study, age ranging from 18-75years living with diabetes with no vascular or neurological abnormalities.

(c) Describe how permission has been obtained from cooperating institution(s)—school, hospital, organization, prison, or other relevant organization. (Append letters.) Is the approval of another Research Ethics Committee required?

The following permissions have been requested and obtained. The request was done in writing to the following persons:

Dean, Faculty of Health Sceinces (Appendix 3)
Head of Department, Podiatry, University of Malta (Appendix 3)

(d) What do subjects do, or what is done to them, or what information is gathered? (Append copies of instructions or tests or questionnaires.) How many times will observations, tests, etc., be conducted? How long will their participation take?

Data collection will take place at the Podiatry Lab, Faculty of Health Sciences (permission granted from the Head of Podiatry Department, Appendix 3). The following procedure will be implemented:

1. Testing will take place in a quiet environment, with room temperature kept constant at 20-23 ^{0}C.

2. Participants will be asked to attend only once and each examination should take around 1.15hrs

3. Demographic Data, including participants' age, gender and BMI will be recorded.

4. Prior to baseline assessment the participant will have a 15 minute equilibration period in the examination room to adjust to the room temperature by lying on an examination couch with bare feet without any surface contact.

5. A physical examination of each participant's foot will be performed by the researcher who is a qualified and experienced podiatrist to ensure that there are no unperceived or unknown problems/conditions present, before and after the trial ensuring safety for the participant.

6. In-shoe sensors will be placed in the participant's shoe as follows:

   Humidity sensor - placed on the dorsal aspect of the foot, between the hallux and the 2nd digit
   Thermistor – placed behind the medial malleolus and under the medial longitudinal arch.
   In-shoe pressure sensors - placed between the shoe and the plantar aspect of the foot.

7. Participants will be asked to walk on a treadmill for 40 minutes at various speeds, within their comfortable psychological and physical limits.

A thermal camera (Fluke Co., Model i25) will be used to monitor plantar temperature at baseline (prior treadmill walking) after foot acclimatization and immediately after the walking trial.

It will be made clear both verbally and in writing that if during the study the participants start feeling uncomfortable they are free to stop at any time, without giving justification.

(e) Which of the following data categories are collected?

Data that reveals – race or ethnic origin	YES / <u>NO</u>
political opinions	YES / <u>NO</u>
religious or philosophical beliefs	YES / <u>NO</u>
trade union memberships	YES / <u>NO</u>
health	<u>YES</u> / NO
sex life	YES / <u>NO</u>
genetic information	YES / <u>NO</u>

3. How do you explain the research to subjects and obtain their informed consent to participate? (If in writing, append a copy of consent form.) If subjects are minors, mentally infirm, or otherwise not legally competent to consent to participation, how is their assent obtained and from whom is proxy consent obtained? How is it made clear to subjects that they can quit the study at any time?

Participants will be invited in writing to participate in the study (please find enclosed copy of letter). A full verbal explanation of the purpose and procedure of the study will also be given. They will be given 2 weeks to consider their response. Participants will be asked to sign in a consent form (please find enclosed copy of the letter). It will be made clear in both the inviting letter and the consent form that participants are free to withdraw from the study any time.

4 .Do subjects risk any harm—physical, psychological, legal, social—by participating in the research? Are the risks necessary? What safeguards do you take to minimize the risks?

There are no perceived risks within this research.

Safeguards to minimize risks: At no time will the participants' names be mentioned during the interpretation of the data. No identifying features will be reported in the public domain when results of this research will be published. Participants which will be conveniently selected will be coded with a number, known only to the researcher. All data will be recorded on a spreadsheet to group together the information required for interpretation of the results. Only the researcher will have access to this computer which is protected with a password known only to them.

5. Are subjects deliberately deceived in any way? If so, what is the nature of the deception? Is it likely to be significant to subjects? Is there any other way to conduct the research that would not involve deception, and, if so, why have you not chosen that alternative? What explanation for the deception do you give to subjects following their participation?

Subjects will not be intentionally deceived in any way. All subjects would have consented to participate in the study.

6. How will participation in this research benefit subjects? If subjects will be "debriefed" or receive information about the research project following its conclusion, how do you ensure the educational value of the process? (Include copies of any debriefing or educational materials)

Consenting participants are unlikely to benefit from the research other than the satisfaction of having contributed to the research. Since this study will be replicated on the diabetic foot, and of primary consideration to the researcher is the vulnerability of this population, the establishment of methods needs to be undertaken.

TERMS AND CONDITIONS FOR APPROVAL IN TERMS OF THE DATA PROTECTION ACT

- Personal data shall only be collected and processed for the specific research purpose.
- The data shall be adequate, relevant and not excessive in relation to the processing purpose.
- All reasonable measures shall be taken to ensure the correctness of personal data.
- Personal data shall not be disclosed to third parties and may only be required by the University or the supervisor for verification purposes. All necessary measures shall be implemented to ensure confidentiality and where possible, data shall be anonymised.
- Unless otherwise authorised by the University Research Ethics Committee, the researcher shall obtain the consent from the data subject (respondent) and provide him with the following information: The researcher's identity and habitual residence, the purpose of processing and the recipients to whom personal data may be disclosed. The data subject shall also be informed about his rights to access, rectify, and where applicable erase the data concerning him.

I, the undersigned hereby undertake to abide by the terms and conditions for approval as attached to this application.

I, the undersigned, also give my consent to the University of Malta's Research Ethics Committee to process my personal data for the purpose of evaluating my request and other matters related to this application. I also understand that, I can request in writing a copy of my personal information. I shall also request rectification, blocking or erasure of such personal data that has not been processed in accordance with the Act.

Signature:

APPLICANT'S SIGNATURE	FACULTY SPONSOR'S SIGNATURE I have reviewed this completed application and I am satisfied with the adequacy of the proposed research design and the measures proposed for the protection of human subjects.
 DATE 29/01/2014	 DATE 29/01/2014

ATTACHMENTS: .

* Recruitment letter, poster * Other institutional approval * Subject instructions
* Tests or questionnaires * Information sheets or debriefing materials
* Written consent form (or script) *Other

Return the completed application to your faculty Research Ethics Committee

To be completed by Faculty Research Ethics Committee

We have examined the above proposal and advise

Acceptance **Refusal** **Conditional acceptance**

For the following reason/s:

Signature [signature] Date 16/5/2012

To be completed by University Research Ethics Committee

We have examined the above proposal and grant

Acceptance **Refusal** **Conditional acceptance**

For the following reason/s:

Signature [signature] Date 1/6/12

Appendix IV

Participant's Information Sheet & Consent Form

Consent Form – English Version

I have been asked to participate in a research study entitled 'The influence of in-shoe mechanical variables and micro-climate in prescribed therapeutic footwear in a Maltese population living with diabetes mellitus.'

The purpose and details of this research have been clearly explained to me by the researcher, Stephen Mizzi. I understand the nature of the study I have been asked to take part in and possible effects for me.

I understand that the result of this study may be used for scientific purposes and that results achieved from this research which I am participating may be reported or published, however, I shall not be personally identified in any way, either individually or collectively without my written permission. I was informed that all data will be destroyed after successful completion of this research and all identification numbers of participants will be destroyed as well.

I am under no obligation to participate in this study and am doing so voluntarily. I may withdraw from the study at any time, without giving any reason. I am not receiving any remuneration for participation in this research.

In case of queries during my participation I may contact Mr Stephen Mizzi on 99440240.

Name of Participant: _____

Contact Number: _____

Signature of Participant: _____

Researcher: Stephen Mizzi **Contact Number**: 99440240

Signature of Researcher:

Name of Supervisor: Prof. Kate Springett

Signature of Supervisor:

Email Address: kate.springett@canterbury.ac.uk

Proposta Għall-Formula tal-Kunsens – Bil- Malti

Jien ġejt mitlub/a nipparteċipa f'riċerka bl-isem ta' 'The influence of in-shoe mechanical variables and micro-climate in prescribed therapeutic footwear in a Maltese population living with diabetes mellitus.'

L-iskop u d-dettalji ta' dan l-istudju ġew spjegati lili minn Stephen Mizzi u xi diffikultajiet li kelli ġew iċċarati.

Jien nagħti l-kunsens tiegħi lir-riċerkatur responsabbli għal din ir-riċerka li ser issir. Nifhem li r-riżultati li jinkisbu minn din ir-riċerka li jien qiegħed/qiegħda nieħu sehem fiha, jista' jsir rapport fuqhom jew jistgħu jiġu ppubblikati. Madanakollu jien bl-ebda mod ma jien se nkun identifikat/a personalment u l-anqas b'mod kollettiv, mingħajr il-permess bil-miktub tiegħi. Jien ġejt mgħarraf/mgħarrfa li l-informazzjoni personali kollha se tinqered wara li din ir-riċerka tintemm b'suċċess u li n-numri ta' identifikazzjoni tal-parteċipanti kollha se jiġu meqruda wkoll.

B'ebda mod ma jien obbligat/obbligata li nipparteċipa f'din ir-riċerka u qed nagħmel hekk mingħajr ma ġegħlni ħadd. Jien nista' nirtira minn din ir-riċerka meta rrid, mingħajr ma nagħti raġuni ghall- irtirar tiegħi. Jien mhux qed nirċievi ħlas għall- parteċipazzjoni tiegħi f'din ir-riċerka.

F'każ li jkolli bżonn nagħmel xi mistoqsija dwar din ir-ricerka, jien nista' nikkuntattja lil Stephen Mizzi fuq 99440240.

Isem il-parteċipant/a: _____

Numru tat-telefown: _____

Firma tall- partiċipant/a: _____

Isem ir-Riċerkatur: Stephen Mizzi **Numru tat- telefown:** 99440240

Firma ta' Riċerkatur:

Isem tas-Supervizor: Prof. Kate Spingett

kate.springett@canterbury.ac.uk

Invitation/Information Letter

Mr Stephen Mizzi

PhD Student

Faculty of Health Sciences,

University of Malta

Date:

Dear Sir/Madam,

I am conducting a study for my doctoral degree entitled "The influence of in-shoe mechanical variables and micro-climate in prescribed therapeutic footwear in a Maltese population living with diabetes mellitus".

You are kindly being invited to take part in this research. Before you decide whether to participate, please take your time to read this letter which provides information about this study. Please do not hesitate to contact me if there is anything that is not clear or if you require any additional information.

The aim of this study is to investigate in-shoe measurements changes in plantar pressure, humidity and temperature that occur within the shoe of a healthy participant, during activity.

If you choose to take part in this study you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw from this research project without giving a reason. Whether you decide to participate or withdraw at any time from this research will not affect you in any way.

Data collection is to be carried out at the Biomechanics Laboratory, Faculty of Health Sciences and it will include:

1. Collection of demographic data
2. A physical examination of your feet
3. Sensors (humidity, thermistor and pressure) will be placed in your shoes.
4. You will be asked to walk on a treadmill at various speeds for 40 minutes.

You will be asked to attend only once during this research. Please note that no harm will be induced during the test, however, if you feel uncomfortable at any time during data collection your participation will be terminated at once.

Therefore, the aim of this study is to investigate in-shoe measurement changes in plantar pressure, humidity and temperature that occur within the shoe of a healthy participant, during activity, which will later be replicated on participants living with diabetes mellitus.

Your name is to remain strictly confidential and it will not be made identifiable when results of this research are published. Ethical approval from the University of Malta has been sought and granted prior to conduction of this research. If you require further information about this research or would like to participate please contact the undersigned on 99440240 or email *stephen.mizzi@um.edu.mt*.

Regards

Stephen Mizzi

Researcher

PhD Student

Name of Supervisor: Prof. Kate Springett ***Email Address:*** kate.springett@can

Ittra ta' Invit u Informazzjoni - Maltese

Stephen Mizzi

Faculty of Health Sciences

University of Malta

Għażiż/a Sinjur/a,

Jien għalliem fi ħdan l-Universita ta' Malta qiegħed nagħmel riċerka b'isem ta' ***'The influence of in-shoe mechanical variables and micro-climate in prescribed therapeutic footwear in a Maltese population living with diabetes mellitus.'***

Int qiegħed/qiegħda tiġi mistieden/mistiedna biex tieħu sehem f'din ir-riċerka. Qabel ma tiddeċiedi jekk tieħux sehem jew le, huwa importanti li tifhem għaliex din ir-riċerka qiegħda ssir kif ukoll x'tinvolvi. Jekk jogħġok ħu l-ħin kollu neċessarju biex taqra sewwa l-informazzjoni li ġejja u jekk tixtieq iddiskutiha ma' ħaddiehor ukoll. Jekk hemm xi ħaġa li mhix ċara jew jekk trid aktar informazzjoni dwar din ir-riċerka, tiddejjaqx tistaqsi. Ħu l-ħin kollu meħtieġ biex tiddeċiedi jekk tridx tipparteċipa jew le.

L-għan prinċipali ta' dan l-istudju hu li jinvestiga l-bidliet fil-klima ta' ġewwa ż-żarbun li jinkludu l'umdità u temperatura, kif ukoll il-pressjoni ta' diversi postijiet ta' taħt is-sieq waqt il-mixi. Ser jiġu magħżula 10 persuni sabiex jieħdu sehem f'din ir-riċerka.

Jekk tiddeċiedi li tieħu sehem f'din ir-riċerka int ser tingħata din il karta ta' informazzjoni u ser tiġi mitlub/mitluba tiffirma formula ta'kunsens. Int liberu/libera li ma tkompliex tieħu sehem f'din ir-riċerka minajr ma tagħti raguni għal dan. Int f'ebda ħin m'int ser tiġi affettwat/affettwata b'xi mod kemm jekk tiddeċiedi li tipparteċipa jew le.

Din ir-riċerka tikkonsisti min :

1. Ġbir ta' informazzjoni demografika
2. Isirlek eżami fiżiku ta' saqajk

3. Tqegħid ta ‘sensors’ (umdità, temperature u pressjoni) se jitpoġġew fiż-żraben tiegħek.
4. Inti ser tiġi mitlub li timxi fuq treadmill b'veloċitajiet differenti għal 40 minuta.

Int mitlub tieħu sehem darba waħda biss waqt din ir-riċerka u jekk f’xi ħin tħossok imħawwad/imħawwda għal xi raġuni, ir-riċerka tintemm.

L-għan ta' dan l-istudju hu li jinvestiga l-bidliet ta’ klima fiż-żarbun li jinkludu umdità u temperatura kif ukoll il-kejl ta’ pressjoni fil-qiegħ tas-sieq, li jseħħu fiż-żarbun ta' parteċipanti b'saħħithom matul l-attività, li iktar tard se jiġu replikati fuq parteċipanti li jbatu mid-dijabete.

L-identita' tiegħek ser tinżamm kunfidenzjali u ħadd ma jkun jista jidentifikak la darba jiġu ppublikati r-riżultati ta’ din ir-riċerka. L-approvazzjoni ta’etika mill-Universita ta’Malta ġiet mogħtija qabel ma bdiet ssir din ir-riċerka.

Jekk tixtieq aktar informazzjoni dwar din ir-riċerka, tista ċċempilli fuq 23401154.

Grażżi bil-quddiem tal-parteċipazzjoni tiegħek f’din ir-riċerka.

Dejjem tiegħek

Stephen Mizzi

Reċerkatur

Name of Supervisor: Prof. Kate Springett ***Email*** ***Address:***
kate.springett@canterbury.ac.uk
302

Appendix V

Raw data for RH Sensor 1_{new} and Thermistor Sensor 1_{new} Before & After Adjustment

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Raw data for RH Sensor 1new Before Adjustment

Table 1: Raw data for **RH sensor 1new** – *Before Adjustment*				
Calibration Point	Nominal Temp ^{0}C	Nominal Humidity %	Mean RH ref - BA	Mean RH Sen 1 new
1	20	50	49.698	53.39
2	20	65	64.466	69.66
3	20	80	79.367	86.31
4	20	95	94.115	103.61
5	30	50	49.815	52.05
6	30	65	64.745	67.76
7	30	80	79.672	84.14
8	30	95	94.556	101.6
9	40	50	49.928	50.72
10	40	65	64.851	66.05
11	40	80	79.784	82.31
12	40	95	94.649	99.63
13	50	50	49.815	49.36
14	50	65	64.819	64.3
15	50	80	79.805	80.2
16	50	95	94.621	96.16

Raw data for RH Sensor 1new After Adjustment

Table 2: Raw data for **RH sensor 1new** – *After Adjustment*				
Calibration Point	Nominal Temp ^{0}C	Nominal Humidity %	Mean RH ref - BA	Mean RH Sen 1 new
1	30	50	49.676	50.42
2	30	65	64.626	64.85
3	30	80	79.544	79.57
4	30	95	94.397	95.79

Raw data for Temp Sensor 1new Before Adjustment

Varying Isotherms and one fixed RH levels of 50%

Table 3: Raw data for Thermistor **sensor 1new** – *Before Adjustment*				
Calibration Point	Nominal Temp ^{0}C	Nominal Humidity %	Mean Tref - BA	Mean T Sen 1 new
1	20	50	20.142	20.10
2	30	50	29.902	31.00
3	40	50	39.737	41.04
4	50	50	49.597	49.53

Varying RH levels at a fixed Isotherms

Table 4: Raw data for **Temperature sensor 1new** – Before *Adjustment*				
Calibration Point	Nominal Temp ^{0}C	Nominal Humidity %	Mean T ref – BA	Mean T Sen 1 new
1	30	50	29.905	31.10
2	30	65	29.932	31.13
3	30	80	29.935	31.14
4	30	95	29.931	31.13

Appendix VI

Data Analysis for New RH Sensors and Thermistors

Correlation and Level of Agreement - **RH** Sensor

Correlation and Level of Agreement **RH Sensor 2_{new}**

Table 1:

Correlation and Level of Agreement RH Sensor 2_{new} – Before Adjustment

	Statistical Test	95% CI	
		Lower Limit	Upper Limit
Temp. Level @ 20°C	**ICC** Single Measures (r) **0.99**	0.85	1.0
	Bland & Altman Mean Difference (±SD) **-6.3 (±2.98)**	-12.2	-0.5

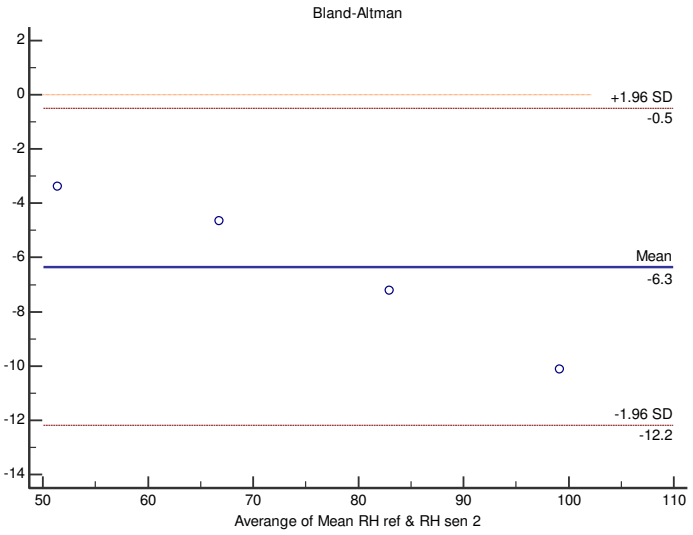


Table 2: Correlation and Level of Agreement RH Sensor 2_{new} – Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **30^{0}C**	**ICC** Single Measures (r) **0.99**	0.89	1.00
	Bland & Altman Mean Difference (±SD) **-4.2 (±2.5)**	-9.1	-0.7

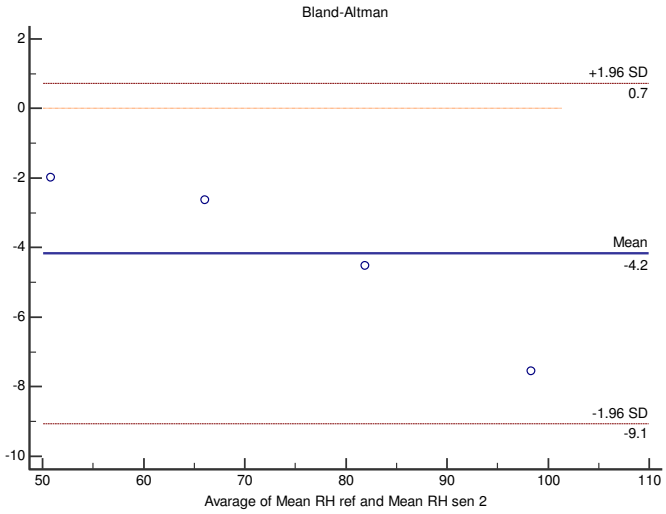


Table 3: Correlation and Level of Agreement RH Sensor 2_{new} – Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **40^{0}C**	**ICC** Single Measures (r) **1.00**	0.94	1.00
	Bland & Altman Mean Difference (±SD) **-2.0 (±1.8)**	-5.6	1.5

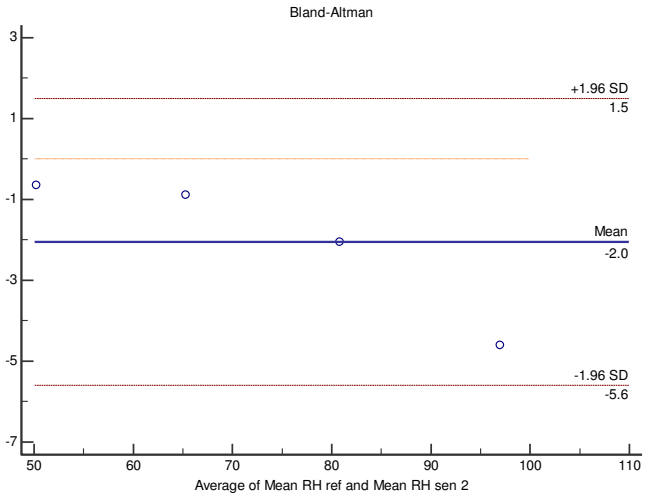


Table 4: Correlation and Level of Agreement RH Sensor 2_{new} – Before Adjustment			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **50^{0}C**	**ICC** Single Measures (r) **1.00**	0.99	1.00
	Bland & Altman Mean Difference (±SD) **- 0.1 (±0.8)**	-1.5	1.7

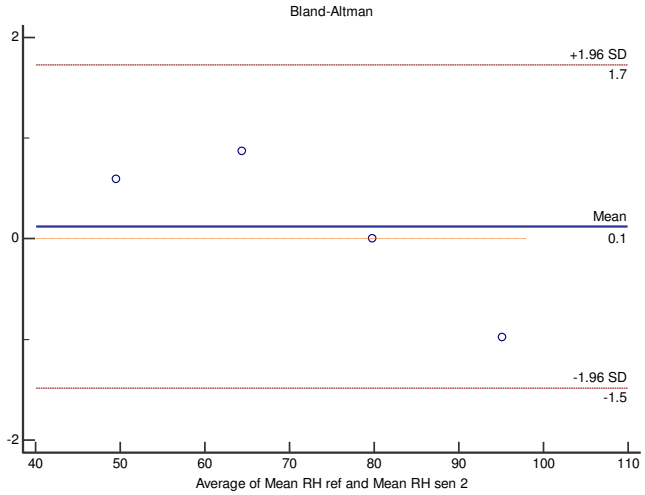
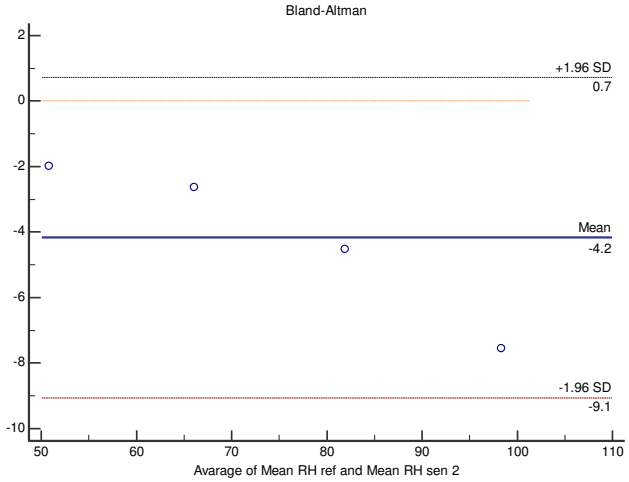
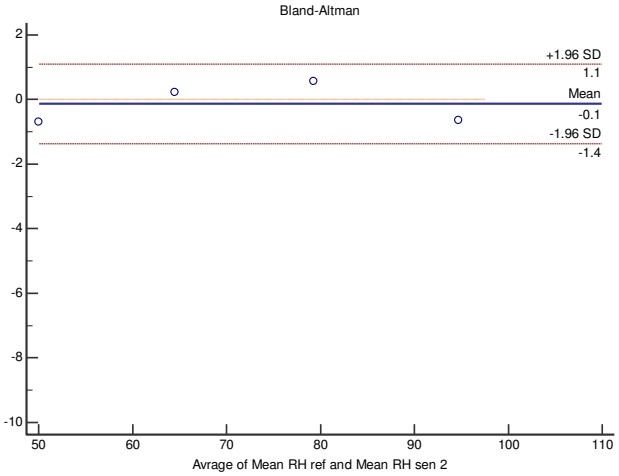


Table 5: Correlation and Level of Agreement RH Sensor 2_{new} – Before & After Calibration					
Before Calibration			After Calibration		
Statistical Test	95% CI		Statistical Test	95% CI	
	Lower Limit	Upper Limit		Lower Limit	Upper Limit
ICC Single Measures (r) **0.99**	0.89	1.00	**ICC** Single Measures (r) 0.9995	1.00	1.00
Bland & Altman Mean Difference (±SD) **- 4.2 (±2.5)**	-9.1	-0.7	**Bland & Altman** Mean Difference (±SD) **- 0.1 (±0.6)**	-1.4	1.1


Bland-Altman
+1.96 SD
0.7
Mean
-4.2
-1.96 SD
-9.1
2
0
-2
-4
-6
-8
-10
50
60
70
80
90
100
110
Avarage of Mean RH ref and Mean RH sen 2


Bland-Altman
+1.96 SD
1.1
Mean
-0.1
-1.96 SD
-1.4
2
0
-2
-4
-6
-8
-10
50
60
70
80
90
100
110
Avrage of Mean RH ref and Mean RH sen 2

Correlation and Level of Agreement **RH Sensor 3$_{new}$**

Table 6: Correlation and Level of Agreement RH Sensor 3$_{new}$ - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **20^{0}C**	**ICC** Single Measures (r) **0.99**	0.91	1.00
	Bland & Altman Mean Difference (±SD) **- 5.8 (±2.2)**	-1.5	10.2

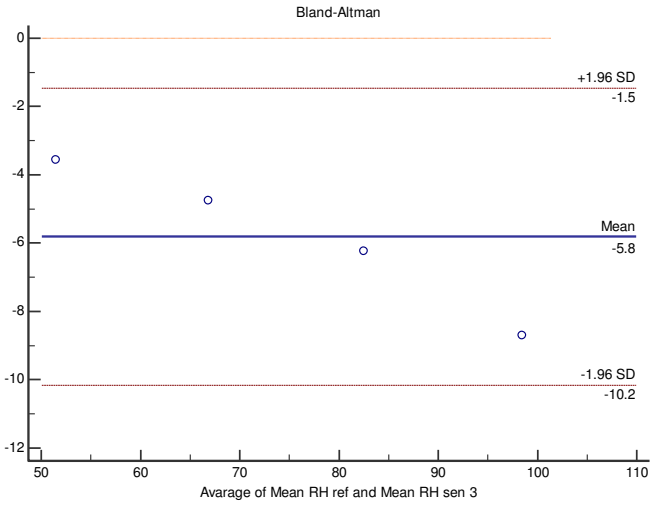


Table 7: Correlation and Level of Agreement RH Sensor 3$_{new}$ - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **30^{0}C**	**ICC** Single Measures (r) **1.00**	0.94	1.00
	Bland & Altman Mean Difference (±SD) **- 3.5 (±1.8)**	-7.0	-0.0

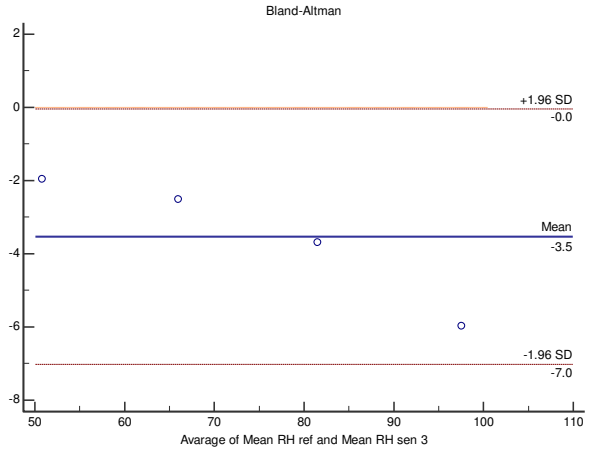


Table 8: Correlation and Level of Agreement RH Sensor 3_{new} - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **40^{0}C**	**ICC** Single Measures (r) **1.00**	0.96	1.00
	Bland & Altman Mean Difference (±SD) **- 1.7 (±1.5)**	-4.5	1.2

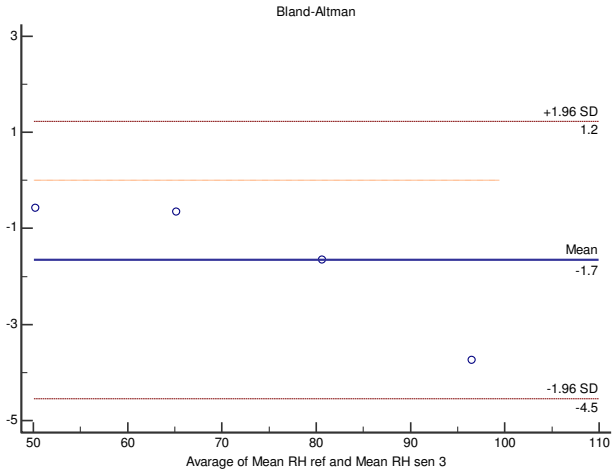


Table 9: Correlation and Level of Agreement RH Sensor 3_{new} - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **50^{0}C**	**ICC** Single Measures (r) **1.00**	0.99	1.00
	Bland & Altman Mean Difference (±SD) **- 0.4 (±0.6)**	-0.8	1.7

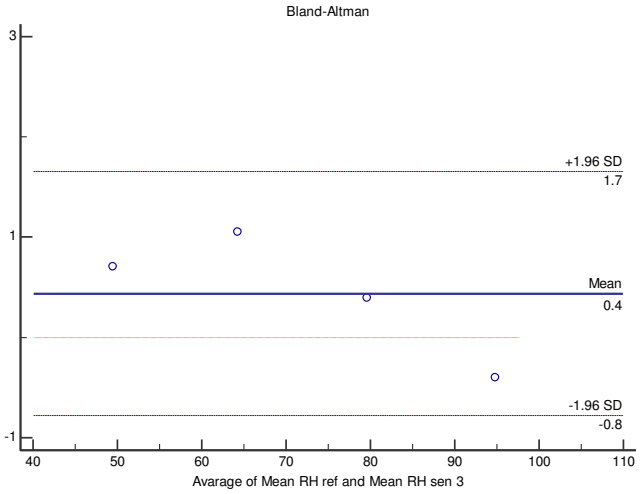
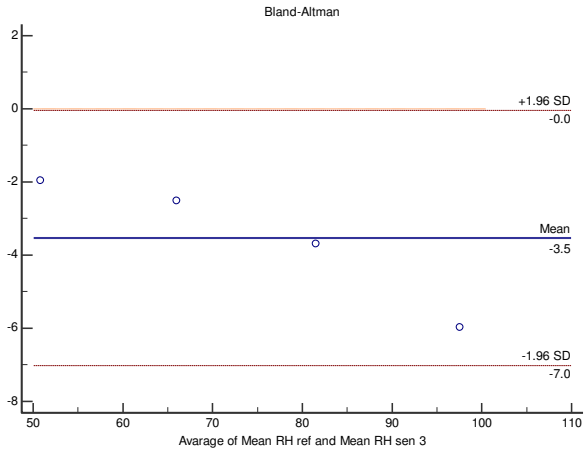
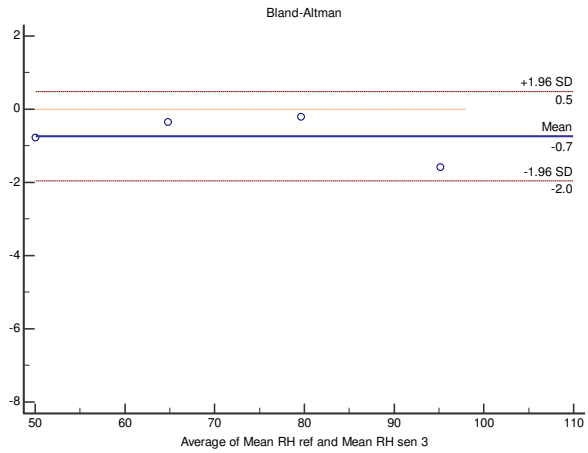


Table 10: Correlation and Level of Agreement RH Sensor 3_{new} – Before & After Calibration					
Before Calibration			After Calibration		
Statistical Test	95% CI		Statistical Test	95% CI	
	Lower Limit	Upper Limit		Lower Limit	Upper Limit
ICC Single Measures (r) **1.00**	0.94	1.00	**ICC** Single Measures (r) **1.00**	0.99	1.00
Bland & Altman Mean Difference (±SD) **- 3.5 (±1.8)**	-7.0	-0.0	**Bland & Altman** Mean Difference (±SD) **- 0.7 (±0.6)**	-2.0	0.5





Correlation and Level of Agreement **RH Sensor 4$_{new}$**

Table 11: Correlation and Level of Agreement RH Sensor 4$_{new}$ - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **20^{0}C**	**ICC** Single Measures (r) **0.99**	0.87	1.00
	Bland & Altman Mean Difference (±SD) **- 7.0 (±2.9)**	-12.7	-1.3

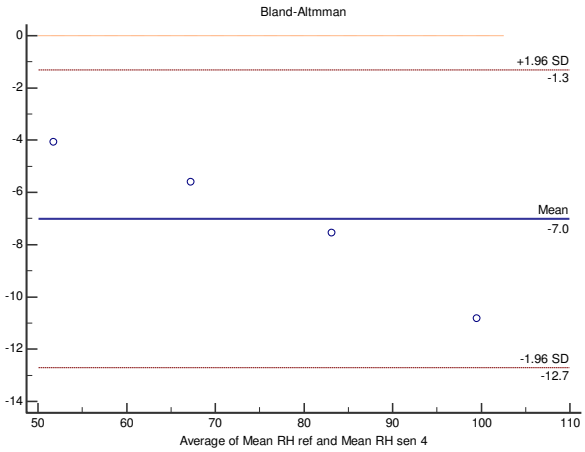


Table 12: Correlation and Level of Agreement RH Sensor 4$_{new}$ - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **30^{0}C**	**ICC** Single Measures (r) **0.99**	0.88	1.00
	Bland & Altman Mean Difference (±SD) **- 5.0 (±2.7)**	-10.2	0.3

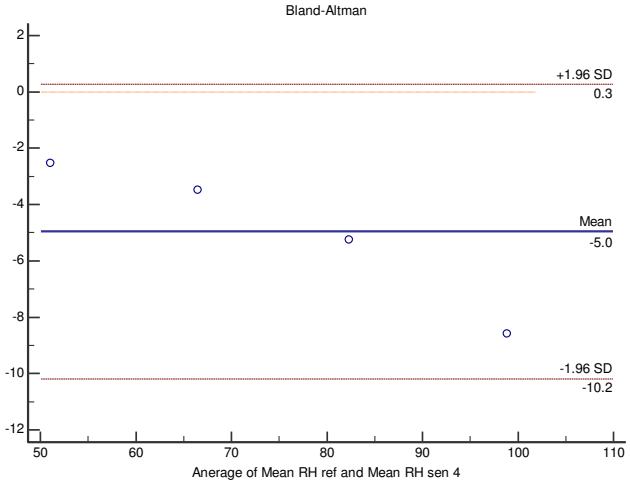


Table 13: Correlation and Level of Agreement RH Sensor 4_{new} - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **40^{0}C**	**ICC** Single Measures (r) **0.99**	0.90	1.00
	Bland & Altman Mean Difference (±SD) **- 3.0 (±2.3)**	-7.5	1.6

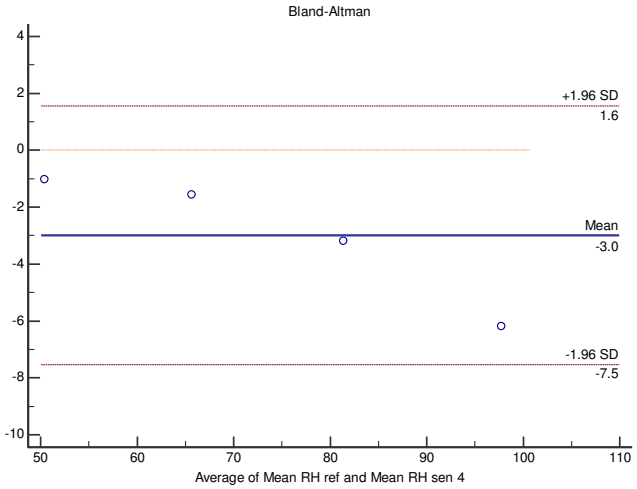


Table 14: Correlation and Level of Agreement RH Sensor 4_{new} - Before Adjustment

Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp. Level @ **50^{0}C**	**ICC** Single Measures (r) **1.00**	0.97	1.00
	Bland & Altman Mean Difference (±SD) **-0.6 (±1.3)**	-3.2	2.0

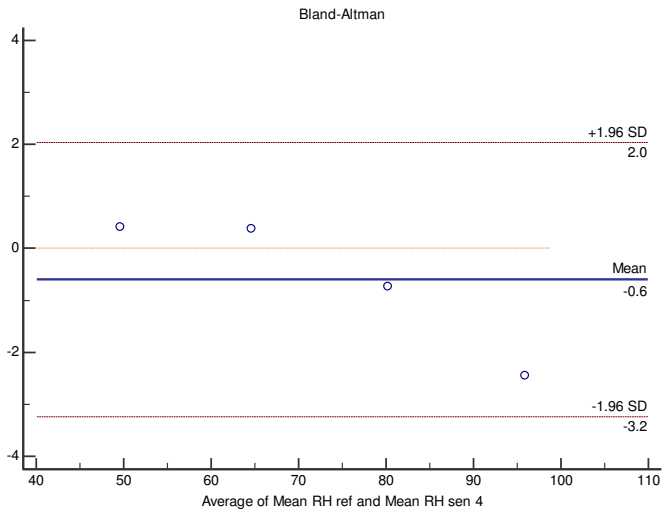
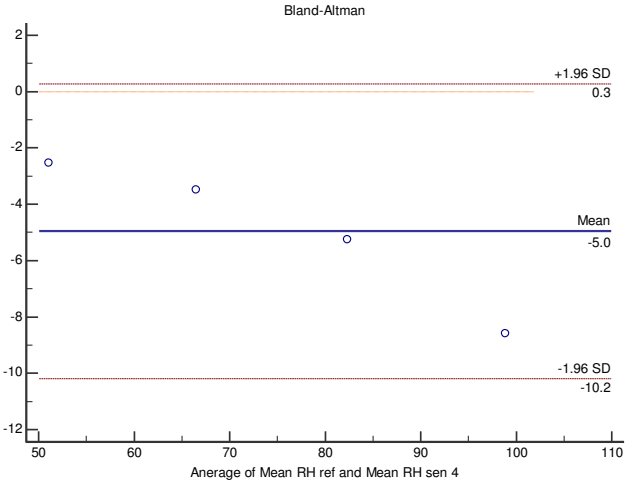
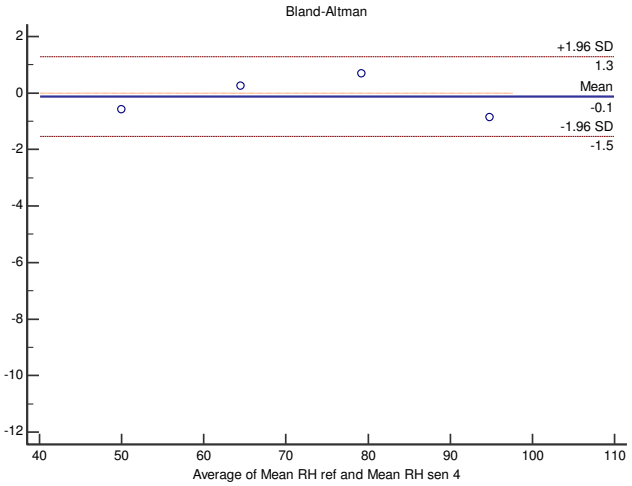


Table 15: Correlation and Level of Agreement RH Sensor 4_{new} – Before & After Calibration					
Before Calibration			After Calibration		
Statistical Test	95% CI		Statistical Test	95% CI	
	Lower Limit	Upper Limit		Lower Limit	Upper Limit
ICC Single Measures (r) **0.99**	0.88	1.00	**ICC** Single Measures (r) **0.99**	0.99	1.00
Bland & Altman Mean Difference (±SD) **- 5.0 (±2.7)**	-10.2	-0.3	**Bland & Altman** Mean Difference (±SD) **- 0.1 (±0.7)**	-1.5	1.3





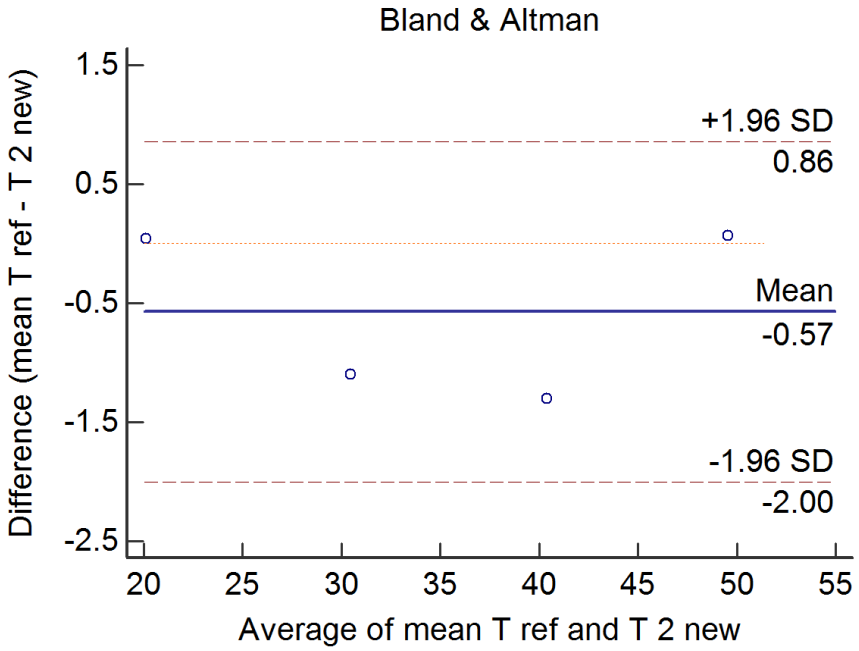
Correlation and Level of Agreement - **Thermistors**

Correlation and Level of Agreement Thermistor **2_{new}**

Varying Isotherms and one fixed RH levels of 50%

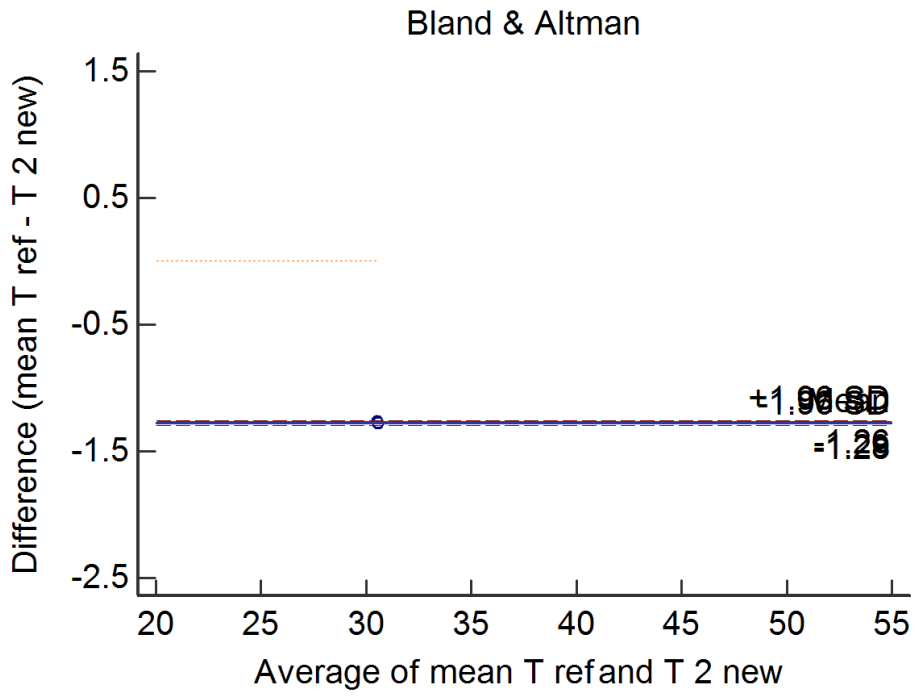
Table 5.3: Correlation and Level of Agreement of T_{ref} & Thermistor **2_{new}**

Statistical Test		95% CI	
		Lower Limit	Upper Limit
RH Level @ 50%	**ICC** *Single Measures r* 1.00	0.97	1.00
	Bland & Altman *Mean Difference (±SD)* -0.6 (±0.7)	-2.00	0.86



Varying RH levels at a fixed Isotherms 30^0C

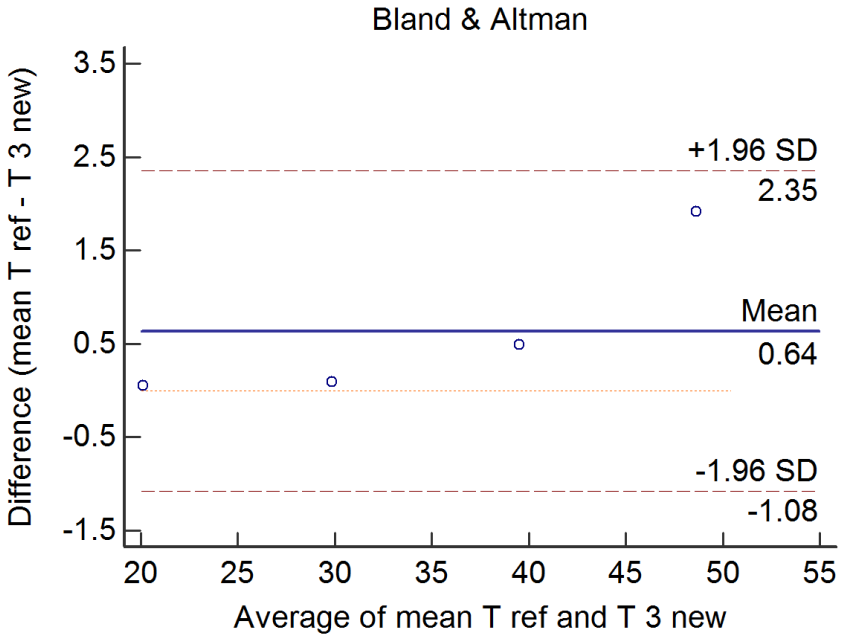
Table 5.4: Correlation and Level of Agreement of T_{ref} & Thermistor **2_{new}**			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp Level @ 30^0C	**ICC** *Single Measures r* 0.91	0.086	1.00
	Bland & Altman *Mean Difference (±SD)* -1.28 (±0.01)	-1.32	-1.30



Correlation and Level of Agreement Thermistor **3$_{new}$**

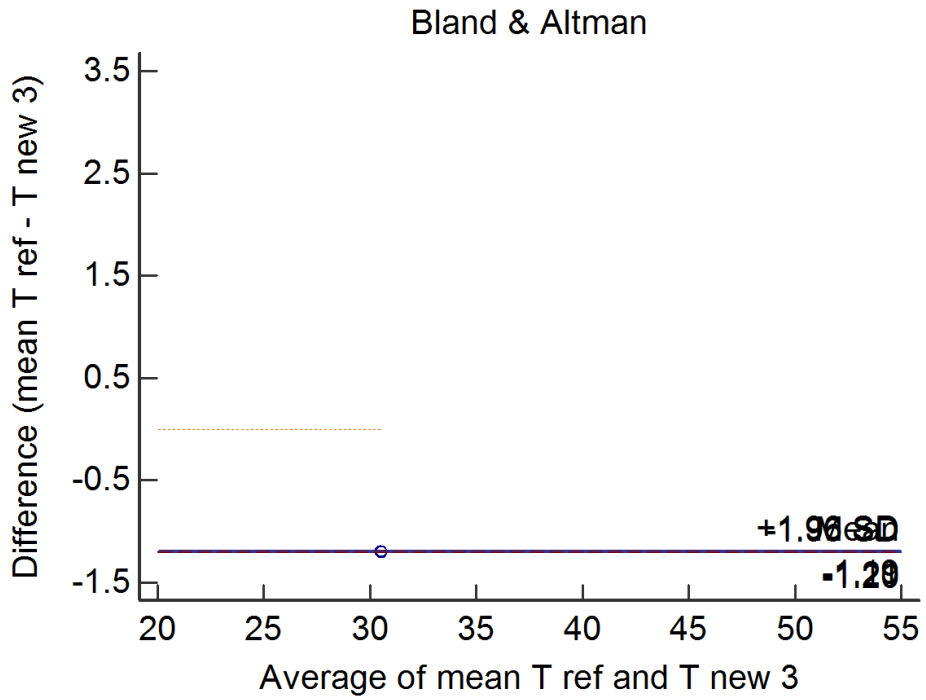
Varying Isotherms and one fixed RH levels of 50%

Table 5.3: Correlation and Level of Agreement of T_{ref} & Thermistor **3$_{new}$**			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
RH Level @ 50%	**ICC** *Single Measures r* 1.00	0.96	1.00
	Bland & Altman *Mean Difference (±SD)* -0.6 (±0.9)	-1.10	2.35



Varying RH levels at a fixed Isotherms 30^0C

Table 5.4: Correlation and Level of Agreement of T_{ref} & Thermistor $\mathbf{3_{new}}$			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp Level @ 30^0C	**ICC** *Single Measures r* 0.96	0.56	1.00
	Bland & Altman *Mean Difference (±SD)* -1.29 (±0.01)	-1.20	-1.19

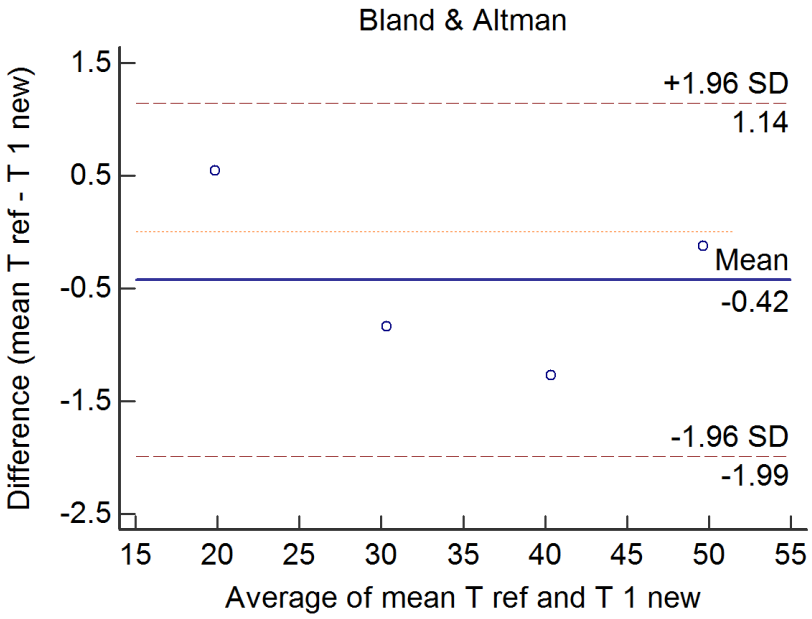


Correlation and Level of Agreement Thermistor **4_{new}**

Varying Isotherms and one fixed RH levels of 50%

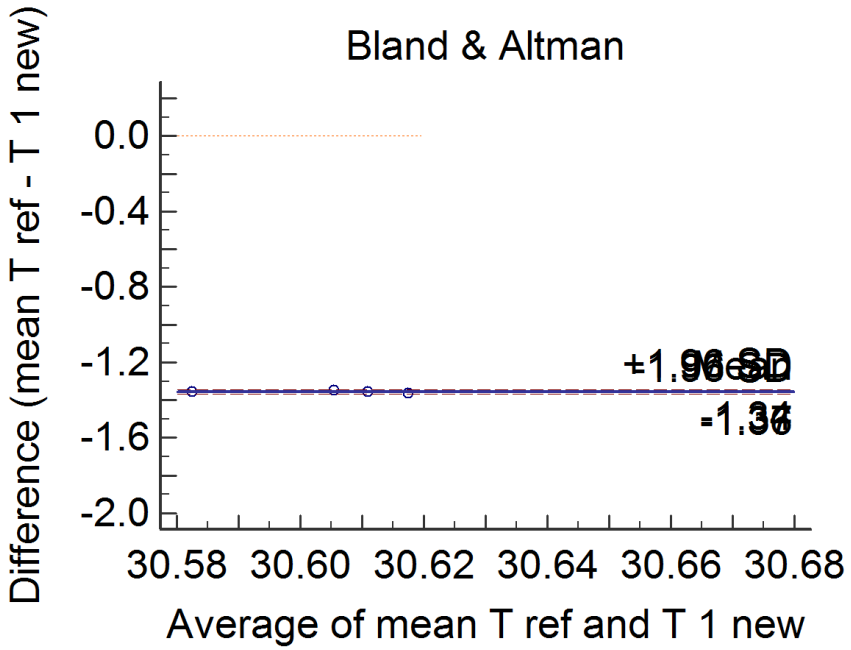
Table 5.3: Correlation and Level of Agreement of T_{ref} & Thermistor **4_{new}**

	Statistical Test	95% CI	
		Lower Limit	Upper Limit
RH Level @ 50%	**ICC** *Single Measures r* 1.00	0.97	1.00
	Bland & Altman *Mean Difference (±SD)* -0.4 (±0.8)	-2.0	1.1



Varying RH levels at a fixed Isotherms 30^0C

Table 5.4: Correlation and Level of Agreement of T_{ref} & Thermistor $\mathbf{4_{new}}$			
Statistical Test		95% CI	
		Lower Limit	Upper Limit
Temp Level @ 30^0C	**ICC** *Single Measures r* 0.91	0.15	.99
	Bland & Altman *Mean Difference (±SD)* -1.37 (±0.01)	-1.4	-1.3



Appendix VII

Test for Normalcy of Data

Healthy Participant Group ***(In-shoe Temperature)***

Table 1: presents the distribution of data obtained from **temperature readings** at the different locations and seasons using individual data measurements (Shapiro-Wilk Normality Test)

Time	**Arch Temp. in Summer**			**Arch Temp. in Winter**			**Toe Temp. in Summer**			**Toe Temp. in Winter**		
/min	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value
0	0.914	22	0.057	0.962	26	0.442	0.968	24	0.627	0.945	26	0.173
1	0.926	22	0.101	0.973	26	0.691	0.988	24	0.991	0.956	26	0.324
2	0.928	22	0.113	0.969	26	0.605	0.988	24	0.987	0.953	26	0.267
3	0.931	22	0.129	0.965	26	0.488	0.977	24	0.836	0.960	26	0.396
4	0.945	22	0.254	0.959	26	0.375	0.988	24	0.989	0.958	26	0.358
5	0.938	22	0.182	0.959	26	0.371	0.978	24	0.865	0.960	26	0.392
6	0.954	22	0.384	0.964	26	0.470	0.975	24	0.782	0.960	26	0.387
7	0.954	22	0.374	0.963	26	0.443	0.972	24	0.711	0.959	26	0.381
8	0.956	22	0.408	0.964	26	0.467	0.954	24	0.325	0.962	26	0.432
9	0.961	22	0.501	0.964	26	0.486	0.945	24	0.209	0.961	26	0.416
10	0.965	22	0.605	0.964	26	0.472	0.956	24	0.368	0.962	26	0.434
11	0.968	22	0.656	0.964	26	0.480	0.965	24	0.551	0.964	26	0.471
12	0.967	22	0.641	0.964	26	0.478	0.933	24	0.113	0.953	26	0.277
13	0.973	22	0.776	0.962	26	0.442	0.940	24	0.160	0.953	26	0.273
14	0.979	22	0.892	0.960	26	0.394	0.929	24	0.094	0.952	26	0.258
15	0.978	22	0.876	0.957	26	0.341	0.929	24	0.094	0.949	26	0.222
16	0.983	22	0.957	0.953	26	0.266	0.920	24	0.058	0.947	26	0.196
17	0.978	22	0.887	0.950	26	0.229	0.911	24	0.038	0.942	26	0.154
18	0.980	22	0.920	0.946	26	0.188	0.928	24	0.087	0.936	26	0.106
19	0.978	22	0.878	0.939	26	0.127	0.927	24	0.082	0.922	26	0.051
20	0.979	22	0.902	0.935	26	0.102	0.934	24	0.120	0.913	26	0.031
21	0.970	22	0.713	0.925	26	0.059	0.967	24	0.598	0.902	26	0.018
22	0.965	22	0.606	0.914	26	0.032	0.934	24	0.122	0.892	26	0.011
23	0.971	22	0.728	0.904	26	0.019	0.929	24	0.093	0.886	26	0.008
24	0.969	22	0.696	0.888	26	0.009	0.953	24	0.309	0.869	26	0.003
25	0.974	22	0.799	0.885	26	0.007	0.979	24	0.872	0.861	26	0.002
26	0.977	22	0.871	0.875	26	0.004	0.970	24	0.676	0.852	26	0.002
27	0.967	22	0.634	0.868	26	0.003	0.947	24	0.228	0.840	26	0.001
28	0.964	22	0.573	0.858	26	0.002	0.970	24	0.658	0.833	26	0.001
29	0.953	22	0.369	0.847	26	0.001	0.955	24	0.354	0.817	26	0.000

30	0.963	22	0.559	0.842	26	0.001	0.951	24	0.279	0.799	26	0.000
31	0.959	22	0.470	0.834	26	0.001	0.965	24	0.540	0.779	26	0.000
32	0.964	22	0.573	0.831	26	0.001	0.984	24	0.959	0.762	26	0.000
33	0.960	22	0.486	0.835	26	0.001	0.984	24	0.957	0.752	26	0.000
34	0.973	22	0.782	0.830	26	0.001	0.988	24	0.988	0.738	26	0.000
35	0.967	22	0.635	0.826	26	0.001	0.990	24	0.996	0.719	26	0.000
36	0.966	22	0.625	0.824	26	0.000	0.977	24	0.843	0.712	26	0.000
37	0.979	22	0.892	0.821	26	0.000	0.975	24	0.791	0.707	26	0.000
38	0.963	22	0.562	0.819	26	0.000	0.974	24	0.776	0.703	26	0.000

Most temperature distributions were found to be normal. It was noted that only temperatures recorded towards the second half of the sampling time in Winter tended to have more skewed distributions than those recorded near the start of the sampling time.

Table 2: presents the distribution of data obtained from **RH readings** at the different locations and seasons using individual data measurements (Shapiro-Wilk Normality Test)

Time	**Arch R.H. in Summer**			**Arch R.H. in Winter**			**Toe R.H. in Summer**			**Toe R.H. in Winter**		
/min	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value
0	0.962	25	0.447	0.897	27	0.012	0.968	27	0.544	0.976	19	0.880
1	0.971	25	0.671	0.922	27	0.044	0.967	27	0.526	0.971	19	0.790
2	0.973	25	0.714	0.933	27	0.080	0.976	27	0.754	0.962	19	0.621
3	0.966	25	0.552	0.935	27	0.094	0.967	27	0.529	0.946	19	0.332
4	0.968	25	0.602	0.942	27	0.134	0.961	27	0.388	0.935	19	0.212
5	0.960	25	0.421	0.947	27	0.180	0.959	27	0.351	0.932	19	0.191
6	0.964	25	0.494	0.944	27	0.150	0.957	27	0.322	0.927	19	0.153
7	0.954	25	0.303	0.943	27	0.148	0.958	27	0.325	0.927	19	0.155
8	0.946	25	0.200	0.940	27	0.123	0.952	27	0.234	0.920	19	0.115
9	0.941	25	0.159	0.931	27	0.073	0.942	27	0.136	0.932	19	0.188
10	0.941	25	0.159	0.931	27	0.074	0.932	27	0.076	0.939	19	0.254
11	0.958	25	0.381	0.937	27	0.103	0.941	27	0.126	0.944	19	0.317
12	0.949	25	0.241	0.930	27	0.067	0.937	27	0.100	0.944	19	0.315
13	0.947	25	0.211	0.921	27	0.043	0.932	27	0.078	0.947	19	0.352
14	0.948	25	0.229	0.924	27	0.050	0.941	27	0.132	0.946	19	0.334
15	0.951	25	0.267	0.923	27	0.046	0.921	27	0.042	0.938	19	0.240
16	0.945	25	0.194	0.929	27	0.066	0.903	27	0.016	0.941	19	0.274
17	0.944	25	0.183	0.933	27	0.081	0.887	27	0.007	0.943	19	0.304
18	0.950	25	0.255	0.921	27	0.043	0.885	27	0.006	0.936	19	0.221
19	0.945	25	0.190	0.928	27	0.063	0.870	27	0.003	0.923	19	0.129
20	0.942	25	0.161	0.929	27	0.067	0.867	27	0.003	0.921	19	0.117
21	0.947	25	0.219	0.928	27	0.063	0.867	27	0.003	0.927	19	0.150
22	0.960	25	0.418	0.921	27	0.041	0.881	27	0.005	0.925	19	0.137
23	0.958	25	0.368	0.924	27	0.048	0.875	27	0.004	0.915	19	0.092
24	0.943	25	0.173	0.919	27	0.038	0.886	27	0.006	0.922	19	0.122
25	0.957	25	0.359	0.918	27	0.035	0.887	27	0.007	0.915	19	0.092
26	0.953	25	0.298	0.918	27	0.036	0.894	27	0.010	0.923	19	0.131
27	0.964	25	0.502	0.907	27	0.019	0.888	27	0.007	0.921	19	0.118
28	0.966	25	0.539	0.912	27	0.025	0.875	27	0.004	0.921	19	0.116
29	0.963	25	0.468	0.910	27	0.023	0.884	27	0.006	0.922	19	0.124
30	0.957	25	0.359	0.909	27	0.021	0.878	27	0.004	0.908	19	0.068
31	0.964	25	0.499	0.909	27	0.022	0.877	27	0.004	0.899	19	0.046
32	0.962	25	0.454	0.913	27	0.027	0.868	27	0.003	0.903	19	0.056
33	0.960	25	0.417	0.910	27	0.022	0.878	27	0.004	0.909	19	0.071
34	0.962	25	0.458	0.922	27	0.044	0.873	27	0.003	0.911	19	0.077
35	0.951	25	0.265	0.925	27	0.053	0.869	27	0.003	0.919	19	0.111
36	0.961	25	0.433	0.916	27	0.032	0.877	27	0.004	0.917	19	0.099
37	0.963	25	0.475	0.912	27	0.026	0.883	27	0.006	0.925	19	0.141
38	0.960	25	0.417	0.911	27	0.024	0.891	27	0.009	0.920	19	0.113

Most RH distributions were found to be normal, particularly those recorded during Summer, arch area and Winter toe area. It was noted that RH recorded towards the second half of the sampling time in Summer toe area and Winter arch area tended to have more skewed distributions than those recorded near the start of the sampling time.

Table 3: presents the distribution of data obtained from **temperature readings** at the different locations and seasons using individual data measurements (Shapiro-Wilk Normality Test)

Time (/min)	Arch Temp. in Summer			Arch Temp. in Winter			Toe Temp. in Summer			Toe Temp. in Winter		
	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value
0	0.960	10	0.786	0.927	10	0.418	0.948	10	0.647	0.949	10	0.661
1	0.943	10	0.584	0.908	10	0.270	0.926	10	0.405	0.918	10	0.337
2	0.943	10	0.588	0.908	10	0.269	0.936	10	0.507	0.944	10	0.594
3	0.944	10	0.604	0.914	10	0.307	0.954	10	0.714	0.949	10	0.658
4	0.958	10	0.757	0.940	10	0.555	0.939	10	0.541	0.967	10	0.861
5	0.957	10	0.749	0.922	10	0.377	0.926	10	0.409	0.949	10	0.652
6	0.961	10	0.796	0.923	10	0.380	0.938	10	0.526	0.960	10	0.785
7	0.967	10	0.865	0.926	10	0.411	0.937	10	0.515	0.956	10	0.739
8	0.969	10	0.880	0.952	10	0.697	0.944	10	0.603	0.958	10	0.765
9	0.960	10	0.786	0.949	10	0.658	0.927	10	0.422	0.958	10	0.762
10	0.945	10	0.614	0.953	10	0.707	0.907	10	0.262	0.957	10	0.755
11	0.954	10	0.713	0.955	10	0.724	0.895	10	0.191	0.943	10	0.592
12	0.936	10	0.511	0.950	10	0.669	0.883	10	0.143	0.947	10	0.635
13	0.921	10	0.368	0.961	10	0.795	0.873	10	0.108	0.941	10	0.563
14	0.918	10	0.339	0.957	10	0.748	0.863	10	0.083	0.936	10	0.504
15	0.924	10	0.395	0.960	10	0.789	0.876	10	0.117	0.927	10	0.421
16	0.964	10	0.830	0.954	10	0.719	0.871	10	0.103	0.928	10	0.424
17	0.946	10	0.624	0.956	10	0.740	0.864	10	0.085	0.920	10	0.356
18	0.952	10	0.692	0.961	10	0.798	0.842	10	0.047	0.907	10	0.259
19	0.956	10	0.736	0.964	10	0.827	0.835	10	0.038	0.903	10	0.235
20	0.945	10	0.607	0.967	10	0.866	0.831	10	0.035	0.902	10	0.232
21	0.952	10	0.696	0.964	10	0.829	0.845	10	0.050	0.903	10	0.239
22	0.953	10	0.708	0.967	10	0.861	0.856	10	0.068	0.909	10	0.272
23	0.959	10	0.776	0.967	10	0.857	0.847	10	0.054	0.899	10	0.213
24	0.955	10	0.727	0.974	10	0.926	0.842	10	0.046	0.900	10	0.217
25	0.953	10	0.703	0.962	10	0.809	0.847	10	0.054	0.900	10	0.218
26	0.948	10	0.642	0.967	10	0.858	0.873	10	0.108	0.895	10	0.194
27	0.954	10	0.711	0.969	10	0.883	0.896	10	0.197	0.887	10	0.159
28	0.950	10	0.669	0.970	10	0.886	0.932	10	0.469	0.884	10	0.146
29	0.957	10	0.752	0.969	10	0.880	0.940	10	0.549	0.897	10	0.202
30	0.954	10	0.716	0.968	10	0.869	0.935	10	0.495	0.892	10	0.181
31	0.950	10	0.664	0.967	10	0.863	0.932	10	0.468	0.903	10	0.236
32	0.932	10	0.464	0.969	10	0.877	0.928	10	0.428	0.898	10	0.208
33	0.931	10	0.462	0.969	10	0.882	0.917	10	0.335	0.895	10	0.192
34	0.931	10	0.453	0.969	10	0.885	0.908	10	0.270	0.899	10	0.215
35	0.927	10	0.415	0.969	10	0.879	0.908	10	0.270	0.905	10	0.250
36	0.919	10	0.349	0.973	10	0.916	0.876	10	0.116	0.905	10	0.247
37	0.922	10	0.374	0.969	10	0.882	0.888	10	0.160	0.911	10	0.285
38	0.913	10	0.305	0.967	10	0.859	0.859	10	0.075	0.914	10	0.310

Table 4: presents the distribution of data obtained from **RH readings** at the different locations and seasons using individual data measurements (Shapiro-Wilk Normality Test)

Time (/min)	Arch R.H. in Summer			Arch R.H. in Winter			Toe R.H. in Summer			Toe R.H. in Winter		
	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value	Statistic	df	P-value
0	0.923	10	0.386	0.917	9	0.365	0.951	9	0.701	0.979	9	0.959
1	0.904	10	0.240	0.945	9	0.631	0.938	9	0.564	0.858	9	0.092
2	0.897	10	0.205	0.939	9	0.569	0.908	9	0.301	0.907	9	0.296
3	0.914	10	0.306	0.940	9	0.583	0.878	9	0.150	0.895	9	0.225
4	0.913	10	0.302	0.938	9	0.561	0.906	9	0.287	0.870	9	0.122
5	0.921	10	0.367	0.938	9	0.557	0.882	9	0.166	0.976	9	0.941
6	0.914	10	0.307	0.925	9	0.435	0.887	9	0.186	0.979	9	0.960
7	0.921	10	0.368	0.917	9	0.365	0.889	9	0.196	0.974	9	0.928
8	0.915	10	0.314	0.910	9	0.315	0.877	9	0.146	0.963	9	0.825
9	0.914	10	0.307	0.919	9	0.382	0.908	9	0.305	0.955	9	0.742
10	0.915	10	0.319	0.920	9	0.393	0.913	9	0.337	0.959	9	0.784
11	0.912	10	0.295	0.920	9	0.391	0.915	9	0.353	0.980	9	0.965
12	0.914	10	0.313	0.927	9	0.454	0.910	9	0.315	0.955	9	0.741
13	0.912	10	0.294	0.941	9	0.593	0.914	9	0.347	0.967	9	0.869
14	0.906	10	0.256	0.941	9	0.590	0.919	9	0.382	0.953	9	0.721
15	0.923	10	0.383	0.929	9	0.471	0.902	9	0.263	0.950	9	0.689
16	0.911	10	0.287	0.933	9	0.513	0.894	9	0.221	0.970	9	0.894
17	0.910	10	0.283	0.924	9	0.429	0.901	9	0.259	0.956	9	0.756
18	0.913	10	0.300	0.923	9	0.418	0.906	9	0.291	0.959	9	0.789
19	0.917	10	0.333	0.924	9	0.429	0.905	9	0.285	0.948	9	0.666
20	0.913	10	0.300	0.921	9	0.402	0.917	9	0.365	0.914	9	0.344
21	0.911	10	0.291	0.923	9	0.420	0.910	9	0.319	0.958	9	0.781
22	0.915	10	0.320	0.913	9	0.334	0.906	9	0.286	0.939	9	0.574
23	0.922	10	0.370	0.913	9	0.340	0.917	9	0.370	0.932	9	0.503
24	0.912	10	0.298	0.885	9	0.179	0.905	9	0.283	0.946	9	0.650
25	0.916	10	0.328	0.905	9	0.280	0.907	9	0.295	0.913	9	0.340
26	0.919	10	0.347	0.893	9	0.212	0.910	9	0.313	0.906	9	0.288
27	0.911	10	0.291	0.914	9	0.342	0.913	9	0.336	0.934	9	0.519
28	0.921	10	0.365	0.893	9	0.212	0.916	9	0.361	0.906	9	0.292
29	0.928	10	0.424	0.890	9	0.198	0.928	9	0.460	0.909	9	0.309
30	0.918	10	0.343	0.888	9	0.192	0.934	9	0.525	0.926	9	0.447
31	0.926	10	0.405	0.875	9	0.138	0.926	9	0.442	0.886	9	0.183
32	0.923	10	0.384	0.891	9	0.204	0.926	9	0.441	0.932	9	0.501
33	0.923	10	0.381	0.873	9	0.133	0.935	9	0.529	0.912	9	0.331
34	0.924	10	0.389	0.874	9	0.135	0.923	9	0.414	0.924	9	0.424
35	0.914	10	0.312	0.857	9	0.088	0.917	9	0.369	0.947	9	0.655
36	0.904	10	0.243	0.863	9	0.104	0.912	9	0.327	0.941	9	0.592
37	0.914	10	0.307	0.871	9	0.127	0.915	9	0.352	0.949	9	0.681
38	0.930	10	0.445	0.885	9	0.176	0.926	9	0.448	0.971	9	0.902

Appendix VIII

Summer vs Winter

Healthy Participant Group In-Shoe Temperature ***(Summer vs Winter)***

Table 1: Difference between Seasons in Arch and Toe temperature

		Arch Temperature Analysis				**Toe** Temperature Analysis			
Time /min	Season	Mean Arch Temp. (°C)	Std. Deviation	P-value Non-Parametric test	P-value	Mean Toe Temp. (°C)	Std. Deviation	P-value Non-Parametric test	P-value
0	Summer	32.85	0.681	0.000	0.000	33.64	0.969	0.000	0.000
	Winter	26.60	1.778			25.42	3.363		
1	Summer	32.97	0.764	0.000	0.000	33.65	1.005	0.000	0.000
	Winter	26.84	1.842			25.41	3.200		
2	Summer	33.10	0.841	0.000	0.000	33.64	1.019	0.000	0.000
	Winter	26.96	1.877			25.32	3.171		
3	Summer	33.23	0.874	0.000	0.000	33.66	0.955	0.000	0.000
	Winter	27.10	1.924			25.31	3.108		
4	Summer	33.43	0.945	0.000	0.000	33.80	0.973	0.000	0.000
	Winter	27.27	1.971			25.39	3.116		
5	Summer	33.60	0.970	0.000	0.000	33.86	1.006	0.000	0.000
	Winter	27.46	2.037			25.56	3.150		
6	Summer	33.76	1.007	0.000	0.000	34.00	0.939	0.000	0.000
	Winter	27.64	2.105			25.71	3.187		
7	Summer	33.90	1.020	0.000	0.000	34.09	0.918	0.000	0.000
	Winter	27.83	2.202			25.84	3.230		
8	Summer	34.06	1.033	0.000	0.000	34.28	0.958	0.000	0.000
	Winter	28.01	2.270			26.04	3.317		
9	Summer	34.21	1.054	0.000	0.000	34.41	0.924	0.000	0.000
	Winter	28.20	2.354			26.20	3.379		
10	Summer	34.37	1.069	0.000	0.000	34.54	0.917	0.000	0.000
	Winter	28.39	2.420			26.41	3.454		
11	Summer	34.40	1.039	0.000	0.000	34.68	0.971	0.000	0.000
	Winter	28.54	2.499			26.52	3.509		
12	Summer	34.60	1.053	0.000	0.000	34.90	0.877	0.000	0.000
	Winter	28.75	2.547			26.82	3.565		
13	Summer	34.72	1.042	0.000	0.000	35.00	0.778	0.000	0.000
	Winter	28.92	2.605			27.08	3.792		
14	Summer	34.83	0.983	0.000	0.000	35.07	0.728	0.000	0.000
	Winter	29.12	2.652			27.40	3.811		
15	Summer	34.95	0.968	0.000	0.000	35.21	0.648	0.000	0.000

	Winter	29.29	2.706			27.68	3.891		
16	Summer	35.05	0.960	0.000	0.000	35.33	0.604	0.000	0.000
	Winter	29.50	2.776			27.95	4.048		
17	Summer	35.16	0.953	0.000	0.000	35.43	0.557	0.000	0.000
	Winter	29.70	2.841			28.24	4.172		
18	Summer	35.26	0.948	0.000	0.000	35.53	0.521	0.000	0.000
	Winter	29.92	2.895			28.61	4.324		
19	Summer	35.34	0.928	0.000	0.000	35.53	0.462	0.000	0.000
	Winter	30.11	2.927			28.91	4.437		
20	Summer	35.41	0.913	0.000	0.000	35.65	0.454	0.000	0.000
	Winter	30.30	2.978			29.25	4.532		
21	Summer	35.49	0.893	0.000	0.000	35.68	0.454	0.000	0.000
	Winter	30.50	3.024			29.59	4.617		
22	Summer	35.52	0.855	0.000	0.000	35.65	0.454	0.000	0.000
	Winter	30.71	3.062			29.92	4.644		
23	Summer	35.62	0.828	0.000	0.000	35.74	0.416	0.000	0.000
	Winter	30.94	3.080			30.30	4.663		
24	Summer	35.72	0.794	0.000	0.000	35.88	0.390	0.000	0.000
	Winter	31.14	3.112			30.56	4.717		
25	Summer	35.78	0.762	0.000	0.000	35.88	0.385	0.000	0.000
	Winter	31.35	3.127			30.86	4.729		
26	Summer	35.83	0.721	0.000	0.000	35.91	0.354	0.000	0.000
	Winter	31.55	3.158			31.13	4.734		
27	Summer	35.89	0.696	0.000	0.000	35.98	0.349	0.000	0.000
	Winter	31.73	3.173			31.37	4.747		
28	Summer	35.94	0.692	0.000	0.000	36.02	0.421	0.000	0.000
	Winter	31.90	3.177			31.58	4.712		
29	Summer	35.99	0.657	0.000	0.000	36.04	0.378	0.000	0.000
	Winter	32.06	3.194			31.84	4.661		
30	Summer	36.02	0.650	0.000	0.000	36.07	0.370	0.000	0.000
	Winter	32.21	3.189			32.12	4.636		
31	Summer	36.04	0.609	0.000	0.000	36.06	0.379	0.000	0.000
	Winter	32.35	3.168			32.35	4.612		
32	Summer	36.07	0.598	0.000	0.000	36.04	0.364	0.000	0.001
	Winter	32.47	3.167			32.58	4.569		
33	Summer	36.07	0.571	0.000	0.000	35.96	0.400	0.000	0.001
	Winter	32.58	3.144			32.68	4.506		
34	Summer	36.10	0.572	0.000	0.000	36.10	0.480	0.000	0.001
	Winter	32.74	3.123			32.91	4.505		
35	Summer	36.13	0.550	0.000	0.000	36.09	0.377	0.000	0.002
	Winter	32.84	3.110			33.02	4.501		
36	Summer	36.16	0.535	0.000	0.000	36.09	0.431	0.001	0.003
	Winter	32.96	3.076			33.25	4.416		
37	Summer	36.15	0.516	0.000	0.000	36.07	0.440	0.003	0.004
	Winter	33.09	3.010			33.39	4.345		
38	Summer	36.18	0.503	0.000	0.000	36.12	0.371	0.003	0.005
	Winter	33.20	2.950			33.53	4.239		

Healthy Participant Group In-Shoe Relative Humidity ***(Summer vs Winter)***

Table 2: Difference between Seasons in Arch and Toe Relative Humidity

		Arch RH Analysis				**Toe** RH Analysis			
Time /min	Season	Mean Arch Temp. (%)	Std. Deviation	P-value Non-Parametric test	P-value	Mean Toe Temp. (%)	Std. Deviation	P-value Non-Parametric test	P-value
0	Summer	71.41	8.787	0.159	0.169	72.68	8.419	0.081	0.092
	Winter	68.30	7.881			68.65	8.658		
1	Summer	71.31	8.888	0.213	0.334	75.30	7.841	0.041	0.062
	Winter	69.06	8.450			71.08	8.438		
2	Summer	71.40	9.235	0.162	0.311	76.00	8.362	0.123	0.241
	Winter	68.91	8.851			73.16	8.860		
3	Summer	71.34	9.637	0.232	0.374	76.67	8.773	0.109	0.208
	Winter	69.03	9.419			73.46	9.486		
4	Summer	71.53	9.556	0.179	0.314	77.11	8.951	0.165	0.270
	Winter	68.93	9.574			74.27	9.579		
5	Summer	72.09	9.691	0.128	0.233	77.87	9.205	0.098	0.205
	Winter	68.99	9.610			74.45	10.203		
6	Summer	72.48	10.137	0.169	0.260	79.08	9.433	0.054	0.111
	Winter	69.34	10.107			74.51	11.058		
7	Summer	72.85	10.300	0.114	0.189	79.80	9.732	0.036	0.085
	Winter	69.17	10.200			74.91	10.761		
8	Summer	73.17	10.474	0.086	0.127	80.66	9.891	0.030	0.051
	Winter	68.87	10.132			74.92	11.219		
9	Summer	73.39	10.686	0.077	0.123	81.23	10.136	0.028	0.036
	Winter	68.96	10.280			74.64	12.272		
10	Summer	73.42	11.102	0.126	0.129	81.86	10.492	0.052	0.043
	Winter	68.95	10.370			75.38	12.232		
11	Summer	73.70	10.763	0.074	0.076	82.49	10.777	0.089	0.057
	Winter	68.52	10.666			76.05	12.577		
12	Summer	74.07	10.818	0.071	0.061	82.85	10.879	0.055	0.042
	Winter	68.56	10.755			76.20	12.278		
13	Summer	74.40	10.869	0.062	0.080	83.42	11.087	0.066	0..046
	Winter	69.16	11.068			76.53	13.129		
14	Summer	74.29	11.019	0.085	0.116	83.38	11.109	0.064	0.048
	Winter	69.56	11.163			76.41	14.067		
15	Summer	74.40	11.295	0.108	0.145	83.64	10.949	0.083	0.070
	Winter	69.87	11.616			77.20	14.518		
16	Summer	74.27	11.768	0.132	0.147	84.19	11.050	0.105	0.078
	Winter	69.59	12.007			77.78	14.808		
17	Summer	74.72	11.330	0.123	0.126	84.52	11.273	0.087	0.063
	Winter	69.90	11.847			77.70	14.963		
18	Summer	74.86	11.604	0.145	0.170	85.08	11.536	0.137	0.103
	Winter	70.35	12.662			79.11	14.753		
	Summer	75.00	11.556	0.159	0.181	85.18	10.959	0.151	0.113

19	Winter	70.59	12.787			79.44	15.033		
20	Summer	75.21	11.659	0.149	0.177	85.35	11.235	0.151	0.112
	Winter	70.70	12.988			79.51	15.183		
21	Summer	75.02	11.899	0.174	0.201	85.20	11.483	0.161	0.155
	Winter	70.63	13.458			79.86	15.551		
22	Summer	75.03	11.995	0.179	0.204	85.20	11.280	0.205	0.223
	Winter	70.70	13.201			80.33	16.812		
23	Summer	75.37	12.366	0.225	0.229	85.61	11.408	0.059	0.092
	Winter	71.14	13.591			79.17	15.779		
24	Summer	75.83	12.568	0.201	0.184	85.90	11.325	0.094	0.144
	Winter	71.10	13.460			80.14	16.678		
25	Summer	76.37	12.310	0.213	0.161	85.56	11.131	0.083	0.166
	Winter	71.37	13.735			80.15	16.836		
26	Summer	75.80	12.705	0.287	0.244	85.83	11.123	0.123	0.167
	Winter	71.60	13.968			80.50	16.510		
27	Summer	76.54	12.695	0.213	0.175	85.60	11.388	0.128	0.197
	Winter	71.61	14.123			80.56	16.596		
28	Summer	76.91	12.791	0.162	0.134	85.34	12.220	0.149	0.221
	Winter	71.28	14.587			80.34	16.984		
29	Summer	77.19	12.906	0.130	0.097	85.91	11.757	0.146	0.162
	Winter	70.94	14.459			80.41	16.467		
30	Summer	77.19	12.941	0.134	0.127	85.80	11.803	0.128	0.177
	Winter	71.33	14.975			80.41	16.898		
31	Summer	78.29	12.016	0.083	0.072	86.22	11.671	0.080	0.128
	Winter	71.50	15.061			79.96	17.688		
32	Summer	78.47	12.058	0.064	0.063	86.25	11.578	0.066	0.138
	Winter	71.38	15.045			80.04	18.193		
33	Summer	77.96	12.319	0.066	0.065	86.34	11.710	0.179	0.282
	Winter	71.03	14.764			81.79	18.030		
34	Summer	77.27	13.483	0.128	0.100	86.55	11.691	0.058	0.125
	Winter	70.92	14.886			80.30	17.199		
35	Summer	78.52	12.687	0.045	0.046	86.79	11.653	0.117	0.194
	Winter	70.85	14.735			81.27	18.490		
36	Summer	78.30	12.380	0.072	0.063	86.92	11.771	0.097	0.175
	Winter	71.19	15.188			81.28	17.959		
37	Summer	78.96	12.275	0.047	0.044	86.96	11.393	0.107	0.212
	Winter	71.06	15.469			81.65	18.303		
38	Summer	77.84	13.162	0.096	0.082	87.19	11.481	0.100	0.204
	Winter	70.93	15.635			81.82	18.133		

DM Participant Group In-Shoe Temperature ***(Summer vs Winter)***

Table 3: Difference between Seasons in Arch and Toe temperature

		Arch Temperature Analysis				**Toe** Temperature Analysis			
Time (/min)	Season	Mean Arch Temp. (°C)	Std. Deviation	P-value Non-Parametric test	P-value	Mean Toe Temp. (°C)	Std. Deviation	P-value Non-Parametric test	P-value
0	Summer	28.93	1.055	0.000	0.000	28.16	1.522	0.003	0.001
	Winter	25.75	1.275			24.79	2.341		
1	Summer	28.82	1.008	0.000	0.000	28.12	1.718	0.000	0.001
	Winter	25.67	1.304			24.40	2.204		
2	Summer	28.86	1.027	0.000	0.000	28.17	1.722	0.001	0.000
	Winter	25.74	1.415			24.39	2.114		
3	Summer	28.93	1.027	0.000	0.000	28.24	1.707	0.001	0.000
	Winter	25.80	1.392			24.47	2.060		
4	Summer	29.05	1.008	0.000	0.000	28.36	1.700	0.001	0.000
	Winter	25.98	1.571			24.58	2.149		
5	Summer	29.15	1.020	0.000	0.000	28.49	1.700	0.000	0.000
	Winter	26.04	1.561			24.67	2.032		
6	Summer	29.24	0.993	0.000	0.000	28.52	1.740	0.001	0.000
	Winter	26.15	1.580			24.78	2.030		
7	Summer	29.36	0.984	0.000	0.000	28.68	1.814	0.000	0.000
	Winter	26.24	1.594			24.87	2.018		
8	Summer	29.46	0.970	0.000	0.000	28.73	1.866	0.000	0.000
	Winter	26.30	1.683			24.86	2.057		
9	Summer	29.59	1.021	0.000	0.000	28.91	1.907	0.000	0.000
	Winter	26.45	1.693			25.02	2.077		
10	Summer	29.71	1.011	0.000	0.000	29.06	1.976	0.000	0.000
	Winter	26.54	1.700			25.09	2.114		
11	Summer	29.82	1.063	0.000	0.000	29.21	1.983	0.000	0.000
	Winter	26.61	1.722			25.11	2.222		
12	Summer	30.00	1.072	0.000	0.000	29.38	1.980	0.000	0.000
	Winter	26.74	1.722			25.25	2.287		
13	Summer	30.12	1.081	0.000	0.000	29.56	2.000	0.000	0.000
	Winter	26.83	1.807			25.38	2.348		
14	Summer	30.27	1.115	0.000	0.000	29.84	2.062	0.000	0.000
	Winter	26.94	1.838			25.49	2.443		
15	Summer	30.38	1.089	0.000	0.000	29.96	2.061	0.001	0.001
	Winter	27.04	1.879			25.63	2.520		
16	Summer	30.48	1.126	0.000	0.000	30.00	2.061	0.000	0.001
	Winter	27.05	1.943			25.61	2.684		
17	Summer	30.67	1.161	0.000	0.000	30.31	2.099	0.001	0.001
	Winter	27.21	1.992			25.95	2.760		
18	Summer	30.81	1.201	0.000	0.000	30.54	2.199	0.001	0.001
	Winter	27.36	2.035			26.11	2.920		
19	Summer	30.94	1.242	0.000	0.000	30.73	2.263	0.002	0.002
	Winter	27.45	2.069			26.28	3.030		
20	Summer	31.11	1.294	0.000	0.000	30.88	2.189	0.003	0.002
	Winter	27.55	2.114			26.46	3.181		
21	Summer	31.23	1.303	0.001	0.000	31.06	2.183	0.003	0.002
	Winter	27.64	2.131			26.57	3.262		
22	Summer	31.40	1.343	0.001	0.000	31.25	2.158	0.004	0.002
	Winter	27.75	2.215			26.72	3.382		

23	Summer	31.60	1.405	0.001	0.000	31.58	2.096	0.004	0.002
	Winter	27.91	2.249			26.90	3.421		
24	Summer	31.74	1.415	0.001	0.000	31.79	2.051	0.007	0.002
	Winter	27.99	2.227			27.14	3.493		
25	Summer	31.86	1.451	0.002	0.000	32.00	2.046	0.007	0.002
	Winter	28.13	2.257			27.34	3.585		
26	Summer	31.99	1.488	0.002	0.000	32.25	2.018	0.007	0.002
	Winter	28.24	2.347			27.46	3.732		
27	Summer	32.14	1.496	0.002	0.000	32.51	1.958	0.007	0.002
	Winter	28.37	2.397			27.64	3.767		
28	Summer	32.29	1.524	0.002	0.001	32.71	1.893	0.007	0.002
	Winter	28.50	2.407			27.86	3.837		
29	Summer	32.46	1.539	0.002	0.001	33.05	1.870	0.005	0.002
	Winter	28.61	2.441			28.00	3.883		
30	Summer	32.58	1.555	0.002	0.000	33.25	1.885	0.005	0.001
	Winter	28.72	2.431			28.13	3.836		
31	Summer	32.72	1.561	0.002	0.001	33.53	1.835	0.004	0.001
	Winter	28.85	2.499			28.27	3.796		
32	Summer	32.80	1.564	0.002	0.001	33.61	1.862	0.003	0.001
	Winter	28.94	2.540			28.31	3.923		
33	Summer	32.97	1.574	0.002	0.001	33.92	1.802	0.003	0.001
	Winter	29.05	2.548			28.51	3.893		
34	Summer	33.10	1.601	0.002	0.001	34.10	1.806	0.003	0.001
	Winter	29.18	2.590			28.60	3.908		
35	Summer	33.20	1.589	0.002	0.001	34.20	1.744	0.003	0.002
	Winter	29.30	2.615			28.80	3.990		
36	Summer	33.31	1.590	0.001	0.001	34.39	1.743	0.003	0.002
	Winter	29.40	2.623			28.93	3.960		
37	Summer	33.41	1.645	0.001	0.001	34.54	1.793	0.002	0.001
	Winter	29.56	2.612			29.03	3.917		
38	Summer	33.56	1.641	0.001	0.001	34.74	1.794	0.002	0.001
	Winter	29.66	2.621			29.13	3.923		

DM Participant Group In-Shoe Relative Humidity ***(Summer vs Winter)***

Table 4: Difference between Seasons in Arch and Toe temperature

		Arch RH Analysis				**Toe** RH Analysis			
Time **(/min)**	Season	Mean Arch RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value	Mean Toe RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value
0	Summer	69.52	11.334	1.000	0.982	65.14	9.810	0.853	0.972
	Winter	69.43	7.067			65.26	3.127		
1	Summer	69.41	13.418	0.853	0.843	70.74	8.093	0.218	0.659
	Winter	70.45	9.186			69.44	4.217		
2	Summer	70.11	13.915	0.912	0.868	72.12	8.614	0.353	0.709
	Winter	71.00	9.200			70.96	4.354		
3	Summer	70.21	14.960	0.971	0.947	73.19	9.139	0.190	0.569
	Winter	70.58	9.403			71.36	3.746		
4	Summer	71.14	15.110	0.796	0.939	73.72	9.241	0.280	0.522
	Winter	70.70	9.528			71.65	3.522		
5	Summer	71.20	15.585	0.684	0.855	74.52	9.250	0.165	0.416
	Winter	70.12	9.494			71.90	3.214		
6	Summer	71.67	15.751	0.739	0.854	74.93	9.550	0.165	0.456
	Winter	70.58	9.798			72.46	3.259		
7	Summer	71.64	15.827	0.631	0.849	75.37	9.484	0.143	0.412
	Winter	70.50	10.137			72.63	3.731		
8	Summer	71.86	16.180	0.684	0.853	75.77	9.987	0.165	0.418
	Winter	70.73	10.138			72.95	4.086		
9	Summer	72.16	16.302	0.684	0.836	76.33	10.280	0.143	0.422
	Winter	70.90	9.861			73.44	4.199		
10	Summer	72.14	16.392	0.796	0.859	77.09	10.384	0.165	0.401
	Winter	71.04	10.334			74.00	4.638		
11	Summer	72.73	17.201	0.853	0.786	77.61	10.786	0.165	0.417
	Winter	71.00	9.978			74.51	4.873		
12	Summer	73.00	17.048	0.912	0.830	77.92	10.930	0.190	0.411
	Winter	71.64	10.176			74.73	4.863		
13	Summer	73.41	16.872	0.912	0.775	78.51	11.134	0.190	0.377
	Winter	71.59	10.419			75.01	4.968		
14	Summer	73.77	17.022	1.000	0.778	78.86	10.920	0.393	0.534
	Winter	71.97	10.367			76.37	5.858		
15	Summer	74.44	16.774	0.853	0.716	79.01	11.301	0.529	0.625
	Winter	72.13	10.517			76.99	6.044		
16	Summer	74.05	17.114	0.912	0.782	78.86	11.668	0.529	0.688
	Winter	72.26	10.722			77.13	6.733		
17	Summer	74.52	17.108	1.000	0.781	79.29	11.412	0.631	0.707
	Winter	72.71	10.814			77.69	6.701		
18	Summer	74.91	16.807	0.971	0.792	79.68	11.512	0.579	0.726
	Winter	73.21	10.988			78.16	6.940		
19	Summer	75.08	17.129	0.971	0.819	80.13	11.585	0.579	0.623
	Winter	73.59	10.932			78.01	6.768		
20	Summer	75.15	17.210	0.971	0.830	79.96	11.980	0.631	0.857
	Winter	73.74	11.017			79.14	7.587		
21	Summer	75.27	17.601	0.971	0.807	80.32	12.255	0.579	0.729
	Winter	73.64	11.083			78.73	7.167		
22	Summer	75.28	17.599	1.000	0.848	80.41	12.281	0.631	0.793
	Winter	73.99	11.431			79.20	7.288		

23	Summer	76.08	17.762	0.912	0.800	80.54	12.756	0.739	0.881
	Winter	74.38	11.188			79.83	7.492		
24	Summer	75.76	17.815	0.912	0.882	80.91	13.189	0.842	0.829
	Winter	74.75	11.558			79.81	7.496		
25	Summer	76.14	17.483	0.971	0.825	81.11	13.285	0.905	0.988
	Winter	74.66	11.302			81.03	8.504		
26	Summer	76.61	17.575	0.912	0.784	81.33	13.487	0.968	0.995
	Winter	74.77	11.480			81.37	8.749		
27	Summer	76.11	18.104	1.000	0.870	81.37	14.025	0.905	0.915
	Winter	74.99	11.390			80.79	8.149		
28	Summer	76.57	18.129	1.000	0.888	81.47	13.822	0.905	0.984
	Winter	75.60	11.693			81.58	8.855		
29	Summer	76.54	17.544	0.912	0.962	81.77	13.827	1.000	0.987
	Winter	76.22	12.232			81.68	9.119		
30	Summer	76.46	17.578	0.912	0.925	81.95	14.058	1.000	0.887
	Winter	75.81	12.104			81.16	8.701		
31	Summer	76.61	17.318	0.912	0.973	82.02	13.919	1.000	0.978
	Winter	76.38	12.143			81.86	9.012		
32	Summer	76.18	17.585	1.000	0.999	81.60	14.102	1.000	0.967
	Winter	76.17	11.510			81.37	8.919		
33	Summer	76.76	17.726	0.971	0.959	81.47	13.918	0.968	0.892
	Winter	76.41	11.770			82.22	9.263		
34	Summer	76.77	17.702	0.971	0.974	81.80	14.744	0.968	0.964
	Winter	76.54	11.833			82.07	9.254		
35	Summer	76.62	18.339	0.971	0.973	79.24	13.378	0.796	0.679
	Winter	76.39	11.836			81.51	9.086		
36	Summer	76.44	18.751	0.912	0.922	81.31	15.085	0.968	0.933
	Winter	75.75	11.805			81.81	9.597		
37	Summer	74.93	17.910	0.905	0.934	79.40	13.683	0.853	0.686
	Winter	75.52	12.073			81.53	9.032		
38	Summer	75.12	18.539	0.842	0.995	79.84	13.504	0.912	0.900
	Winter	75.07	11.826			80.49	9.108		

Appendix IX

Left vs Right Foot

Summer/Toe/Temperature (Healthy)

Summer Temperature Toe Analysis (Healthy Group, **Left vs Right**)

Table 1: Summer Temperature Analysis (Healthy Participant Group)

Time (/**min**)	Location	Mean Temperature (ºC)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	33.43	.850	0.401	0.348
	Right	33.81	1.061		
1	Left	33.41	.971	0.339	0.301
	Right	33.85	1.028		
2	Left	33.37	.951	0.311	0.230
	Right	33.88	1.052		
3	Left	33.42	.915	0.369	0.267
	Right	33.86	.976		
4	Left	33.55	.943	0.339	0.267
	Right	34.01	.986		
5	Left	33.56	1.012	0.284	0.193
	Right	34.11	.970		
6	Left	33.78	.921	0.339	0.301
	Right	34.18	.948		
7	Left	33.81	.913	0.259	0.173
	Right	34.33	.887		
8	Left	34.05	1.024	0.434	0.294
	Right	34.47	.894		
9	Left	34.17	.985	0.369	0.244
	Right	34.62	.853		
10	Left	34.29	.945	0.401	0.215
	Right	34.76	.869		
11	Left	34.38	.977	0.259	0.172
	Right	34.93	.928		
12	Left	34.59	.925	0.259	0.122
	Right	35.15	.780		
13	Left	34.75	.794	0.311	0.151
	Right	35.21	.727		
14	Left	34.83	.725	0.284	0.143
	Right	35.27	.695		
15	Left	34.98	.642	0.235	0.116
	Right	35.40	.612		
16	Left	35.15	.666	0.284	0.195
	Right	35.47	.526		

17	Left	35.24	.606	0.235	0.136
	Right	35.59	.579		
18	Left	35.31	.556	0.199	0.158
	Right	35.71	.527		
19	Left	35.29	.544	0.140	0.115
	Right	35.73	.581		
20	Left	35.45	.506	0.187	0.147
	Right	35.81	.540		
21	Left	35.41	.579	0.110	0.105
	Right	35.90	.596		
22	Left	35.41	.509	0.140	0.111
	Right	35.86	.577		
23	Left	35.50	.458	0.116	0.108
	Right	35.94	.556		
24	Left	35.69	.425	0.140	0.129
	Right	36.03	.588		
25	Left	35.62	.419	0.102	0.100
	Right	36.10	.485		
26	Left	35.70	.418	0.110	0.104
	Right	36.09	.479		
27	Left	35.76	.432	0.102	0.102
	Right	36.17	.446		
28	Left	35.79	.477	0.106	0.108
	Right	36.22	.437		
29	Left	35.83	.466	0.108	0.107
	Right	36.23	.489		
30	Left	35.85	.424	0.107	0.104
	Right	36.26	.403		
31	Left	35.87	.459	0.122	0.117
	Right	36.23	.422		
32	Left	35.81	.448	0.105	0.102
	Right	36.24	.433		
33	Left	35.82	.422	0.256	0.207
	Right	36.08	.430		
34	Left	35.95	.516	0.168	0.286
	Right	36.22	.430		
35	Left	35.89	.460	0.107	0.112
	Right	36.27	.483		
36	Left	35.86	.419	0.112	0.148
	Right	36.29	.425		
37	Left	35.87	.406	0.146	0.136
	Right	36.25	.405		
38	Left	35.92	.453	0.114	0.143
	Right	36.28	.481		

Winter/Toe/Temperature (Healthy)

Winter Temperature Analysis (Healthy Participant Group, **Left vs Right**)

Table 2: Winter Temperature Analysis (Healthy Participant Group)

Time (/**min**)	Location	Mean Temperature (**ºC**)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	25.43	3.155	0.939	0.991
	Right	25.41	3.688		
1	Left	25.43	3.035	0.898	0.978
	Right	25.39	3.481		
2	Left	25.39	3.015	0.817	0.918
	Right	25.26	3.443		
3	Left	25.37	2.936	0.858	0.916
	Right	25.24	3.390		
4	Left	25.47	2.960	0.939	0.895
	Right	25.30	3.384		
5	Left	25.69	3.001	0.898	0.848
	Right	25.44	3.410		
6	Left	25.86	3.049	0.858	0.823
	Right	25.57	3.438		
7	Left	25.99	3.110	0.858	0.826
	Right	25.70	3.468		
8	Left	26.19	3.202	0.739	0.813
	Right	25.88	3.551		
9	Left	26.39	3.258	0.701	0.781
	Right	26.01	3.619		
10	Left	26.63	3.351	0.663	0.758
	Right	26.20	3.677		
11	Left	26.77	3.425	0.626	0.725
	Right	26.27	3.714		
12	Left	27.11	3.481	0.663	0.683
	Right	26.52	3.765		
13	Left	27.40	3.712	0.590	0.668
	Right	26.75	3.993		
14	Left	27.77	3.740	0.555	0.629
	Right	27.03	3.995		
15	Left	28.13	3.816	0.522	0.569
	Right	27.24	4.067		
16	Left	28.41	4.051	0.522	0.571
	Right	27.49	4.154		
17	Left	28.72	4.202	0.489	0.565
	Right	27.75	4.255		
18	Left	29.13	4.351	0.427	0.553
	Right	28.10	4.409		
19	Left	29.44	4.490	0.369	0.552
	Right	28.38	4.499		
20	Left	29.78	4.598	0.427	0.562
	Right	28.72	4.586		
21	Left	30.15	4.682	0.343	0.549
	Right	29.04	4.671		

22	Left	30.47	4.726	0.397	0.558
	Right	29.37	4.685		
23	Left	30.84	4.717	0.457	0.564
	Right	29.76	4.735		
24	Left	31.05	4.805	0.343	0.605
	Right	30.07	4.770		
25	Left	31.32	4.820	0.427	0.628
	Right	30.40	4.786		
26	Left	31.58	4.823	0.427	0.640
	Right	30.68	4.796		
27	Left	31.78	4.851	0.397	0.669
	Right	30.96	4.801		
28	Left	31.97	4.828	0.457	0.684
	Right	31.19	4.755		
29	Left	32.17	4.788	0.489	0.723
	Right	31.50	4.701		
30	Left	32.43	4.760	0.457	0.736
	Right	31.80	4.679		
31	Left	32.66	4.750	0.397	0.740
	Right	32.04	4.641		
32	Left	32.86	4.679	0.427	0.755
	Right	32.29	4.627		
33	Left	32.95	4.602	0.457	0.768
	Right	32.41	4.578		
34	Left	33.17	4.604	0.343	0.774
	Right	32.65	4.576		
35	Left	33.27	4.606	0.317	0.779
	Right	32.76	4.567		
36	Left	33.52	4.501	0.369	0.767
	Right	32.99	4.496		
37	Left	33.65	4.447	0.293	0.763
	Right	33.12	4.406		
38	Left	33.76	4.362	0.369	0.782
	Right	33.29	4.276		

Summer/Arch/Temperature (Healthy)

Summer Temperature Arch Analysis (Healthy Group, **Left vs Right**)

Table 1: Summer Temperature Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean Temperature **(ºC)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	32.77	.587	0.815	0.516
	Right	32.97	.820		
1	Left	32.88	.683	0.483	0.536
	Right	33.09	.895		
2	Left	33.01	.736	0.616	0.568
	Right	33.23	1.006		
3	Left	33.15	.783	0.713	0.649
	Right	33.33	1.031		
4	Left	33.33	.830	0.616	0.561
	Right	33.57	1.128		
5	Left	33.49	.860	0.764	0.565
	Right	33.74	1.148		
6	Left	33.67	.880	0.867	0.619
	Right	33.89	1.211		
7	Left	33.80	.901	0.920	0.603
	Right	34.04	1.213		
8	Left	33.96	.914	0.920	0.618
	Right	34.20	1.230		
9	Left	34.13	.928	0.920	0.653
	Right	34.34	1.262		
10	Left	34.28	.939	0.815	0.633
	Right	34.51	1.282		
11	Left	34.29	.926	0.867	0.584
	Right	34.55	1.226		
12	Left	34.46	.944	0.616	0.474
	Right	34.79	1.224		
13	Left	34.59	.927	0.664	0.502
	Right	34.90	1.223		
14	Left	34.73	.859	0.713	0.568
	Right	34.98	1.178		
15	Left	34.84	.856	0.616	0.552
	Right	35.10	1.147		
16	Left	34.95	.847	0.570	0.559
	Right	35.20	1.140		
17	Left	35.06	.852	0.570	0.544
	Right	35.32	1.119		
18	Left	35.16	.850	0.443	0.538
	Right	35.42	1.108		
19	Left	35.23	.845	0.526	0.541
	Right	35.49	1.070		
20	Left	35.31	.829	0.443	0.539
	Right	35.56	1.056		
21	Left	35.39	.809	0.333	0.525
	Right	35.64	1.034		

22	Left	35.45	.794	0.616	0.636
	Right	35.63	.977		
23	Left	35.53	.768	0.443	0.562
	Right	35.75	.939		
24	Left	35.62	.733	0.333	0.513
	Right	35.85	.902		
25	Left	35.68	.709	0.333	0.469
	Right	35.92	.855		
26	Left	35.75	.676	0.404	0.529
	Right	35.95	.808		
27	Left	35.81	.661	0.483	0.521
	Right	36.01	.768		
28	Left	35.85	.643	0.367	0.481
	Right	36.07	.778		
29	Left	35.90	.625	0.367	0.443
	Right	36.12	.717		
30	Left	35.92	.610	0.333	0.384
	Right	36.17	.714		
31	Left	35.95	.578	0.367	0.404
	Right	36.17	.663		
32	Left	35.98	.557	0.333	0.408
	Right	36.20	.663		
33	Left	36.01	.532	0.570	0.531
	Right	36.17	.643		
34	Left	36.03	.532	0.526	0.505
	Right	36.20	.644		
35	Left	36.06	.508	0.442	0.469
	Right	36.24	.621		
36	Left	36.08	.490	0.333	0.431
	Right	36.27	.607		
37	Left	36.10	.477	0.483	0.566
	Right	36.23	.588		
38	Left	36.11	.472	0.442	0.469
	Right	36.28	.559		

Winter/Arch/Temperature (Healthy)

Winter Temperature Arch Analysis (Healthy Participant Group, **Left vs Right**)

Table 2: Winter Temperature Analysis (Healthy Participant Group)					
Time (/**min**)	Location	Mean Temperature (**ºC**)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	26.62	1.471	0.980	0.964
	Right	26.59	2.103		
1	Left	26.87	1.562	0.980	0.946
	Right	26.82	2.152		
2	Left	27.04	1.577	0.898	0.838
	Right	26.89	2.200		
3	Left	27.22	1.625	0.980	0.772
	Right	26.99	2.245		
4	Left	27.39	1.670	0.898	0.757
	Right	27.15	2.295		
5	Left	27.59	1.741	0.939	0.742
	Right	27.32	2.362		
6	Left	27.79	1.826	0.980	0.731
	Right	27.50	2.419		
7	Left	28.00	1.926	0.939	0.712
	Right	27.67	2.517		
8	Left	28.18	2.021	0.858	0.719
	Right	27.85	2.567		
9	Left	28.36	2.110	0.739	0.728
	Right	28.03	2.652		
10	Left	28.55	2.180	0.739	0.748
	Right	28.23	2.719		
11	Left	28.74	2.262	0.817	0.699
	Right	28.35	2.795		
12	Left	28.89	2.336	0.858	0.789
	Right	28.61	2.831		
13	Left	29.06	2.392	0.817	0.782
	Right	28.77	2.894		
14	Left	29.23	2.457	0.817	0.827
	Right	29.00	2.931		
15	Left	29.41	2.523	0.939	0.833
	Right	29.18	2.977		
16	Left	29.60	2.615	0.858	0..851
	Right	29.39	3.031		
17	Left	29.79	2.685	0.898	0.881
	Right	29.62	3.096		
18	Left	29.99	2.745	0.858	0.914
	Right	29.86	3.149		
19	Left	30.16	2.772	0.817	0.933
	Right	30.06	3.187		
20	Left	30.33	2.827	0.858	0.964
	Right	30.28	3.238		
21	Left	30.50	2.877	0.939	0.991
	Right	30.49	3.283		

22	Left	30.70	2.922	0.939	0.996
	Right	30.71	3.316		
23	Left	30.89	2.926	0.858	0.934
	Right	30.99	3.346		
24	Left	31.07	2.929	0.817	0.919
	Right	31.20	3.405		
25	Left	31.23	2.950	0.663	0.846
	Right	31.48	3.412		
26	Left	31.41	2.961	0.633	0.824
	Right	31.69	3.459		
27	Left	31.57	2.963	0.701	0.801
	Right	31.89	3.485		
28	Left	31.72	2.954	0.590	0.781
	Right	32.08	3.498		
29	Left	31.87	2.950	0.590	0.765
	Right	32.25	3.531		
30	Left	32.02	2.926	0.590	0.768
	Right	32.40	3.542		
31	Left	32.16	2.886	0.522	0.766
	Right	32.54	3.536		
32	Left	32.26	2.879	0.489	0.748
	Right	32.67	3.539		
33	Left	32.39	2.833	0.522	0.768
	Right	32.77	3.535		
34	Left	32.54	2.784	0.457	0.751
	Right	32.94	3.533		
35	Left	32.65	2.769	0.397	0.762
	Right	33.03	3.522		
36	Left	32.74	2.731	0.270	0.716
	Right	33.19	3.484		
37	Left	32.86	2.648	0.293	0.705
	Right	33.32	3.427		
38	Left	32.99	2.571	0.270	0.716
	Right	33.42	3.379		

Summer/Toe/Relative Humidity (Healthy)

Summer RH Toe Analysis (Healthy Participant Group, **Left vs Right**)

Table 3: Summer RH Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	74.70	8.623	0.215	0.211
	Right	70.66	8.004		
1	Left	77.05	8.453	0.198	0.244
	Right	73.55	7.045		
2	Left	77.58	8.950	0.335	0.327
	Right	74.42	7.730		
3	Left	78.31	9.408	0.291	0.331
	Right	75.02	8.095		
4	Left	78.69	9.645	0.291	0.358
	Right	75.52	8.244		
5	Left	79.37	9.731	0.335	0.400
	Right	76.38	8.745		
6	Left	80.43	9.845	0.358	0.460
	Right	77.73	9.164		
7	Left	81.30	10.049	0.291	0.425
	Right	78.30	9.534		
8	Left	81.81	10.291	0.383	0.548
	Right	79.51	9.719		
9	Left	82.29	10.577	0.435	0.590
	Right	80.18	9.953		
10	Left	82.85	10.797	0.435	0.627
	Right	80.87	10.485		
11	Left	84.26	10.845	0.332	0.421
	Right	80.84	10.847		
12	Left	84.76	10.716	0.286	0.388
	Right	81.07	11.119		
13	Left	85.35	10.774	0.308	0.395
	Right	81.63	11.469		
14	Left	84.39	11.173	0.550	0.642
	Right	82.38	11.371		
15	Left	84.45	10.815	0.646	0.705
	Right	82.84	11.428		
16	Left	84.72	10.979	0.783	0.804
	Right	83.65	11.509		
17	Left	85.09	11.118	0.818	0.795
	Right	83.95	11.817		
18	Left	85.69	11.299	0.783	0.784
	Right	84.47	12.162		
19	Left	86.11	11.042	0.550	0.661
	Right	84.24	11.208		
20	Left	86.35	11.268	0.462	0.646
	Right	84.35	11.533		
21	Left	86.12	11.614	0.646	0.679
	Right	84.28	11.711		

22	Left	86.59	11.319	0.462	0.524
	Right	83.81	11.489		
23	Left	86.84	11.572	0.581	0.577
	Right	84.37	11.537		
24	Left	87.15	11.455	0.520	0.568
	Right	84.65	11.479		
25	Left	86.30	10.912	0.713	0.733
	Right	84.83	11.708		
26	Left	86.82	10.678	0.613	0.647
	Right	84.84	11.868		
27	Left	86.12	11.345	0.713	0.813
	Right	85.07	11.835		
28	Left	85.33	12.835	1.000	0.997
	Right	85.35	12.058		
29	Left	86.38	11.832	0.783	0.836
	Right	85.43	12.108		
30	Left	86.12	11.980	0.890	0.889
	Right	85.48	12.067		
31	Left	86.76	11.709	0.713	0.811
	Right	85.68	12.048		
32	Left	86.80	11.481	0.679	0.806
	Right	85.69	12.081		
33	Left	87.07	11.212	0.783	0.750
	Right	85.62	12.568		
34	Left	87.40	11.137	0.713	0.705
	Right	85.69	12.581		
35	Left	87.85	11.151	0.550	0.638
	Right	85.72	12.459		
36	Left	88.04	11.388	0.491	0.624
	Right	85.80	12.465		
37	Left	88.03	10.892	0.520	0.626
	Right	85.88	12.185		
38	Left	88.33	11.002	0.520	0.611
	Right	86.06	12.246		

Winter/Toe/Relative Humidity (Healthy)

Winter RH Toe Analysis (Healthy Participant Group, **Left vs Right**)

Table 4: Winter Relative Humidity Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	67.39	7.890	1.000	0.533
	Right	69.63	9.388		
1	Left	69.15	6.561	0.537	0.290
	Right	72.73	9.703		
2	Left	70.66	6.892	0.369	0.210
	Right	75.27	10.014		
3	Left	70.52	6.864	0.231	0.139
	Right	76.19	10.957		
4	Left	71.54	6.633	0.301	0.178
	Right	76.78	11.356		
5	Left	71.60	7.003	0.231	0.185
	Right	77.08	12.149		
6	Left	71.30	8.501	0.328	0.168
	Right	77.47	12.591		
7	Left	72.59	7.191	0.572	0.318
	Right	76.90	13.027		
8	Left	72.72	6.877	0.572	0.344
	Right	76.81	13.918		
9	Left	71.86	9.512	0.471	0.293
	Right	77.03	14.135		
10	Left	72.99	8.928	0.584	0.371
	Right	77.27	14.361		
11	Left	74.20	9.193	0.753	0.544
	Right	77.25	14.552		
12	Left	74.54	7.633	0.877	0.517
	Right	77.61	15.359		
13	Left	74.92	8.913	0.870	0.575
	Right	77.79	15.913		
14	Left	73.91	10.222	0.681	0.395
	Right	78.56	16.774		
15	Left	74.86	9.728	0.797	0.440
	Right	79.21	17.773		
16	Left	75.38	10.096	0.870	0.456
	Right	79.68	17.823		
17	Left	74.51	11.873	0.607	0.325
	Right	80.43	17.142		
18	Left	75.73	10.752	0.758	0.272
	Right	82.02	17.351		
19	Left	75.68	10.946	0643	0.230
	Right	82.67	17.575		
20	Left	75.54	10.710	0.572	0.209
	Right	82.91	17.869		
21	Left	75.69	11.266	0.440	0.197
	Right	83.43	18.106		

22	Left	75.78	13.575	0.614	0.277
	Right	83.25	18.477		
23	Left	73.47	8.137	0.381	0.087
	Right	83.65	18.963		
24	Left	75.77	11.905	0.547	0.229
	Right	83.57	19.383		
25	Left	75.77	11.715	0.572	0.210
	Right	83.90	19.901		
26	Left	76.97	11.770	0.797	0.306
	Right	83.52	19.637		
27	Left	77.05	12.334	0.837	0.313
	Right	83.57	19.489		
28	Left	76.75	12.624	0.661	0.334
	Right	83.17	19.758		
29	Left	77.17	11.555	0.758	0.345
	Right	83.20	19.758		
30	Left	76.76	12.685	0.607	0.318
	Right	83.54	19.751		
31	Left	76.28	12.515	0.572	0.320
	Right	83.11	21.118		
32	Left	76.71	13.223	0.607	0.383
	Right	82.89	21.669		
33	Left	78.02	14.039	0.571	0.435
	Right	84.21	20.315		
34	Left	75.12	9.395	0.511	0.157
	Right	84.37	20.922		
35	Left	76.28	14.694	0.352	0.240
	Right	85.19	20.675		
36	Left	77.26	12.503	0.537	0.283
	Right	84.73	21.454		
37	Left	77.65	13.935	0.504	0.298
	Right	85.07	21.275		
38	Left	78.31	12.927	0.572	0.355
	Right	84.82	21.676		

Summer/Arch/Relative Humidity (Healthy)

Summer RH Arch Analysis (Healthy Participant Group, **Left vs Right**)

Table 3: Summer RH Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	73.10	8.381	0.270	0.318
	Right	69.72	9.164		
1	Left	72.60	8.395	0.335	0.453
	Right	70.03	9.487		
2	Left	72.44	8.947	0.438	0.584
	Right	70.44	9.726		
3	Left	72.35	9.146	0.467	0.609
	Right	70.40	10.323		
4	Left	72.30	8.920	0.435	0.678
	Right	70.76	10.431		
5	Left	72.71	9.087	0.462	0.745
	Right	71.48	10.567		
6	Left	73.33	9.574	0.497	0.684
	Right	71.70	10.932		
7	Left	73.66	9.796	0.409	0.700
	Right	72.09	11.060		
8	Left	74.05	9.906	0.438	0.684
	Right	72.36	11.286		
9	Left	74.12	10.263	0.438	0.740
	Right	72.71	11.407		
10	Left	74.50	10.302	0.409	0.637
	Right	72.42	12.096		
11	Left	74.56	9.989	0.581	0.682
	Right	72.85	11.801		
12	Left	74.55	10.198	0.679	0.820
	Right	73.60	11.770		
13	Left	75.00	10.034	0.581	0.775
	Right	73.79	11.995		
14	Left	74.75	10.453	0.713	0.831
	Right	73.83	11.936		
15	Left	74.66	10.638	0.679	0.904
	Right	74.13	12.314		
16	Left	74.81	10.906	0.713	0.814
	Right	73.73	12.964		
17	Left	74.99	10.670	0.890	0.902
	Right	74.44	12.353		
18	Left	75.08	10.938	0.963	0.922
	Right	74.64	12.646		
19	Left	75.27	10.969	0.927	0.905
	Right	74.74	12.526		
20	Left	75.53	11.086	0.963	0.890
	Right	74.90	12.618		
21	Left	75.35	11.131	1.000	0.887
	Right	74.69	13.036		

22	Left	75.20	11.236	0.890	0.942
	Right	74.86	13.135		
23	Left	75.76	11.154	0.890	0.870
	Right	74.97	13.886		
24	Left	75.95	11.285	0.771	0.963
	Right	75.71	14.291		
25	Left	75.97	11.432	0.593	0.864
	Right	76.81	13.650		
26	Left	76.06	11.534	0.818	0.916
	Right	75.54	14.214		
27	Left	76.52	11.482	0.783	0.994
	Right	76.56	14.243		
28	Left	76.19	11.630	0.627	0.765
	Right	77.70	14.376		
29	Left	76.43	11.697	0.593	0. 759
	Right	78.00	14.536		
30	Left	76.37	11.691	0.662	0.741
	Right	78.07	14.600		
31	Left	76.43	11.668	0.411	0.405
	Right	80.46	12.558		
32	Left	76.63	11.694	0.382	0.412
	Right	80.62	12.628		
33	Left	76.43	11.358	0.497	0.513
	Right	79.61	13.540		
34	Left	76.93	11.876	0.783	0.896
	Right	77.61	15.372		
35	Left	76.71	11.684	0.328	0.444
	Right	80.63	13.983		
36	Left	76.85	11.522	0.438	0.539
	Right	79.85	13.534		
37	Left	76.81	11.574	0.280	0.344
	Right	81.48	13.089		
38	Left	76.92	11.545	0.593	0.714
	Right	78.83	15.129		

Winter/Arch/Relative Humidity (Healthy)

Winter RH Arch (Healthy Participant Group, **Left vs Right**)

Table 4: Winter Relative Humidity Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	68.25	7.826	0.818	0.974
	Right	68.35	8.230		
1	Left	69.77	8.142	0.408	0.665
	Right	68.35	8.996		
2	Left	69.70	8.422	0.462	0.644
	Right	68.11	9.509		
3	Left	70.10	8.998	0.383	0.557
	Right	67.96	10.042		
4	Left	70.10	9.192	0.435	0.529
	Right	67.76	10.146		
5	Left	70.24	9.252	0.408	0.502
	Right	67.74	10.142		
6	Left	70.38	9.550	0.467	0.592
	Right	68.23	10.951		
7	Left	70.62	9.743	0.335	0.460
	Right	67.71	10.797		
8	Left	70.22	9.827	0.335	0.489
	Right	67.51	10.615		
9	Left	70.26	9.902	0.358	0.513
	Right	67.65	10.852		
10	Left	70.30	10.049	0.358	0.503
	Right	67.61	10.884		
11	Left	69.92	10.705	0.435	0.498
	Right	67.12	10.838		
12	Left	69.98	10.771	0.462	0.495
	Right	67.14	10.948		
13	Left	70.50	10.935	0.462	0.531
	Right	67.82	11.445		
14	Left	71.20	11.111	0.312	0.446
	Right	67.91	11.380		
15	Left	71.40	11.499	0.335	0.497
	Right	68.35	11.958		
16	Left	71.28	11.629	0.312	0.466
	Right	67.90	12.572		
17	Left	71.85	11.651	0.251	0.393
	Right	67.94	12.145		
18	Left	72.13	12.289	0.312	0.467
	Right	68.57	13.234		
19	Left	72.44	12.485	0.312	0.454
	Right	68.73	13.278		
20	Left	72.63	12.653	0.270	0.441
	Right	68.76	13.498		
21	Left	72.56	13.342	0.408	0.457
	Right	68.69	13.787		

22	Left	72.64	13.159	0.383	0.447
	Right	68.75	13.441		
23	Left	73.39	13.630	0.312	0.391
	Right	68.89	13.674		
24	Left	73.28	13.505	0.335	0.402
	Right	68.93	13.553		
25	Left	73.70	13.864	0.358	0.379
	Right	69.04	13.707		
26	Left	74.02	14.041	0.358	0.369
	Right	69.18	13.980		
27	Left	74.17	14.252	0.358	0.346
	Right	69.04	14.033		
28	Left	73.74	14.842	0.358	0.381
	Right	68.81	14.442		
29	Left	73.46	14.821	0.312	0.367
	Right	68.43	14.175		
30	Left	73.85	15.400	0.335	0.382
	Right	68.80	14.657		
31	Left	73.98	15.243	0.435	0.394
	Right	69.03	15.018		
32	Left	74.16	15.510	0.408	0.337
	Right	68.59	14.590		
33	Left	73.77	15.388	0.358	0.335
	Right	68.29	14.133		
34	Left	73.52	15.650	0.383	0.366
	Right	68.32	14.171		
35	Left	73.47	15.703	0.408	0.358
	Right	68.24	13.772		
36	Left	73.79	16.066	0.462	0.373
	Right	68.58	14.364		
37	Left	73.50	16.545	0.408	0.414
	Right	68.62	14.507		
38	Left	73.40	16.741	0.491	0.412
	Right	68.45	14.638		

Summer/Toe/Temperature (Diabetic)

Summer Temperature Toe Analysis (Diabetic Group, **Left vs Right**)

Table 5: Summer Temperature Analysis (Diabetic Group)					
Time (/**min**)	Location	Mean Temperature (ºC)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	27.57	1.714	0.117	0.241
	Right	28.75	1.186		
1	Left	27.36	1.771	0.117	0.175
	Right	28.88	1.437		
2	Left	27.44	1.783	0.117	0.198
	Right	28.89	1.474		
3	Left	27.52	1.834	0.117	0.198
	Right	28.96	1.377		
4	Left	27.70	1.820	0.175	0.240
	Right	29.02	1.447		
5	Left	27.81	1.796	0.117	0.224
	Right	29.17	1.457		
6	Left	27.83	1.898	0.117	0.227
	Right	29.21	1.416		
7	Left	28.00	1.962	0.117	0.254
	Right	29.37	1.542		
8	Left	28.08	2.061	0.117	0.294
	Right	29.39	1.586		
9	Left	28.24	2.093	0.175	0.297
	Right	29.57	1.644		
10	Left	28.41	2.133	0.175	0.323
	Right	29.72	1.779		
11	Left	28.54	2.151	0.175	0.316
	Right	29.88	1.765		
12	Left	28.80	2.225	0.251	0.390
	Right	29.95	1.744		
13	Left	28.98	2.248	0.251	0.390
	Right	30.14	1.761		
14	Left	29.24	2.254	0.175	0.395
	Right	30.43	1.900		
15	Left	29.33	2.277	0.117	0.366
	Right	30.58	1.840		
16	Left	29.44	2.352	0.251	0.422
	Right	30.57	1.799		
17	Left	29.71	2.391	0.175	0.394
	Right	30.92	1.812		
18	Left	29.93	2.414	0.175	0.409
	Right	31.16	2.028		
19	Left	30.11	2.491	0.175	0.421
	Right	31.35	2.089		
20	Left	30.31	2.452	0.251	0.445
	Right	31.45	1.990		
21	Left	30.46	2.454	0.175	0.420
	Right	31.66	1.952		

22	Left	30.71	2.495	0.175	0.458
	Right	31.79	1.876		
23	Left	31.02	2.351	0.175	0.435
	Right	32.13	1.893		
24	Left	31.18	2.266	0.117	0.381
	Right	32.39	1.846		
25	Left	31.44	2.275	0.347	0.421
	Right	32.56	1.861		
26	Left	31.62	2.253	0.175	0.354
	Right	32.88	1.760		
27	Left	31.89	2.190	0.251	0.346
	Right	33.13	1.696		
28	Left	32.15	2.118	0.251	0.378
	Right	33.27	1.668		
29	Left	32.50	2.083	0.347	0.379
	Right	33.60	1.661		
30	Left	32.73	2.094	0.347	0.415
	Right	33.77	1.713		
31	Left	33.01	2.027	0.347	0.403
	Right	34.05	1.672		
32	Left	33.17	2.044	0.347	0.494
	Right	34.04	1.775		
33	Left	33.51	1.989	0.602	0.507
	Right	34.33	1.713		
34	Left	33.77	2.030	0.602	0.589
	Right	34.44	1.715		
35	Left	33.92	1.994	0.602	0.634
	Right	34.49	1.633		
36	Left	34.09	1.901	0.602	0.606
	Right	34.70	1.728		
37	Left	34.35	2.050	0.754	0.759
	Right	34.73	1.717		
38	Left	34.54	2.022	0.754	0.752
	Right	34.93	1.748		

Winter/Toe/Temperature (Diabetic)

Winter Temperature Toe Analysis (Diabetic Group, **Left vs Right**)

Table 6: Winter Temperature Analysis (Diabetic Group)

Time (/**min**)	Location	Mean Temperature (ºC)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	24.06	2.116	0.347	0.353
	Right	25.52	2.552		
1	Left	23.67	1.725	0.347	0.319
	Right	25.14	2.570		
2	Left	23.62	1.705	0.251	0.272
	Right	25.16	2.377		
3	Left	23.73	1.656	0.251	0.285
	Right	25.20	2.335		
4	Left	23.82	1.788	0.251	0.288
	Right	25.34	2.397		
5	Left	23.94	1.656	0.251	0.279
	Right	25.40	2.282		
6	Left	24.06	1.720	0.251	0.290
	Right	25.49	2.243		
7	Left	24.15	1.706	0.251	0.284
	Right	25.59	2.227		
8	Left	24.12	1.742	0.251	0.278
	Right	25.60	2.261		
9	Left	24.31	1.766	0.251	0.306
	Right	25.73	2.308		
10	Left	24.33	1.789	0.251	0.280
	Right	25.85	2.325		
11	Left	24.35	1.806	0.251	0.307
	Right	25.87	2.532		
12	Left	24.47	1.910	0.347	0.311
	Right	26.02	2.572		
13	Left	24.58	1.942	0.347	0.303
	Right	26.19	2.646		
14	Left	24.66	1.982	0.347	0.307
	Right	26.33	2.786		
15	Left	24.77	2.027	0.347	0.310
	Right	26.49	2.889		
16	Left	24.72	2.128	0.347	0.324
	Right	26.50	3.118		
17	Left	25.01	2.184	0.347	0.309
	Right	26.88	3.189		
18	Left	25.22	2.353	0.347	0.364
	Right	27.01	3.414		
19	Left	25.34	2.452	0.347	0.361
	Right	27.21	3.532		
20	Left	25.54	2.691	0.347	0.396
	Right	27.37	3.666		
21	Left	25.67	2.795	0.347	0.415
	Right	27.47	3.756		

22	Left	25.81	2.998	0.347	0.424
	Right	27.64	3.827		
23	Left	26.03	3.066	0.347	0.453
	Right	27.77	3.878		
24	Left	26.32	3.213	0.347	0.485
	Right	27.97	3.926		
25	Left	26.53	3.370	0.347	0.512
	Right	28.14	3.993		
26	Left	26.67	3.671	0.347	0.539
	Right	28.24	4.039		
27	Left	26.95	3.686	0.465	0.595
	Right	28.33	4.143		
28	Left	27.15	3.932	0.465	0.590
	Right	28.57	4.050		
29	Left	27.25	4.041	0.465	0.577
	Right	28.74	4.028		
30	Left	27.50	3.971	0.465	0.634
	Right	28.76	4.044		
31	Left	27.53	4.018	0.465	0.570
	Right	29.01	3.862		
32	Left	27.67	4.118	0.465	0.634
	Right	28.96	4.078		
33	Left	27.85	4.176	0.465	0.618
	Right	29.18	3.944		
34	Left	27.95	4.187	0.465	0.628
	Right	29.25	3.971		
35	Left	28.19	4.268	0.465	0.658
	Right	29.40	4.085		
36	Left	28.30	4.274	0.465	0.641
	Right	29.56	4.001		
37	Left	28.48	4.208	0.465	0.680
	Right	29.59	4.007		
38	Left	28.53	4.233	0.465	0.654
	Right	29.74	3.974		

Summer/Arch/Temperature (Diabetic)

Summer Temperature Arch Analysis (Diabetic Group, **Left vs Right**)

Table 5: Summer Temperature Analysis (Diabetic Group)

Time (/**min**)	Location	Mean Temperature (**ºC**)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	29.03	.534	0.917	0.779
	Right	28.83	1.481		
1	Left	28.88	.661	0.917	0.871
	Right	28.77	1.357		
2	Left	28.91	.690	0.917	0.885
	Right	28.81	1.375		
3	Left	28.96	.692	0.917	0.916
	Right	28.89	1.376		
4	Left	29.03	.672	0.602	0.950
	Right	29.07	1.354		
5	Left	29.11	.635	0.465	0.894
	Right	29.20	1.390		
6	Left	29.18	.617	0.465	0.863
	Right	29.30	1.352		
7	Left	29.27	.586	0.465	0.786
	Right	29.46	1.346		
8	Left	29.35	.583	0.602	0.735
	Right	29.58	1.320		
9	Left	29.42	.590	0.602	0.631
	Right	29.76	1.388		
10	Left	29.50	.591	0.602	0.553
	Right	29.92	1.357		
11	Left	29.59	.595	0.602	0.548
	Right	30.04	1.437		
12	Left	29.70	.615	0.602	0.432
	Right	30.29	1.412		
13	Left	29.81	.630	0.602	0.401
	Right	30.44	1.409		
14	Left	29.93	.666	0.602	0.375
	Right	30.61	1.436		
15	Left	30.05	.687	0.465	0.387
	Right	30.70	1.391		
16	Left	30.16	.733	0.347	0.405
	Right	30.79	1.437		
17	Left	30.30	.776	0.347	0.352
	Right	31.03	1.449		
18	Left	30.44	.830	0.347	0.351
	Right	31.19	1.484		
19	Left	30.57	.889	0.347	0.368
	Right	31.32	1.525		
20	Left	30.73	.946	0.251	0.389
	Right	31.49	1.587		
21	Left	30.88	1.009	0.346	0.416
	Right	31.59	1.575		

22	Left	31.01	1.029	0.251	0.391
	Right	31.79	1.619		
23	Left	31.18	1.113	0.251	0.373
	Right	32.02	1.660		
24	Left	31.34	1.173	0.251	0.403
	Right	32.14	1.653		
25	Left	31.45	1.207	0.251	0.402
	Right	32.27	1.690		
26	Left	31.61	1.244	0.251	0.452
	Right	32.37	1.753		
27	Left	31.76	1.298	0.347	0.455
	Right	32.52	1.730		
28	Left	31.92	1.352	0.347	0.472
	Right	32.66	1.746		
29	Left	32.08	1.385	0.465	0.464
	Right	32.84	1.745		
30	Left	32.23	1.426	0.465	0.500
	Right	32.94	1.758		
31	Left	32.36	1.451	0.465	0.502
	Right	33.08	1.748		
32	Left	32.50	1.457	0.465	0.576
	Right	33.09	1.776		
33	Left	32.63	1.477	0.463	0.531
	Right	33.30	1.764		
34	Left	32.76	1.493	0.465	0.541
	Right	33.43	1.805		
35	Left	32.91	1.526	0.465	0.588
	Right	33.50	1.772		
36	Left	33.03	1.561	0.465	0.604
	Right	33.59	1.747		
37	Left	33.19	1.594	0.602	0.690
	Right	33.64	1.849		
38	Left	33.32	1.621	0.465	0.673
	Right	33.80	1.814		

Winter/Arch/Temperature (Diabetic)

Winter Temperature Arch Analysis (Diabetic Group, **Left vs Right**)

Table 6: Winter Temperature Analysis (Diabetic Group)					
Time (/**min**)	Location	Mean Temperature (**ºC**)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	25.72	.500	0.602	0.946
	Right	25.78	1.845		
1	Left	25.72	.797	0.754	0.909
	Right	25.62	1.784		
2	Left	25.83	.877	0.917	0.858
	Right	25.65	1.928		
3	Left	25.92	.901	0.917	0.810
	Right	25.68	1.875		
4	Left	26.02	.960	0.917	0.942
	Right	25.94	2.152		
5	Left	26.08	1.005	0.917	0.945
	Right	26.00	2.114		
6	Left	26.15	1.029	0.754	1.000
	Right	26.15	2.134		
7	Left	26.22	1.063	0.754	0.978
	Right	26.25	2.141		
8	Left	26.29	1.092	0.754	0.982
	Right	26.31	2.277		
9	Left	26.37	1.113	0.754	0.883
	Right	26.54	2.278		
10	Left	26.44	1.130	0.754	0.868
	Right	26.64	2.281		
11	Left	26.52	1.166	0.754	0.886
	Right	26.69	2.301		
12	Left	26.61	1.183	0.754	0.827
	Right	26.87	2.287		
13	Left	26.68	1.221	0.754	0.807
	Right	26.99	2.408		
14	Left	26.77	1.252	0.754	0.787
	Right	27.12	2.442		
15	Left	26.84	1.294	0.754	0.758
	Right	27.24	2.484		
16	Left	26.91	1.349	0.917	0.840
	Right	27.18	2.574		
17	Left	27.01	1.388	0.754	0.764
	Right	27.42	2.625		
18	Left	27.11	1.423	0.602	0.721
	Right	27.61	2.671		
19	Left	27.18	1.476	0.602	0.712
	Right	27.71	2.698		
20	Left	27.28	1.529	0.602	0.715
	Right	27.81	2.745		
21	Left	27.36	1.558	0.754	0.706
	Right	27.91	2.756		

22	Left	27.45	1.607	0.754	0.695
	Right	28.05	2.869		
23	Left	27.56	1.644	0.602	0.654
	Right	28.26	2.895		
24	Left	27.67	1.702	0.754	0.678
	Right	28.30	2.831		
25	Left	27.76	1.778	0.754	0.629
	Right	28.51	2.820		
26	Left	27.88	1.848	0.754	0.659
	Right	28.60	2.944		
27	Left	27.99	1.901	0.917	0.649
	Right	28.74	2.993		
28	Left	28.13	1.950	0.754	0.653
	Right	28.87	2.982		
29	Left	28.23	2.009	0.754	0.651
	Right	28.99	3.002		
30	Left	28.31	2.056	0.754	0.625
	Right	29.13	2.941		
31	Left	28.45	2.138	0.754	0.641
	Right	29.25	3.013		
32	Left	28.54	2.190	0.754	0.650
	Right	29.33	3.055		
33	Left	28.65	2.221	0.754	0.647
	Right	29.46	3.044		
34	Left	28.77	2.282	0.602	0.645
	Right	29.59	3.076		
35	Left	28.87	2.301	0.602	0.639
	Right	29.72	3.106		
36	Left	28.99	2.361	0.602	0.643
	Right	29.82	3.078		
37	Left	29.24	2.393	0.602	0.729
	Right	29.87	3.063		
38	Left	29.36	2.436	0.602	0.742
	Right	29.95	3.049		

Summer/Toe/Relative Humidity (Diabetic)

Summer RH Toe Analysis (Diabetic Group, **Left vs Right**)

Table 7: Summer RH Analysis (Diabetic Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	64.75	8.559	0.917	0.908
	Right	65.54	11.953		
1	Left	69.97	7.155	0.754	0.784
	Right	71.50	9.731		
2	Left	70.77	7.641	0.754	0.648
	Right	73.47	10.198		
3	Left	71.84	8.362	0.754	0.667
	Right	74.55	10.651		
4	Left	72.43	7.848	0.465	0.686
	Right	75.00	11.243		
5	Left	72.69	7.844	0.347	0.565
	Right	76.34	11.076		
6	Left	72.95	7.858	0.347	0.544
	Right	76.91	11.560		
7	Left	73.24	7.576	0.347	0.509
	Right	77.51	11.558		
8	Left	73.47	7.948	0.347	0.499
	Right	78.08	12.165		
9	Left	73.78	8.036	0.347	0.466
	Right	78.87	12.530		
10	Left	74.13	7.659	0.347	0.399
	Right	80.05	12.732		
11	Left	74.42	7.674	0.347	0.380
	Right	80.81	13.319		
12	Left	74.39	7.554	0.251	0.336
	Right	81.45	13.438		
13	Left	74.71	7.399	0.175	0.307
	Right	82.30	13.714		
14	Left	74.99	7.146	0.117	0.289
	Right	82.72	13.415		
15	Left	74.73	7.297	0.117	0.254
	Right	83.28	13.727		
16	Left	74.47	7.471	0.117	0.257
	Right	83.25	14.226		
17	Left	74.83	7.160	0.117	0.237
	Right	83.75	13.862		
18	Left	74.99	7.237	0.117	0.215
	Right	84.37	13.812		
19	Left	75.17	6.577	0.117	0.191
	Right	85.09	14.044		
20	Left	74.64	6.934	0.117	0.172
	Right	85.29	14.281		
21	Left	74.89	6.984	0.117	0.173
	Right	85.75	14.677		

22	Left	74.77	7.146	0.117	0.156
	Right	86.05	14.448		
23	Left	74.80	7.328	0.117	0.166
	Right	86.28	15.165		
24	Left	74.89	7.858	0.117	0.159
	Right	86.93	15.460		
25	Left	74.89	7.789	0.117	0.147
	Right	87.33	15.485		
26	Left	74.75	7.417	0.117	0.128
	Right	87.92	15.678		
27	Left	74.63	7.923	0.117	0.135
	Right	88.11	16.318		
28	Left	74.98	7.641	0.117	0.146
	Right	87.96	16.316		
29	Left	74.88	6.871	0.117	0.119
	Right	88.66	16.256		
30	Left	74.64	6.763	0.117	0.101
	Right	89.26	16.287		
31	Left	74.79	6.997	0.117	0.102
	Right	89.24	16.014		
32	Left	74.22	7.365	0.117	0.099
	Right	88.97	16.037		
33	Left	74.23	7.191	0.117	0.101
	Right	88.71	15.904		
34	Left	74.10	7.779	0.117	0.099
	Right	89.51	16.736		
35	Left	73.82	7.766	0.221	0.190
	Right	86.01	16.931		
36	Left	73.56	8.498	0.117	0.106
	Right	89.06	17.020		
37	Left	72.95	8.445	0.117	0.144
	Right	85.84	15.688		
38	Left	73.38	7.933	0.117	0.137
	Right	86.30	15.591		

Winter/Toe/Relative Humidity (Diabetic)

Winter RH Toe Analysis (Diabetic Group, **Left vs Right**)

Table 7: Summer RH Analysis (Diabetic Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	64.55	3.353	0.602	0.508
	Right	65.97	3.086		
1	Left	68.40	3.546	0.754	0.469
	Right	70.48	4.973		
2	Left	70.05	4.121	0.465	0.539
	Right	71.88	4.857		
3	Left	70.09	3.033	0.465	0.308
	Right	72.64	4.278		
4	Left	70.41	2.360	0.602	0.290
	Right	72.90	4.298		
5	Left	70.75	2.777	0.251	0.283
	Right	73.05	3.497		
6	Left	71.45	3.033	0.347	0.357
	Right	73.48	3.485		
7	Left	71.16	2.896	0.222	0.230
	Right	74.11	4.181		
8	Left	71.21	2.464	0.175	0.195
	Right	74.68	4.898		
9	Left	71.84	2.533	0.251	0.248
	Right	75.05	5.178		
10	Left	72.40	3.405	0.347	0.302
	Right	75.60	5.514		
11	Left	72.69	3.656	0.251	0.261
	Right	76.32	5.639		
12	Left	72.59	3.273	0.175	0.177
	Right	76.87	5.571		
13	Left	72.93	3.186	0.117	0.201
	Right	77.10	5.878		
14	Left	74.99	5.842	0.347	0.489
	Right	77.75	6.191		
15	Left	75.59	5.919	0.465	0.496
	Right	78.39	6.500		
16	Left	75.51	6.455	0.465	0.480
	Right	78.74	7.335		
17	Left	75.94	6.298	0.465	0.440
	Right	79.45	7.327		
18	Left	76.29	6.385	0.347	0.426
	Right	80.03	7.670		
19	Left	75.70	5.472	0.347	0.308
	Right	80.32	7.733		
20	Left	77.68	7.607	0.465	0.573
	Right	80.60	8.143		
21	Left	76.47	5.842	0.251	0.346
	Right	81.00	8.282		

22	Left	76.98	6.312	0.347	0.365
	Right	81.43	8.206		
23	Left	78.14	7.875	0.602	0.507
	Right	81.52	7.556		
24	Left	77.29	6.318	0.221	0.287
	Right	82.97	8.531		
25	Left	78.94	8.321	0.327	0.446
	Right	83.64	9.172		
26	Left	79.14	8.436	0.327	0.428
	Right	84.16	9.514		
27	Left	77.93	6.733	0.142	0.265
	Right	84.36	9.272		
28	Left	79.33	8.556	0.327	0.431
	Right	84.39	9.619		
29	Left	79.23	8.520	0.221	0.404
	Right	84.74	10.122		
30	Left	77.99	6.647	0.142	0.245
	Right	85.13	10.256		
31	Left	79.27	8.137	0.142	0.368
	Right	85.11	10.151		
32	Left	78.01	6.723	0.142	0.229
	Right	85.56	10.473		
33	Left	79.17	7.713	0.142	0.298
	Right	86.05	10.697		
34	Left	78.86	7.151	0.142	0.271
	Right	86.08	11.025		
35	Left	78.07	6.676	0.142	0.226
	Right	85.80	10.789		
36	Left	78.57	7.660	0.142	0.285
	Right	85.86	11.311		
37	Left	79.23	7.591	0.347	0.455
	Right	83.82	10.620		
38	Left	76.97	6.256	0.251	0.242
	Right	84.02	10.792		

Summer/Arch/Relative Humidity (Diabetic)

Summer RH Arch Analysis (Diabetic Group, **Left vs Right**)

Table 7: Summer RH Analysis (Diabetic Group)					
Time (/**min**)	Location	Mean RH (%)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	67.81	10.963	0.602	0.659
	Right	71.24	12.707		
1	Left	68.40	12.415	0.917	0.827
	Right	70.43	15.760		
2	Left	69.28	13.156	0.917	0.863
	Right	70.94	16.151		
3	Left	69.14	13.922	0.754	0.837
	Right	71.27	17.519		
4	Left	70.30	14.105	0.917	0.873
	Right	71.97	17.693		
5	Left	70.48	14.373	0.917	0.894
	Right	71.91	18.402		
6	Left	71.17	14.809	0.917	0.926
	Right	72.18	18.392		
7	Left	71.21	14.533	0.917	0.937
	Right	72.07	18.759		
8	Left	71.39	15.273	0.754	0.933
	Right	72.33	18.848		
9	Left	71.64	15.393	0.754	0.926
	Right	72.68	18.983		
10	Left	71.87	15.589	0.754	0.962
	Right	72.42	19.010		
11	Left	72.66	16.174	0.754	0.990
	Right	72.81	20.103		
12	Left	72.96	16.093	0.754	0.995
	Right	73.04	19.873		
13	Left	73.55	16.064	0.754	0.981
	Right	73.28	19.554		
14	Left	73.77	16.231	0.754	1.000
	Right	73.77	19.710		
15	Left	74.22	16.043	0.917	0.969
	Right	74.67	19.380		
16	Left	74.37	16.524	0.754	0.957
	Right	73.73	19.640		
17	Left	74.61	16.501	0.754	0.988
	Right	74.43	19.654		
18	Left	75.15	16.189	0.754	0.966
	Right	74.66	19.321		
19	Left	75.58	16.652	0.602	0.934
	Right	74.59	19.550		
20	Left	75.33	17.072	0.602	0.976
	Right	74.97	19.361		
21	Left	75.65	17.224	0.602	0.951
	Right	74.90	20.001		

22	Left	75.28	17.207	0.917	1.000
	Right	75.28	20.021		
23	Left	75.87	17.947	0.917	0.973
	Right	76.29	19.689		
24	Left	75.72	17.886	0.917	0.995
	Right	75.80	19.854		
25	Left	75.85	17.525	0.917	0.962
	Right	76.42	19.505		
26	Left	76.51	17.638	0.917	0.987
	Right	76.72	19.593		
27	Left	75.88	18.449	0.917	0.970
	Right	76.34	19.925		
28	Left	76.53	18.122	0.917	0.995
	Right	76.61	20.275		
29	Left	76.60	17.186	0.917	0.992
	Right	76.48	19.929		
30	Left	76.30	17.300	0.917	0.979
	Right	76.62	19.896		
31	Left	76.45	17.010	0.917	0.979
	Right	76.76	19.631		
32	Left	75.84	17.209	0.917	0.957
	Right	76.51	19.984		
33	Left	76.42	17.459	0.917	0.956
	Right	77.11	20.046		
34	Left	76.52	17.936	0.917	0.968
	Right	77.01	19.576		
35	Left	76.29	18.457	0.917	0.959
	Right	76.94	20.391		
36	Left	75.85	19.201	0.917	0.927
	Right	77.03	20.532		
37	Left	75.36	19.428	0.917	0.944
	Right	74.49	18.541		
38	Left	75.61	20.104	0.917	0.940
	Right	74.64	19.199		

Winter/Arch/Relative Humidity (Diabetic)

Winter RH Arch Analysis (Diabetic Group, **Left vs Right**)

Table 7: Summer RH Analysis (Diabetic Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Left	68.76	7.936	0.602	0.783
	Right	70.10	6.947		
1	Left	68.99	9.377	0.465	0.645
	Right	71.90	9.831		
2	Left	69.64	9.232	0.465	0.668
	Right	72.36	10.032		
3	Left	69.23	8.868	0.465	0.676
	Right	71.93	10.757		
4	Left	69.67	9.081	0.602	0.755
	Right	71.72	10.917		
5	Left	68.80	8.867	0.347	0.685
	Right	71.45	10.946		
6	Left	69.19	9.162	0.347	0.680
	Right	71.97	11.279		
7	Left	69.08	9.766	0.347	0.684
	Right	71.91	11.438		
8	Left	69.32	9.735	0.465	0.687
	Right	72.13	11.470		
9	Left	69.51	9.518	0.347	0.682
	Right	72.29	11.106		
10	Left	69.59	9.667	0.465	0.684
	Right	72.48	11.900		
11	Left	69.54	9.400	0.465	0.671
	Right	72.46	11.416		
12	Left	70.05	9.455	0.347	0.650
	Right	73.22	11.717		
13	Left	69.87	9.455	0.465	0.631
	Right	73.32	12.144		
14	Left	70.36	9.417	0.602	0.652
	Right	73.57	12.112		
15	Left	70.80	9.851	0.602	0.715
	Right	73.45	12.143		
16	Left	70.96	9.914	0.754	0.725
	Right	73.56	12.496		
17	Left	71.32	10.204	0.602	0.709
	Right	74.10	12.416		
18	Left	71.70	10.342	0.602	0.691
	Right	74.71	12.611		
19	Left	72.11	10.233	0.754	0.695
	Right	75.06	12.599		
20	Left	72.41	10.725	0.754	0.727
	Right	75.07	12.396		
21	Left	72.68	10.609	0.917	0.802
	Right	74.60	12.709		

22	Left	72.78	11.170	0.754	0.758
	Right	75.21	12.867		
23	Left	73.13	10.946	0.754	0.746
	Right	75.63	12.566		
24	Left	73.58	11.521	0.602	0.770
	Right	75.91	12.825		
25	Left	73.58	11.282	0.754	0.781
	Right	75.75	12.538		
26	Left	73.84	11.288	0.917	0.815
	Right	75.70	12.922		
27	Left	74.02	11.407	0.754	0.806
	Right	75.96	12.627		
28	Left	74.49	11.420	0.917	0.784
	Right	76.70	13.197		
29	Left	75.05	12.231	0.754	0.782
	Right	77.38	13.552		
30	Left	74.90	12.231	0.602	0.828
	Right	76.72	13.340		
31	Left	75.21	12.110	0.602	0.781
	Right	77.54	13.480		
32	Left	75.11	11.658	0.465	0.790
	Right	77.23	12.624		
33	Left	75.50	12.078	0.602	0.823
	Right	77.32	12.797		
34	Left	75.48	11.931	0.602	0.795
	Right	77.60	13.034		
35	Left	75.14	11.709	0.602	0.761
	Right	77.63	13.200		
36	Left	74.62	11.836	0.602	0.782
	Right	76.87	13.050		
37	Left	74.98	11.555	1.000	0.891
	Right	76.20	14.476		
38	Left	74.25	11.316	0.624	0.832
	Right	76.11	14.129		

Appendix X

Toes vs Arches

Summer/Temperature (Healthy)

Summer Temperature Analysis (Healthy Group, **Toes vs Arch**)

Table 1: Summer Temperature Analysis (Healthy Participant Group)

Time (/**min**)	Location	Mean Temperature (**ºC**)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	33.64	0.969	0.005	0.003
	Arch	32.85	0.681		
1	Toe	33.65	1.005	0.011	0.013
	Arch	32.97	0.764		
2	Toe	33.64	1.019	0.062	0.058
	Arch	33.10	0.841		
3	Toe	33.66	0.955	0.147	0.115
	Arch	33.23	0.874		
4	Toe	33.80	0.973	0.180	0.196
	Arch	33.43	0.945		
5	Toe	33.86	1.006	0.272	0.375
	Arch	33.60	0.970		
6	Toe	34.00	0.939	0.403	0.407
	Arch	33.76	1.007		
7	Toe	34.09	0.918	0.429	0.512
	Arch	33.90	1.020		
8	Toe	34.28	0.958	0.379	0.459
	Arch	34.06	1.033		
9	Toe	34.41	0.924	0.356	0.498
	Arch	34.21	1.054		
10	Toe	34.54	0.917	0.391	0.572
	Arch	34.37	1.069		
11	Toe	34.68	0.971	0.333	0.349
	Arch	34.40	1.039		
12	Toe	34.90	0.877	0.253	0.295
	Arch	34.60	1.053		
13	Toe	35.00	0.778	0.312	0.302
	Arch	34.72	1.042		
14	Toe	35.07	0.728	0.333	0.364
	Arch	34.83	0.983		
15	Toe	35.21	0.648	0.333	0.293
	Arch	34.95	0.968		
16	Toe	35.33	0.604	0.322	0.264
	Arch	35.05	0.960		
17	Toe	35.43	0.557	0.367	0.262

	Arch	35.16	0.953		
18	Toe	35.53	0.521	0.344	0.261
	Arch	35.26	0.948		
19	Toe	35.53	0.462	0.692	0.389
	Arch	35.34	0.928		
20	Toe	35.65	0.454	0.403	0.285
	Arch	35.41	0.913		
21	Toe	35.68	0.454	0.567	0.387
	Arch	35.49	0.893		
22	Toe	35.65	0.454	0.742	0.525
	Arch	35.52	0.855		
23	Toe	35.74	0.416	0.826	0.546
	Arch	35.62	0.828		
24	Toe	35.88	0.390	0.582	0.393
	Arch	35.72	0.794		
25	Toe	35.88	0.385	0.843	0.574
	Arch	35.78	0.762		
26	Toe	35.91	0.354	0.809	0.642
	Arch	35.83	0.721		
27	Toe	35.98	0.349	0.878	0.592
	Arch	35.89	0.696		
28	Toe	36.02	0.421	0.878	0.644
	Arch	35.94	0.692		
29	Toe	36.04	0.378	0.912	0.734
	Arch	35.99	0.657		
30	Toe	36.07	0.370	0.965	0.761
	Arch	36.02	0.650		
31	Toe	36.06	0.379	0.965	0.868
	Arch	36.04	0.609		
32	Toe	36.04	0.364	0.742	0.860
	Arch	36.07	0.598		
33	Toe	35.96	0.400	0.429	0.449
	Arch	36.07	0.571		
34	Toe	36.10	0.480	0.843	0.981
	Arch	36.10	0.572		
35	Toe	36.09	0.377	0.692	0.790
	Arch	36.13	0.550		
36	Toe	36.09	0.431	0.567	0.647
	Arch	36.16	0.535		
37	Toe	36.07	0.440	0.582	0.596
	Arch	36.15	0.516		
38	Toe	36.12	0.371	0.495	0.625
	Arch	36.18	0.503		

Winter/Temperature (Healthy)

Winter Temperature Analysis (Healthy Participant Group, **Toes vs Arch**)

Table 2: Winter Temperature Analysis (Healthy Participant Group)					
Time (/**min**)	Location	Mean Temperature (ºC)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	25.42	3.363	0.176	0.120
	Arch	26.60	1.778		
1	Toe	25.41	3.200	0.092	0.055
	Arch	26.84	1.842		
2	Toe	25.32	3.171	0.046	0.029
	Arch	26.96	1.877		
3	Toe	25.31	3.108	0.027	0.016
	Arch	27.10	1.924		
4	Toe	25.39	3.116	0.023	0.013
	Arch	27.27	1.971		
5	Toe	25.56	3.150	0.027	0.014
	Arch	27.46	2.037		
6	Toe	25.71	3.187	0.028	0.013
	Arch	27.64	2.105		
7	Toe	25.84	3.230	0.021	0.013
	Arch	27.83	2.202		
8	Toe	26.04	3.317	0.028	0.016
	Arch	28.01	2.270		
9	Toe	26.20	3.379	0.032	0.017
	Arch	28.20	2.354		
10	Toe	26.41	3.454	0.042	0.021
	Arch	28.39	2.420		
11	Toe	26.52	3.509	0.035	0.021
	Arch	28.54	2.499		
12	Toe	26.82	3.565	0.045	0.029
	Arch	28.75	2.547		
13	Toe	27.08	3.792	0.111	0.048
	Arch	28.92	2.605		
14	Toe	27.40	3.811	0.129	0.066
	Arch	29.12	2.652		
15	Toe	27.68	3.891	0.164	0.091
	Arch	29.29	2.706		
16	Toe	27.95	4.048	0.194	0.115
	Arch	29.50	2.776		
17	Toe	28.24	4.172	0.241	0.146
	Arch	29.70	2.841		
18	Toe	28.61	4.324	0.370	0.206
	Arch	29.92	2.895		
19	Toe	28.91	4.437	0.558	0.259
	Arch	30.11	2.927		
20	Toe	29.25	4.532	0.687	0.330
	Arch	30.30	2.978		
21	Toe	29.59	4.617	0.927	0.409
	Arch	30.50	3.024		
22	Toe	29.92	4.644	0.956	0.473
	Arch	30.71	3.062		

23	Toe	30.30	4.663	0.798	0.562
	Arch	30.94	3.080		
24	Toe	30.56	4.717	0.742	0.603
	Arch	31.14	3.112		
25	Toe	30.86	4.729	0.674	0.660
	Arch	31.35	3.127		
26	Toe	31.13	4.734	0.596	0.711
	Arch	31.55	3.158		
27	Toe	31.37	4.747	0.487	0.750
	Arch	31.73	3.173		
28	Toe	31.58	4.712	0.442	0.775
	Arch	31.90	3.177		
29	Toe	31.84	4.661	0.360	0.843
	Arch	32.06	3.194		
30	Toe	32.12	4.636	0.314	0.937
	Arch	32.21	3.189		
31	Toe	32.35	4.612	0.272	0.998
	Arch	32.35	3.168		
32	Toe	32.58	4.569	0.213	0.920
	Arch	32.47	3.167		
33	Toe	32.68	4.506	0.249	0.929
	Arch	32.58	3.144		
34	Toe	32.91	4.505	0.164	0.872
	Arch	32.74	3.123		
35	Toe	33.02	4.501	0.124	0.871
	Arch	32.84	3.110		
36	Toe	33.25	4.416	0.092	0.783
	Arch	32.96	3.076		
37	Toe	33.39	4.345	0.073	0.777
	Arch	33.09	3.010		
38	Toe	33.53	4.239	0.067	0.751
	Arch	33.20	2.950		

Summer/Relative Humidity (Healthy)

Summer RH Analysis (Healthy Participant Group, **Toes vs Arch**)

Table 3: Summer RH Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	72.68	8.419	0.544	0.583
	Arch	71.41	8.787		
1	Toe	75.30	7.841	0.057	0.081
	Arch	71.31	8.888		
2	Toe	76.00	8.362	0.040	0.058
	Arch	71.40	9.235		
3	Toe	76.67	8.773	0.033	0.037
	Arch	71.34	9.637		
4	Toe	77.11	8.951	0.026	0.028
	Arch	71.53	9.556		
5	Toe	77.87	9.205	0.029	0.026
	Arch	72.09	9.691		
6	Toe	79.08	9.433	0.018	0.016
	Arch	72.48	10.137		
7	Toe	79.80	9.732	0.019	0.013
	Arch	72.85	10.300		
8	Toe	80.66	9.891	0.017	0.009
	Arch	73.17	10.474		
9	Toe	81.23	10.136	0.017	0.007
	Arch	73.39	10.686		
10	Toe	81.86	10.492	0.012	0.005
	Arch	73.42	11.102		
11	Toe	82.49	10.777	0.007	0.004
	Arch	73.70	10.763		
12	Toe	82.85	10.879	0.007	0.004
	Arch	74.07	10.818		
13	Toe	83.42	11.087	0.005	0.004
	Arch	74.40	10.869		
14	Toe	83.38	11.109	0.007	0.003
	Arch	74.29	11.019		
15	Toe	83.64	10.949	0.007	0.003
	Arch	74.40	11.295		
16	Toe	84.19	11.050	0.003	0.002
	Arch	74.27	11.768		
17	Toe	84.52	11.273	0.004	0.002
	Arch	74.72	11.330		
18	Toe	85.08	11.536	0.002	0.002
	Arch	74.86	11.604		
19	Toe	85.18	10.959	0.002	0.001
	Arch	75.00	11.556		
20	Toe	85.35	11.235	0.002	0.002
	Arch	75.21	11.659		
21	Toe	85.20	11.483	0.002	0.002
	Arch	75.02	11.899		
22	Toe	85.20	11.280	0.002	0.002
	Arch	75.03	11.995		

23	Toe	85.61	11.408	0.002	0.002
	Arch	75.37	12.366		
24	Toe	85.90	11.325	0.003	0.003
	Arch	75.83	12.568		
25	Toe	85.56	11.131	0.005	0.005
	Arch	76.37	12.310		
26	Toe	85.83	11.123	0.003	0.003
	Arch	75.80	12.705		
27	Toe	85.60	11.388	0.006	0.007
	Arch	76.54	12.695		
28	Toe	85.34	12.220	0.013	0.016
	Arch	76.91	12.791		
29	Toe	85.91	11.757	0.012	0.011
	Arch	77.19	12.906		
30	Toe	85.80	11.803	0.012	0.013
	Arch	77.19	12.941		
31	Toe	86.22	11.671	0.014	0.017
	Arch	78.29	12.016		
32	Toe	86.25	11.578	0.015	0.019
	Arch	78.47	12.058		
33	Toe	86.34	11.710	0.017	0.012
	Arch	77.96	12.319		
34	Toe	86.55	11.691	0.012	0.008
	Arch	77.27	13.483		
35	Toe	86.79	11.653	0.023	0.016
	Arch	78.52	12.687		
36	Toe	86.92	11.771	0.012	0.011
	Arch	78.30	12.380		
37	Toe	86.96	11.393	0.019	0.016
	Arch	78.96	12.275		
38	Toe	87.19	11.481	0.010	0.007
	Arch	77.84	13.162		

Winter/Relative Humidity (Healthy)

Winter RH (Healthy Participant Group, **Toes vs Arch**)

Table 4: Winter Relative Humidity Analysis (Healthy Participant Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	68.65	8.658	0.581	0.878
	Arch	68.30	7.881		
1	Toe	71.08	8.438	0.275	0.384
	Arch	69.06	8.450		
2	Toe	73.16	8.860	0.091	0.091
	Arch	68.91	8.851		
3	Toe	73.46	9.486	0.081	0.094
	Arch	69.03	9.419		
4	Toe	74.27	9.579	0.036	0.048
	Arch	68.93	9.574		
5	Toe	74.45	10.203	0.031	0.050
	Arch	68.99	9.610		
6	Toe	74.51	11.058	0.066	0.084
	Arch	69.34	10.107		
7	Toe	74.91	10.761	0.055	0.049
	Arch	69.17	10.200		
8	Toe	74.92	11.219	0.066	0.042
	Arch	68.87	10.132		
9	Toe	74.64	12.272	0.097	0.070
	Arch	68.96	10.280		
10	Toe	75.38	12.232	0.054	0.043
	Arch	68.95	10.370		
11	Toe	76.05	12.577	0.026	0.025
	Arch	68.52	10.666		
12	Toe	76.20	12.278	0.023	0.018
	Arch	68.56	10.755		
13	Toe	76.53	13.129	0.052	0.031
	Arch	69.16	11.068		
14	Toe	76.41	14.067	0.080	0.052
	Arch	69.56	11.163		
15	Toe	77.20	14.518	0.062	0.045
	Arch	69.87	11.616		
16	Toe	77.78	14.808	0.044	0.031
	Arch	69.59	12.007		
17	Toe	77.70	14.963	0.066	0.038
	Arch	69.90	11.847		
18	Toe	79.11	14.753	0.045	0.023
	Arch	70.35	12.662		
19	Toe	79.44	15.033	0.055	0.023
	Arch	70.59	12.787		
20	Toe	79.51	15.183	0.057	0.026
	Arch	70.70	12.988		
21	Toe	79.86	15.551	0.064	0.023
	Arch	70.63	13.458		
22	Toe	80.33	16.812	0.085	0.026
	Arch	70.70	13.201		

23	Toe	79.17	15.779	0.139	0.052
	Arch	71.14	13.591		
24	Toe	80.14	16.678	0.094	0.034
	Arch	71.10	13.460		
25	Toe	80.15	16.836	0.100	0.040
	Arch	71.37	13.735		
26	Toe	80.50	16.510	0.093	0.037
	Arch	71.60	13.968		
27	Toe	80.56	16.596	0.090	0.037
	Arch	71.61	14.123		
28	Toe	80.34	16.984	0.105	0.042
	Arch	71.28	14.587		
29	Toe	80.41	16.467	0.097	0.029
	Arch	70.94	14.459		
30	Toe	80.41	16.898	0.115	0.041
	Arch	71.33	14.975		
31	Toe	79.96	17.688	0.115	0.064
	Arch	71.50	15.061		
32	Toe	80.04	18.193	0.141	0.061
	Arch	71.38	15.045		
33	Toe	81.79	18.030	0.048	0.023
	Arch	71.03	14.764		
34	Toe	80.30	17.199	0.047	0.038
	Arch	70.92	14.886		
35	Toe	81.27	18.490	0.045	0.027
	Arch	70.85	14.735		
36	Toe	81.28	17.959	0.049	0.030
	Arch	71.19	15.188		
37	Toe	81.65	18.303	0.042	0.025
	Arch	71.06	15.469		
38	Toe	81.82	18.133	0.038	0.022
	Arch	70.93	15.635		

Summer/Temperature (Diabetic)

Summer Temperature Analysis (Diabetic Group, **Toes vs Arch**)

Table 5: Summer Temperature Analysis (Diabetic Group)					
Time **(/min)**	Location	Mean Temperature **(ºC)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	28.16	1.522	0.280	0.208
	Arch	28.93	1.055		
1	Toe	28.12	1.718	0.353	0.279
	Arch	28.82	1.008		
2	Toe	28.17	1.722	0.353	0.286
	Arch	28.86	1.027		
3	Toe	28.24	1.707	0.280	0.290
	Arch	28.93	1.027		
4	Toe	28.36	1.700	0.315	0.280
	Arch	29.05	1.008		
5	Toe	28.49	1.700	0.393	0.303
	Arch	29.15	1.020		
6	Toe	28.52	1.740	0.315	0.270
	Arch	29.24	0.993		
7	Toe	28.68	1.814	0.315	0.312
	Arch	29.36	0.984		
8	Toe	28.73	1.866	0.218	0.286
	Arch	29.46	0.970		
9	Toe	28.91	1.907	0.218	0.334
	Arch	29.59	1.021		
10	Toe	29.06	1.976	0.280	0.373
	Arch	29.71	1.011		
11	Toe	29.21	1.983	0.247	0.404
	Arch	29.82	1.063		
12	Toe	29.38	1.980	0.280	0.399
	Arch	30.00	1.072		
13	Toe	29.56	2.000	0.247	0.443
	Arch	30.12	1.081		
14	Toe	29.84	2.062	0.315	0.565
	Arch	30.27	1.115		
15	Toe	29.96	2.061	0.280	0.574
	Arch	30.38	1.089		
16	Toe	30.00	2.061	0.280	0.533
	Arch	30.48	1.126		
17	Toe	30.31	2.099	0.280	0.644
	Arch	30.67	1.161		
18	Toe	30.54	2.199	0.315	0.734
	Arch	30.81	1.201		
19	Toe	30.73	2.263	0.393	0.796
	Arch	30.94	1.242		
20	Toe	30.88	2.189	0.481	0.781
	Arch	31.11	1.294		
21	Toe	31.06	2.183	0.579	0.832
	Arch	31.23	1.303		
22	Toe	31.25	2.158	0.631	0.856
	Arch	31.40	1.343		
23	Toe	31.58	2.096	0.796	0.979
	Arch	31.60	1.405		

24	Toe	31.79	2.051	0.796	0.947
	Arch	31.74	1.415		
25	Toe	32.00	2.046	0.853	0.863
	Arch	31.86	1.451		
26	Toe	32.25	2.018	0.971	0.751
	Arch	31.99	1.488		
27	Toe	32.51	1.958	0.853	0.640
	Arch	32.14	1.496		
28	Toe	32.71	1.893	0.579	0.590
	Arch	32.29	1.524		
29	Toe	33.05	1.870	0.529	0.452
	Arch	32.46	1.539		
30	Toe	33.25	1.885	0.481	0.402
	Arch	32.58	1.555		
31	Toe	33.53	1.835	0.393	0.303
	Arch	32.72	1.561		
32	Toe	33.61	1.862	0.353	0.306
	Arch	32.80	1.564		
33	Toe	33.92	1.802	0.280	0.223
	Arch	32.97	1.574		
34	Toe	34.10	1.806	0.218	0.205
	Arch	33.10	1.601		
35	Toe	34.20	1.744	0.190	0.196
	Arch	33.20	1.589		
36	Toe	34.39	1.743	0.143	0.163
	Arch	33.31	1.590		
37	Toe	34.54	1.793	0.143	0.160
	Arch	33.41	1.645		
38	Toe	34.74	1.794	0.105	0.143
	Arch	33.56	1.641		

Winter/Temperature (Diabetic)

Winter Temperature Analysis (Diabetic Group, **Toes vs Arch**)

Table 6: Winter Temperature Analysis (Diabetic Group)					
Time (/**min**)	Location	Mean Temperature (**ºC**)	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	24.79	2.341	0.247	0.272
	Arch	25.75	1.275		
1	Toe	24.40	2.204	0.123	0.135
	Arch	25.67	1.304		
2	Toe	24.39	2.114	0.105	0.111
	Arch	25.74	1.415		
3	Toe	24.47	2.060	0.089	0.108
	Arch	25.80	1.392		
4	Toe	24.58	2.149	0.105	0.115
	Arch	25.98	1.571		
5	Toe	24.67	2.032	0.105	0.109
	Arch	26.04	1.561		
6	Toe	24.78	2.030	0.105	0.107
	Arch	26.15	1.580		
7	Toe	24.87	2.018	0.105	0.109
	Arch	26.24	1.594		
8	Toe	24.86	2.057	0.105	0.104
	Arch	26.30	1.683		
9	Toe	25.02	2.077	0.105	0.107
	Arch	26.45	1.693		
10	Toe	25.09	2.114	0.105	0.108
	Arch	26.54	1.700		
11	Toe	25.11	2.222	0.105	0.110
	Arch	26.61	1.722		
12	Toe	25.25	2.287	0.089	0.117
	Arch	26.74	1.722		
13	Toe	25.38	2.348	0.089	0.139
	Arch	26.83	1.807		
14	Toe	25.49	2.443	0.105	0.151
	Arch	26.94	1.838		
15	Toe	25.63	2.520	0.105	0.173
	Arch	27.04	1.879		
16	Toe	25.61	2.684	0.105	0.187
	Arch	27.05	1.943		
17	Toe	25.95	2.760	0.143	0.254
	Arch	27.21	1.992		
18	Toe	26.11	2.920	0.123	0.284
	Arch	27.36	2.035		
19	Toe	26.28	3.030	0.143	0.327
	Arch	27.45	2.069		
20	Toe	26.46	3.181	0.218	0.378
	Arch	27.55	2.114		
21	Toe	26.57	3.262	0.247	0.398
	Arch	27.64	2.131		
22	Toe	26.72	3.382	0.247	0.434
	Arch	27.75	2.215		
23	Toe	26.90	3.421	0.247	0.447
	Arch	27.91	2.249		

24	Toe	27.14	3.493	0.280	0.528
	Arch	27.99	2.227		
25	Toe	27.34	3.585	0.315	0.560
	Arch	28.13	2.257		
26	Toe	27.46	3.732	0.353	0.582
	Arch	28.24	2.347		
27	Toe	27.64	3.767	0.393	0.613
	Arch	28.37	2.397		
28	Toe	27.86	3.837	0.436	0.661
	Arch	28.50	2.407		
29	Toe	28.00	3.883	0.481	0.675
	Arch	28.61	2.441		
30	Toe	28.13	3.836	0.529	0.688
	Arch	28.72	2.431		
31	Toe	28.27	3.796	0.631	0.690
	Arch	28.85	2.499		
32	Toe	28.31	3.923	0.481	0.678
	Arch	28.94	2.540		
33	Toe	28.51	3.893	0.631	0.717
	Arch	29.05	2.548		
34	Toe	28.60	3.908	0.579	0.703
	Arch	29.18	2.590		
35	Toe	28.80	3.990	0.529	0.745
	Arch	29.30	2.615		
36	Toe	28.93	3.960	0.684	0.756
	Arch	29.40	2.623		
37	Toe	29.03	3.917	0.684	0.730
	Arch	29.56	2.612		
38	Toe	29.13	3.923	0.684	0.729
	Arch	29.66	2.621		

Summer/Relative Humidity (Diabetic)

Summer RH Analysis (Diabetic Group, **Toes vs Arch**)

Table 7: Summer RH Analysis (Diabetic Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	65.14	9.810	0.579	0.368
	Arch	69.52	11.334		
1	Toe	70.74	8.093	0.853	0.793
	Arch	69.41	13.418		
2	Toe	72.12	8.614	0.796	0.703
	Arch	70.11	13.915		
3	Toe	73.19	9.139	0.796	0.596
	Arch	70.21	14.960		
4	Toe	73.72	9.241	0.796	0.651
	Arch	71.14	15.110		
5	Toe	74.52	9.250	0.796	0.570
	Arch	71.20	15.585		
6	Toe	74.93	9.550	0.796	0.583
	Arch	71.67	15.751		
7	Toe	75.37	9.484	0.739	0.531
	Arch	71.64	15.827		
8	Toe	75.77	9.987	0.739	0.524
	Arch	71.86	16.180		
9	Toe	76.33	10.280	0.739	0.503
	Arch	72.16	16.302		
10	Toe	77.09	10.384	0.684	0.431
	Arch	72.14	16.392		
11	Toe	77.61	10.786	0.684	0.457
	Arch	72.73	17.201		
12	Toe	77.92	10.930	0.631	0.452
	Arch	73.00	17.048		
13	Toe	78.51	11.134	0.684	0.436
	Arch	73.41	16.872		
14	Toe	78.86	10.920	0.684	0.437
	Arch	73.77	17.022		
15	Toe	79.01	11.301	0.684	0.485
	Arch	74.44	16.774		
16	Toe	78.86	11.668	0.684	0.472
	Arch	74.05	17.114		
17	Toe	79.29	11.412	0.684	0.473
	Arch	74.52	17.108		
18	Toe	79.68	11.512	0.684	0.468
	Arch	74.91	16.807		
19	Toe	80.13	11.585	0.684	0.450
	Arch	75.08	17.129		
20	Toe	79.96	11.980	0.684	0.477
	Arch	75.15	17.210		
21	Toe	80.32	12.255	0.739	0.467
	Arch	75.27	17.601		
22	Toe	80.41	12.281	0.684	0.460
	Arch	75.28	17.599		
23	Toe	80.54	12.756	0.631	0.527
	Arch	76.08	17.762		

24	Toe	80.91	13.189	0.631	0.472
	Arch	75.76	17.815		
25	Toe	81.11	13.285	0.684	0.483
	Arch	76.14	17.483		
26	Toe	81.33	13.487	0.684	0.509
	Arch	76.61	17.575		
27	Toe	81.37	14.025	0.631	0.477
	Arch	76.11	18.104		
28	Toe	81.47	13.822	0.684	0.505
	Arch	76.57	18.129		
29	Toe	81.77	13.827	0.631	0.468
	Arch	76.54	17.544		
30	Toe	81.95	14.058	0.684	0.451
	Arch	76.46	17.578		
31	Toe	82.02	13.919	0.684	0.451
	Arch	76.61	17.318		
32	Toe	81.60	14.102	0.739	0.457
	Arch	76.18	17.585		
33	Toe	81.47	13.918	0.739	0.517
	Arch	76.76	17.726		
34	Toe	81.80	14.744	0.684	0.498
	Arch	76.77	17.702		
35	Toe	79.24	13.378	0.968	0.729
	Arch	76.62	18.339		
36	Toe	81.31	15.085	0.739	0.530
	Arch	76.44	18.751		
37	Toe	79.40	13.683	0.739	0.538
	Arch	74.93	17.910		
38	Toe	79.84	13.504	0.684	0.524
	Arch	75.12	18.539		

Winter/Relative Humidity (Diabetic)

Winter RH Analysis (Diabetic Group, **Toes vs Arch**)

Table 7: Summer RH Analysis (Diabetic Group)					
Time **(/min)**	Location	Mean RH **(%)**	Std. Deviation	P-value Non-Parametric test	P-value Parametric test
0	Toe	65.26	3.127	0.190	0.113
	Arch	69.43	7.067		
1	Toe	69.44	4.217	0.912	0.758
	Arch	70.45	9.186		
2	Toe	70.96	4.354	0.796	0.991
	Arch	71.00	9.200		
3	Toe	71.36	3.746	0.436	0.811
	Arch	70.58	9.403		
4	Toe	71.65	3.522	0.481	0.772
	Arch	70.70	9.528		
5	Toe	71.90	3.214	0.529	0.586
	Arch	70.12	9.494		
6	Toe	72.46	3.259	0.579	0.575
	Arch	70.58	9.798		
7	Toe	72.63	3.731	0.481	0.544
	Arch	70.50	10.137		
8	Toe	72.95	4.086	0.436	0.533
	Arch	70.73	10.138		
9	Toe	73.44	4.199	0.436	0.467
	Arch	70.90	9.861		
10	Toe	74.00	4.638	0.436	0.424
	Arch	71.04	10.334		
11	Toe	74.51	4.873	0.529	0.336
	Arch	71.00	9.978		
12	Toe	74.73	4.863	0.529	0.401
	Arch	71.64	10.176		
13	Toe	75.01	4.968	0.529	0.366
	Arch	71.59	10.419		
14	Toe	76.37	5.858	0.315	0.257
	Arch	71.97	10.367		
15	Toe	76.99	6.044	0.315	0.221
	Arch	72.13	10.517		
16	Toe	77.13	6.733	0.353	0.240
	Arch	72.26	10.722		
17	Toe	77.69	6.701	0.393	0.231
	Arch	72.71	10.814		
18	Toe	78.16	6.940	0.393	0.244
	Arch	73.21	10.988		
19	Toe	78.01	6.768	0.481	0.291
	Arch	73.59	10.932		
20	Toe	79.14	7.587	0.393	0.218
	Arch	73.74	11.017		
21	Toe	78.73	7.167	0.315	0.238
	Arch	73.64	11.083		
22	Toe	79.20	7.288	0.247	0.240
	Arch	73.99	11.431		
23	Toe	79.83	7.492	0.218	0.216
	Arch	74.38	11.188		

24	Toe	79.81	7.496	0.447	0.279
	Arch	74.75	11.558		
25	Toe	81.03	8.504	0.211	0.187
	Arch	74.66	11.302		
26	Toe	81.37	8.749	0.182	0.180
	Arch	74.77	11.480		
27	Toe	80.79	8.149	0.278	0.224
	Arch	74.99	11.390		
28	Toe	81.58	8.855	0.315	0.230
	Arch	75.60	11.693		
29	Toe	81.68	9.119	0.356	0.290
	Arch	76.22	12.232		
30	Toe	81.16	8.701	0.447	0.289
	Arch	75.81	12.104		
31	Toe	81.86	9.012	0.315	0.284
	Arch	76.38	12.143		
32	Toe	81.37	8.919	0.497	0.290
	Arch	76.17	11.510		
33	Toe	82.22	9.263	0.356	0.252
	Arch	76.41	11.770		
34	Toe	82.07	9.254	0.497	0.277
	Arch	76.54	11.833		
35	Toe	81.51	9.086	0.549	0.309
	Arch	76.39	11.836		
36	Toe	81.81	9.597	0.315	0.240
	Arch	75.75	11.805		
37	Toe	81.53	9.032	0.315	0.233
	Arch	75.52	12.073		
38	Toe	80.49	9.108	0.400	0.276
	Arch	75.07	11.826		

Appendix XI

Individual curves including 95% CI

Healthy Participant Group

In-shoe Skin Temperature – **Summer, left & right toe**

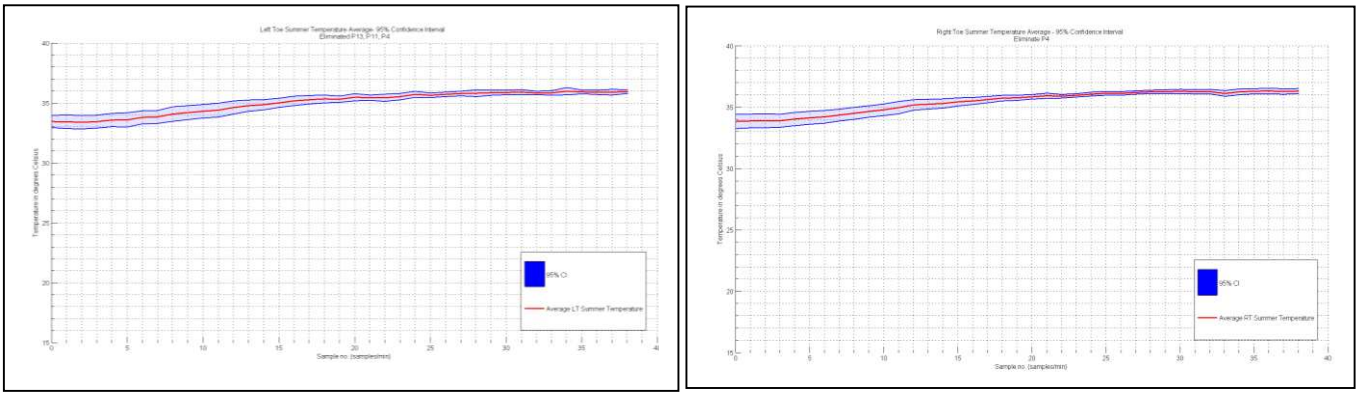


Figure 1: Line graph illustrating in-shoe mean skin toe temperature for healthy participant group at the toes for Left & Right toe including 95% CI

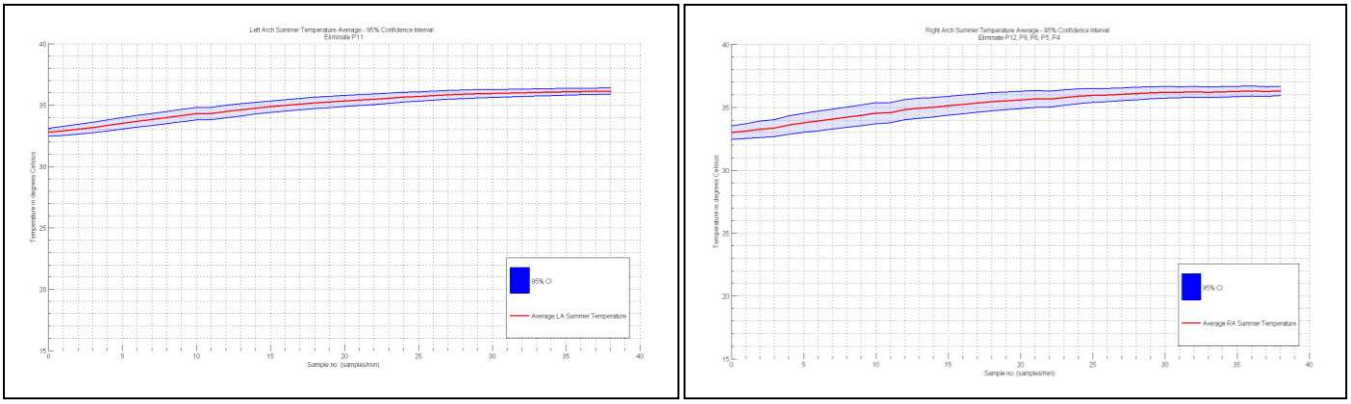


Figure 2: Line graph illustrating in-shoe mean skin temperature arch for healthy participant group at the toes for Left & Right foot including 95% CI

In-shoe Skin Temperature – **Winter, left & right toe**

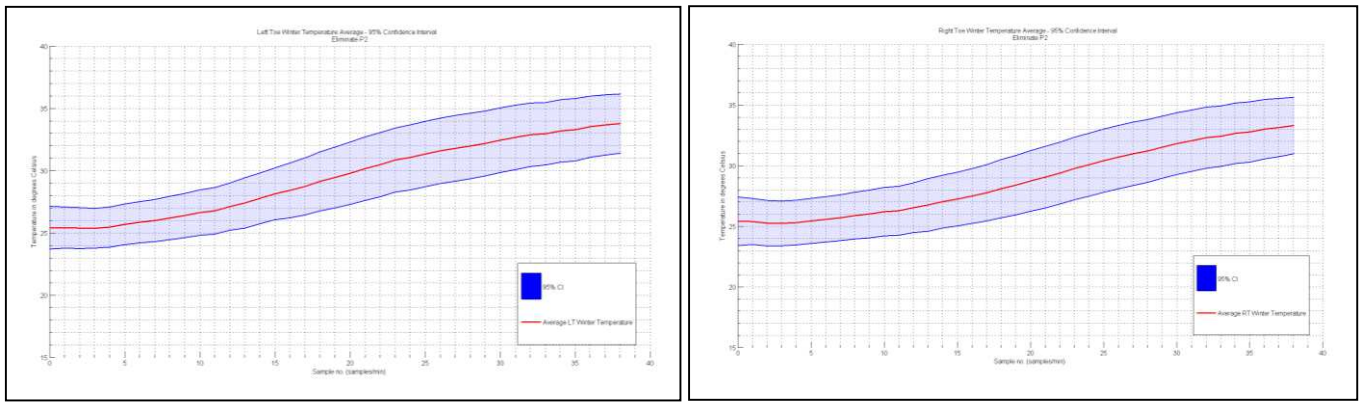


Figure 3: Line graph illustrating in-shoe mean skin toe temperature for healthy participant group at the toes for Left & Right foot including 95% CI

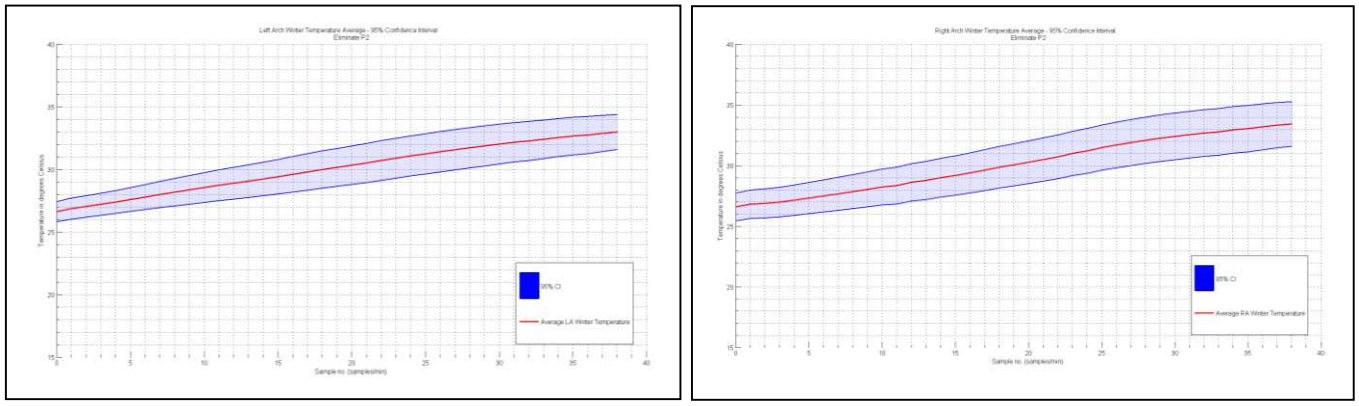


Figure 4: Line graph illustrating in-shoe mean skin temperature arch for healthy participant group at the toes for Left & Right foot including 95% CI

In-shoe Skin RH – **Summer, left & right toe**

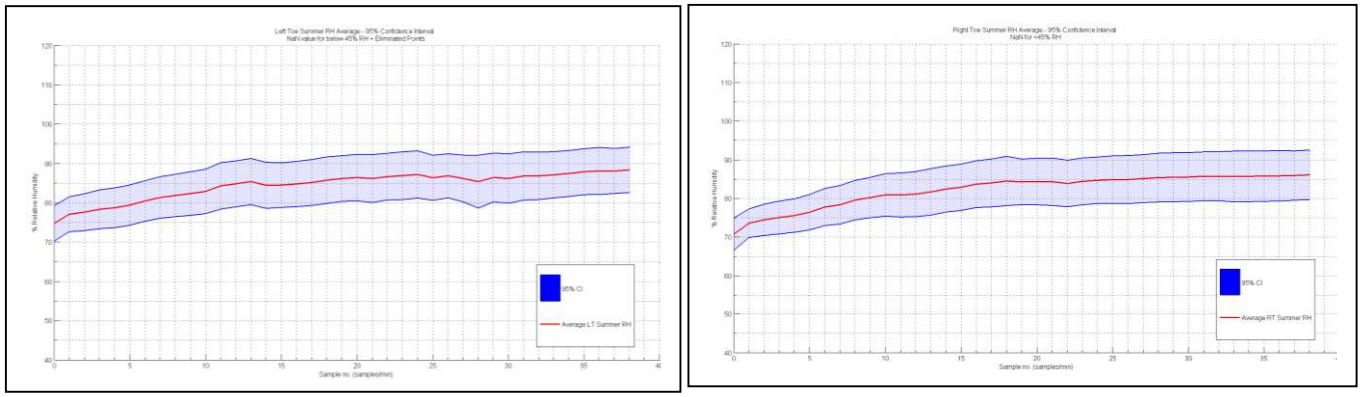


Figure 5: Line graph illustrating in-shoe mean skin toe RH for healthy participant group at the toes for Left & Right toe including 95% CI

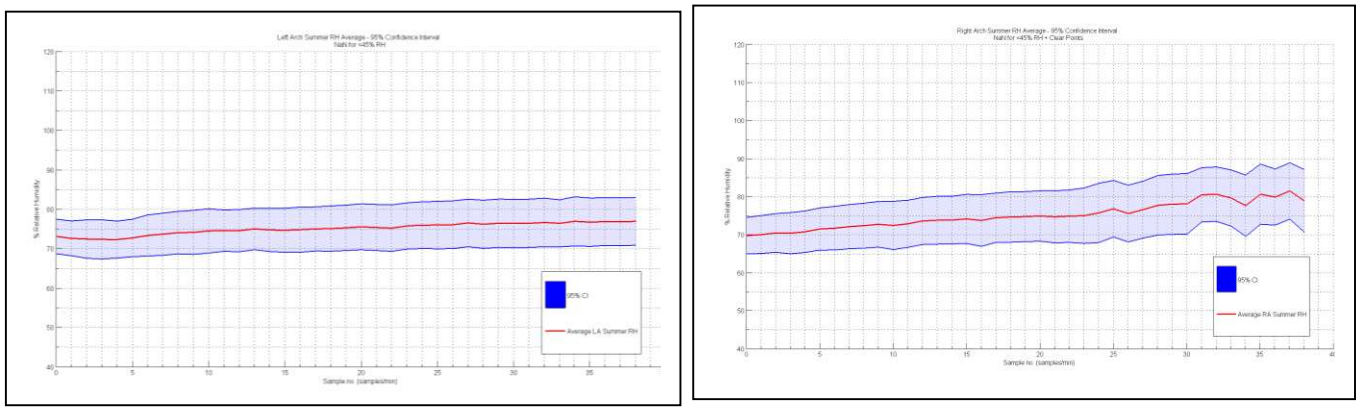


Figure 6: Line graph illustrating in-shoe mean skin RH arch for healthy participant group at the toes for Left & Right foot including 95% CI

In-shoe Skin RH – **Winter, left & right toe**

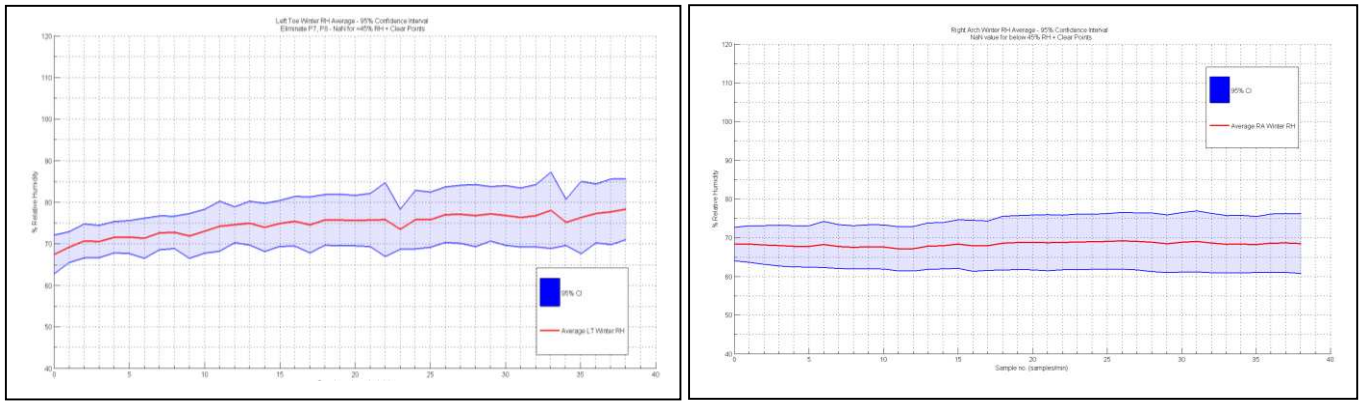


Figure 7: Line graph illustrating in-shoe mean skin toe RH for healthy participant group at the toes for Left & Right foot including 95% CI

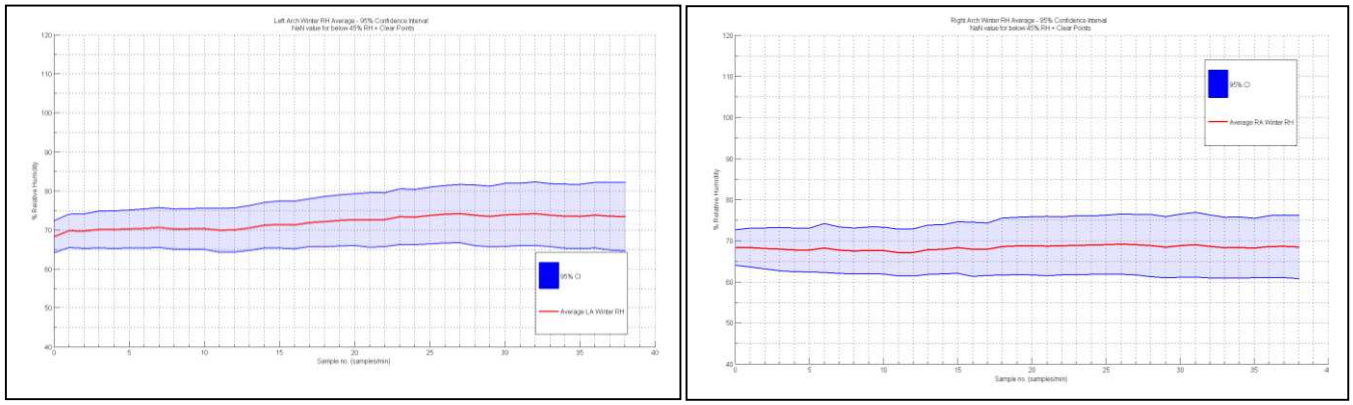


Figure 8: Line graph illustrating in-shoe mean skin RH arch for healthy participant group at the toes for Left & Right foot including 95% CI

Individual curves for DM Subjects including 95% CI

In-shoe Skin Temperature – **Summer**

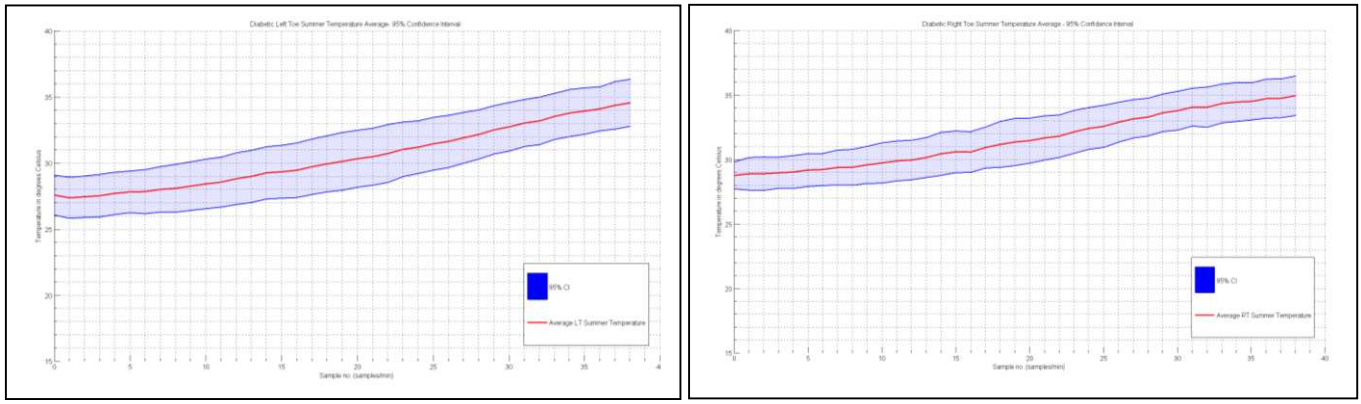


Figure 9: Line graph illustrating in-shoe mean skin toe temperature for DM participant group at the toes for Left & Right foot including 95% CI

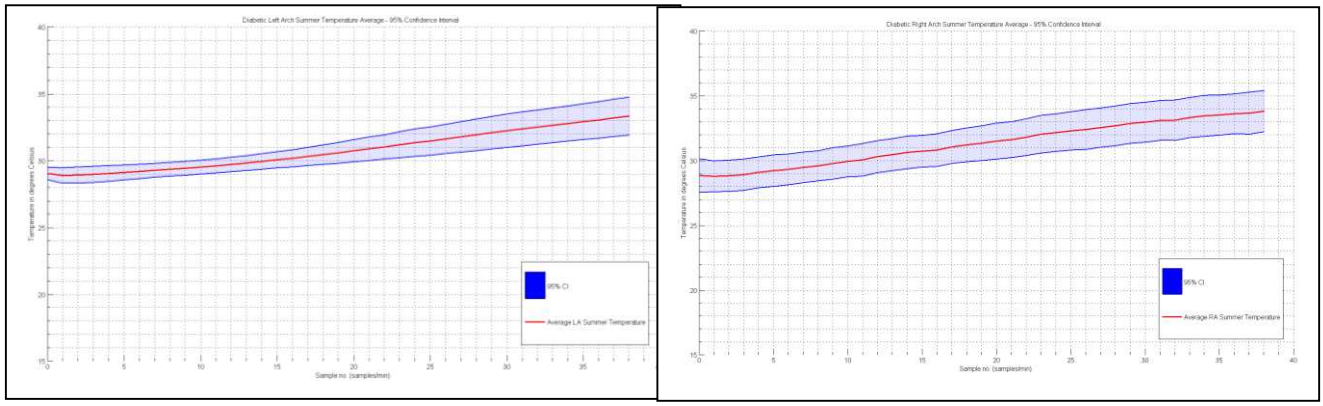


Figure 10: Line graph illustrating in-shoe mean skin temperature arch for DM participant group at the toes for Left & Right foot including 95% CI

In-shoe Skin Temperature – **Winter**

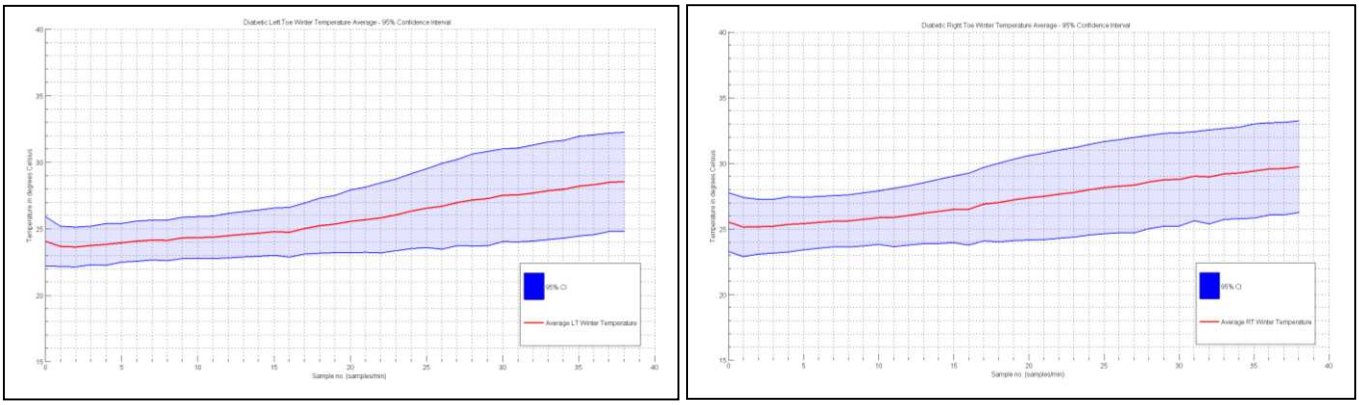


Figure 11: Line graph illustrating in-shoe mean skin toe temperature for DM participant group at the toes for Left & Right foot including 95% CI

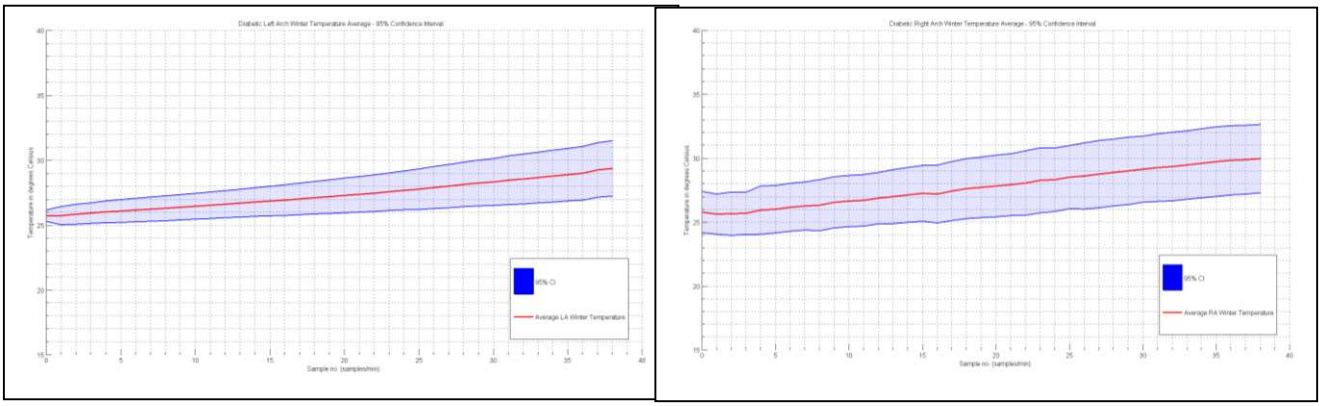


Figure 12: Line graph illustrating in-shoe mean skin temperature arch for DM participant group at the toes for Left & Right foot including 95% CI

In-shoe Skin RH – **Summer**

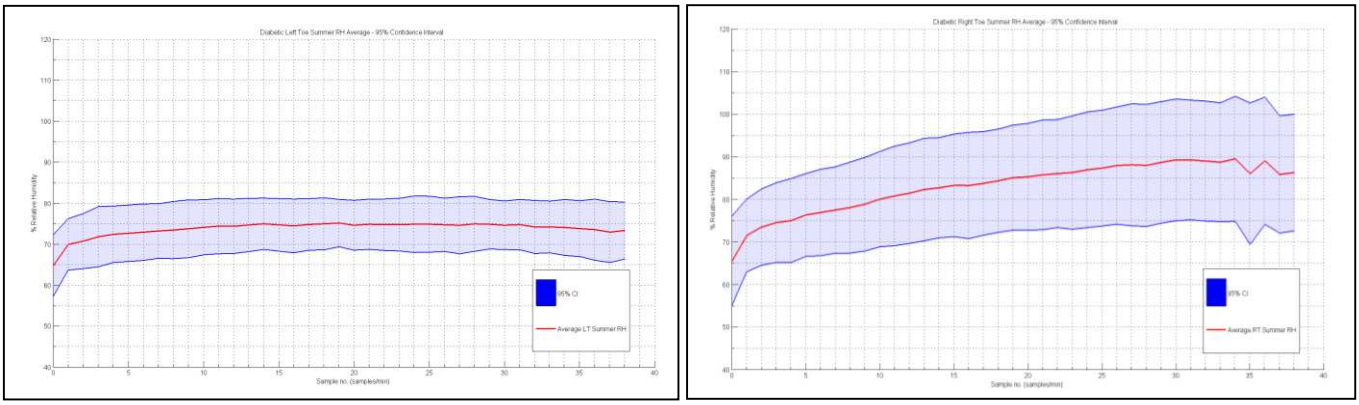


Figure 13: Line graph illustrating in-shoe mean skin toe RH for DM participant group at the toes for Left & Right foot including 95% CI

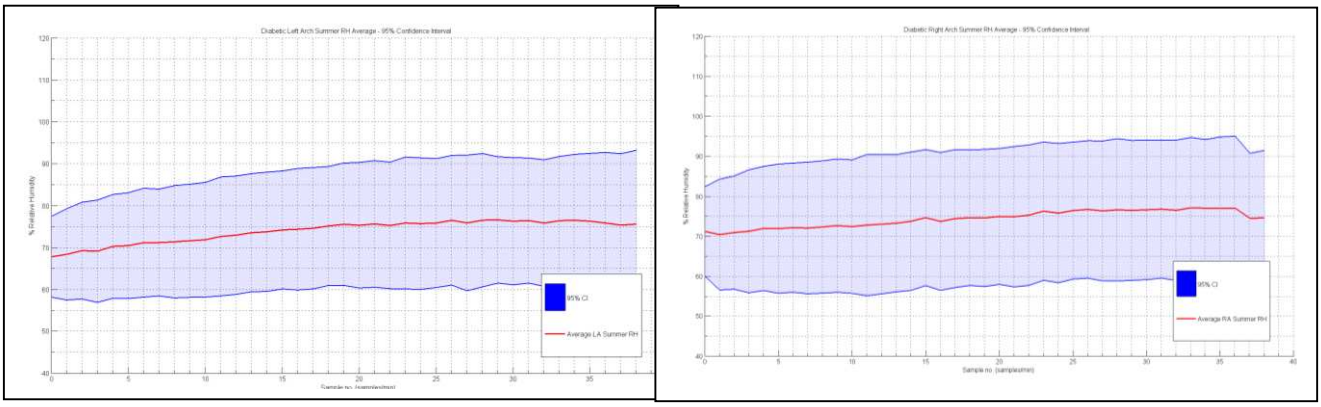


Figure 14: Line graph illustrating in-shoe mean skin RH arch for DM participant group at the toes for Left & Right foot including 95% CI

In-shoe Skin RH – **Winter**

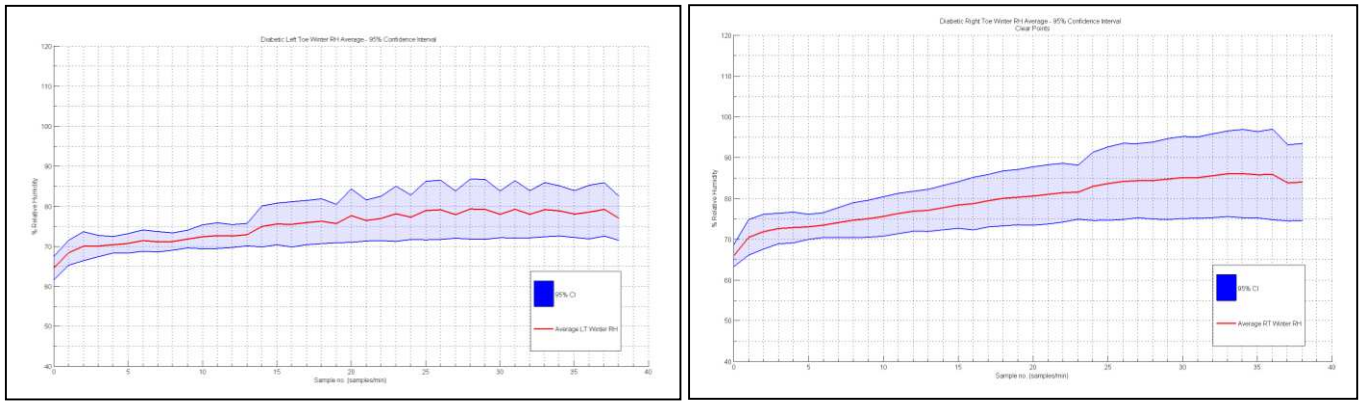


Figure 15: Line graph illustrating in-shoe mean RH toe temperature for DM participant group at the toes for Left & Right foot including 95% CI

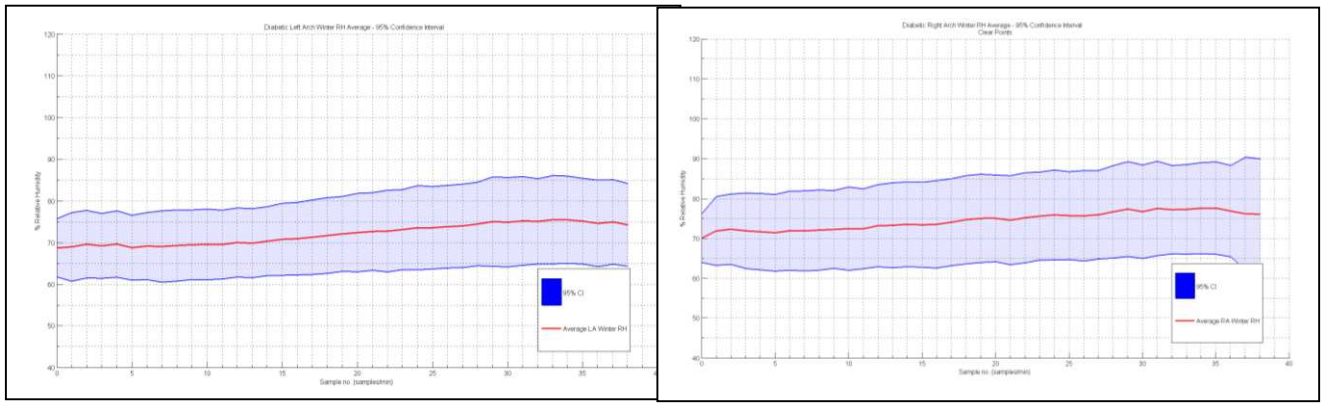


Figure 16: Line graph illustrating in-shoe mean skin RH arch for DM participant group at the toes for Left & Right foot including 95% CI

Appendix XII

Generalized Estimating Equations

Healthy Participant Group

In-Shoe Temperature Analysis

QIC values for the five correlation structures.

Table 1: Quasi Likelihood under Independence for model 1 Criterion (QIC)

Correlation Structure	QIC Value
Independent	32199.8
Autoregressive (Lag 1)	32788.3
Exchangeable	32199.3
M-dependent	32209.5
Unstructured	32567.7

In-Shoe RH Analysis

QIC values for the five correlation structures.

Table 2: Quasi Likelihood under Independence for model 2 Criterion (QIC)

Correlation Structure	QIC Value
Independent	634492.05
Autoregressive (Lag 1)	634903.96
Exchangeable	634667.84
M-dependent	636477.73
Unstructured	639925.99

Diabetic Participant Group

In-Shoe Temperature Analysis

QIC values for the five correlation structures.

Table 3: Quasi Likelihood under Independence for model 1 Criterion (QIC)	
Correlation Structure	QIC Value
Independent	34595.05
Autoregressive (Lag 1)	34528.62
Exchangeable	34506.54
M-dependent (M = 1)	34532.30
Unstructured	36865.51

In-Shoe RH Analysis

QIC values for the five correlation structures

Table 4: Quasi Likelihood under Independence for model 2 Criterion (QIC)	
Correlation Structure	QIC Value
Independent	852320.2
Autoregressive (Lag 1)	852654.6
Exchangeable	852189.3
M-dependent (M = 1)	890623.6
Unstructured	885026.2

Appendix XIII

In-Shoe Temperature Analysis – Healthy Vs DM

Normal vs DM *(TOE)*

Table 1: In-Shoe Temperature Analysis – Normal vs DM (TOE)

		In-shoe Toe Temperature **Summer**				In-shoe Toe Temperature **Winter**			
Time **(/min)**	Location	Mean Temp **(^{0}C)**	SD	P-value Non-Parametric test	P-value Parametric test	Mean Temp **(^{0}C)**	SD	P-value Non-Parametric test	P-value Parametric test
0	Normal	33.64	0.969	0.000	0.000	25.42	3.363	0.639	0.590
	Diabetic	28.16	1.522			24.79	2.341		
1	Normal	33.65	1.005	0.000	0.000	25.41	3.200	0.454	0.369
	Diabetic	28.12	1.718			24.40	2.204		
2	Normal	33.64	1.019	0.000	0.000	25.32	3.171	0.413	0.399
	Diabetic	28.17	1.722			24.39	2.114		
3	Normal	33.66	0.955	0.000	0.000	25.31	3.108	0.454	0.439
	Diabetic	28.24	1.707			24.47	2.060		
4	Normal	33.80	0.973	0.000	0.000	25.39	3.116	0.520	0.460
	Diabetic	28.36	1.700			24.58	2.149		
5	Normal	33.86	1.006	0.000	0.000	25.56	3.150	0.433	0.413
	Diabetic	28.49	1.700			24.67	2.032		
6	Normal	34.00	0.939	0.000	0.000	25.71	3.187	0.337	0.306
	Diabetic	28.52	1.740			24.78	2.030		
7	Normal	34.09	0.918	0.000	0.000	25.84	3.230	0.355	0.287
	Diabetic	28.68	1.814			24.87	2.018		
8	Normal	34.28	0.958	0.000	0.000	26.04	3.317	0.355	0.213
	Diabetic	28.73	1.866			24.86	2.057		
9	Normal	34.41	0.924	0.000	0.000	26.20	3.379	0.393	0.215
	Diabetic	28.91	1.907			25.02	2.077		
10	Normal	34.54	0.917	0.000	0.000	26.41	3.454	0.320	0.176
	Diabetic	29.06	1.976			25.09	2.114		
11	Normal	34.68	0.971	0.000	0.000	26.52	3.509	0.286	0.247
	Diabetic	29.21	1.983			25.11	2.222		
12	Normal	34.90	0.877	0.000	0.000	26.82	3.565	0.241	0.131
	Diabetic	29.38	1.980			25.25	2.287		
13	Normal	35.00	0.778	0.000	0.000	27.08	3.792	0.189	0.198
	Diabetic	29.56	2.000			25.38	2.348		
14	Normal	35.07	0.728	0.000	0.000	27.40	3.811	0.166	0.153
	Diabetic	29.84	2.062			25.49	2.443		
15	Normal	35.21	0.648	0.000	0.000	27.68	3.891	0.155	0.132
	Diabetic	29.96	2.061			25.63	2.520		
16	Normal	35.33	0.604	0.000	0.000	27.95	4.048	0.117	0.055
	Diabetic	30.00	2.061			25.61	2.684		
17	Normal	35.43	0.557	0.000	0.000	28.24	4.172	0.155	0.067

	Diabetic	30.31	2.099			25.95	2.760		
18	Normal	35.53	0.521	0.000	0.000	28.61	4.324	0.135	0.058
	Diabetic	30.54	2.199			26.11	2.920		
19	Normal	35.53	0.462	0.000	0.000	28.91	4.437	0.155	0.053
	Diabetic	30.73	2.263			26.28	3.030		
20	Normal	35.65	0.454	0.000	0.000	29.25	4.532	0.126	0.048
	Diabetic	30.88	2.189			26.46	3.181		
21	Normal	35.68	0.454	0.000	0.000	29.59	4.617	0.101	0.038
	Diabetic	31.06	2.183			26.57	3.262		
22	Normal	35.65	0.454	0.000	0.000	29.92	4.644	0.101	0.043
	Diabetic	31.25	2.158			26.72	3.382		
23	Normal	35.74	0.416	0.000	0.000	30.30	4.663	0.063	0.044
	Diabetic	31.58	2.096			26.90	3.421		
24	Normal	35.88	0.390	0.000	0.000	30.56	4.717	0.063	0.046
	Diabetic	31.79	2.051			27.14	3.493		
25	Normal	35.88	0.385	0.000	0.000	30.86	4.729	0.053	0.041
	Diabetic	32.00	2.046			27.34	3.585		
26	Normal	35.91	0.354	0.000	0.000	31.13	4.734	0.045	0.035
	Diabetic	32.25	2.018			27.46	3.732		
27	Normal	35.98	0.349	0.000	0.000	31.37	4.747	0.045	0.033
	Diabetic	32.51	1.958			27.64	3.767		
28	Normal	36.02	0.421	0.000	0.000	31.58	4.712	0.041	0.033
	Diabetic	32.71	1.893			27.86	3.837		
29	Normal	36.04	0.378	0.000	0.001	31.84	4.661	0.037	0.027
	Diabetic	33.05	1.870			28.00	3.883		
30	Normal	36.07	0.370	0.000	0.001	32.12	4.636	0.031	0.021
	Diabetic	33.25	1.885			28.13	3.836		
31	Normal	36.06	0.379	0.000	0.002	32.35	4.612	0.023	0.018
	Diabetic	33.53	1.835			28.27	3.796		
32	Normal	36.04	0.364	0.000	0.003	32.58	4.569	0.021	0.014
	Diabetic	33.61	1.862			28.31	3.923		
33	Normal	35.96	0.400	0.000	0.006	32.68	4.506	0.021	0.015
	Diabetic	33.92	1.802			28.51	3.893		
34	Normal	36.10	0.480	0.000	0.007	32.91	4.505	0.017	0.012
	Diabetic	34.10	1.806			28.60	3.908		
35	Normal	36.09	0.377	0.000	0.007	33.02	4.501	0.021	0.014
	Diabetic	34.20	1.744			28.80	3.990		
36	Normal	36.09	0.431	0.001	0.013	33.25	4.416	0.015	0.011
	Diabetic	34.39	1.743			28.93	3.960		
37	Normal	36.07	0.440	0.009	0.024	33.39	4.345	0.011	0.009
	Diabetic	34.54	1.793			29.03	3.917		
38	Normal	36.12	0.371	0.046	0.038	33.53	4.239	0.009	0.008
	Diabetic	34.74	1.794			29.13	3.923		

Table 2: In-Shoe Temperature Analysis – Normal vs DM (ARCH)									
		In-shoe Arch Temperature **Summer**				In-shoe Arch Temperature **Winter**			
Time **(/min)**	Location	Mean Temp **(^{0}C)**	SD	P-value Non-Parametric test	P-value Parametric test	Mean Temp **(^{0}C)**	SD	P-value Non-Parametric test	P-value Parametric test
0	Normal	32.85	0.681	0.000	0.000	26.60	1.778	0.117	0.176
	Diabetic	28.93	1.055			25.75	1.275		
1	Normal	32.97	0.764	0.000	0.000	26.84	1.842	0.034	0.075
	Diabetic	28.82	1.008			25.67	1.304		
2	Normal	33.10	0.841	0.000	0.000	26.96	1.877	0.037	0.072
	Diabetic	28.86	1.027			25.74	1.415		
3	Normal	33.23	0.874	0.000	0.000	27.10	1.924	0.031	0.060
	Diabetic	28.93	1.027			25.80	1.392		
4	Normal	33.43	0.945	0.000	0.000	27.27	1.971	0.041	0.072
	Diabetic	29.05	1.008			25.98	1.571		
5	Normal	33.60	0.970	0.000	0.000	27.46	2.037	0.037	0.056
	Diabetic	29.15	1.020			26.04	1.561		
6	Normal	33.76	1.007	0.000	0.000	27.64	2.105	0.028	0.051
	Diabetic	29.24	0.993			26.15	1.580		
7	Normal	33.90	1.020	0.000	0.000	27.83	2.202	0.021	0.045
	Diabetic	29.36	0.984			26.24	1.594		
8	Normal	34.06	1.033	0.000	0.000	28.01	2.270	0.015	0.038
	Diabetic	29.46	0.970			26.30	1.683		
9	Normal	34.21	1.054	0.000	0.000	28.20	2.354	0.023	0.040
	Diabetic	29.59	1.021			26.45	1.693		
10	Normal	34.37	1.069	0.000	0.000	28.39	2.420	0.019	0.034
	Diabetic	29.71	1.011			26.54	1.700		
11	Normal	34.40	1.039	0.000	0.000	28.54	2.499	0.019	0.032
	Diabetic	29.82	1.063			26.61	1.722		
12	Normal	34.60	1.053	0.000	0.000	28.75	2.547	0.015	0.028
	Diabetic	30.00	1.072			26.74	1.722		
13	Normal	34.72	1.042	0.000	0.000	28.92	2.605	0.012	0.027
	Diabetic	30.12	1.081			26.83	1.807		
14	Normal	34.83	0.983	0.000	0.000	29.12	2.652	0.012	0.024
	Diabetic	30.27	1.115			26.94	1.838		
15	Normal	34.95	0.968	0.000	0.000	29.29	2.706	0.012	0.022
	Diabetic	30.38	1.089			27.04	1.879		
16	Normal	35.05	0.960	0.000	0.000	29.50	2.776	0.010	0.015
	Diabetic	30.48	1.126			27.05	1.943		
17	Normal	35.16	0.953	0.000	0.000	29.70	2.841	0.011	0.016
	Diabetic	30.67	1.161			27.21	1.992		
18	Normal	35.26	0.948	0.000	0.000	29.92	2.895	0.010	0.015
	Diabetic	30.81	1.201			27.36	2.035		
19	Normal	35.34	0.928	0.000	0.000	30.11	2.927	0.009	0.013
	Diabetic	30.94	1.242			27.45	2.069		
20	Normal	35.41	0.913	0.000	0.000	30.30	2.978	0.007	0.012
	Diabetic	31.11	1.294			27.55	2.114		
21	Normal	35.49	0.893	0.000	0.000	30.50	3.024	0.008	0.010

	Diabetic	31.23	1.303			27.64	2.131		
22	Normal	35.52	0.855	0.000	0.000	30.71	3.062	0.008	0.009
	Diabetic	31.40	1.343			27.75	2.215		
23	Normal	35.62	0.828	0.000	0.000	30.94	3.080	0.008	0.008
	Diabetic	31.60	1.405			27.91	2.249		
24	Normal	35.72	0.794	0.000	0.000	31.14	3.112	0.005	0.006
	Diabetic	31.74	1.415			27.99	2.227		
25	Normal	35.78	0.762	0.000	0.000	31.35	3.127	0.005	0.006
	Diabetic	31.86	1.451			28.13	2.257		
26	Normal	35.83	0.721	0.000	0.000	31.55	3.158	0.004	0.005
	Diabetic	31.99	1.488			28.24	2.347		
27	Normal	35.89	0.696	0.000	0.000	31.73	3.173	0.004	0.005
	Diabetic	32.14	1.496			28.37	2.397		
28	Normal	35.94	0.692	0.000	0.000	31.90	3.177	0.004	0.004
	Diabetic	32.29	1.524			28.50	2.407		
29	Normal	35.99	0.657	0.000	0.000	32.06	3.194	0.004	0.004
	Diabetic	32.46	1.539			28.61	2.441		
30	Normal	36.02	0.650	0.000	0.000	32.21	3.189	0.002	0.004
	Diabetic	32.58	1.555			28.72	2.431		
31	Normal	36.04	0.609	0.000	0.000	32.35	3.168	0.002	0.004
	Diabetic	32.72	1.561			28.85	2.499		
32	Normal	36.07	0.598	0.000	0.000	32.47	3.167	0.002	0.003
	Diabetic	32.80	1.564			28.94	2.540		
33	Normal	36.07	0.571	0.000	0.000	32.58	3.144	0.002	0.003
	Diabetic	32.97	1.574			29.05	2.548		
34	Normal	36.10	0.572	0.000	0.000	32.74	3.123	0.002	0.003
	Diabetic	33.10	1.601			29.18	2.590		
35	Normal	36.13	0.550	0.000	0.000	32.84	3.110	0.002	0.003
	Diabetic	33.20	1.589			29.30	2.615		
36	Normal	36.16	0.535	0.000	0.000	32.96	3.076	0.002	0.003
	Diabetic	33.31	1.590			29.40	2.623		
37	Normal	36.15	0.516	0.000	0.000	33.09	3.010	0.001	0.002
	Diabetic	33.41	1.645			29.56	2.612		
38	Normal	36.18	0.503	0.000	0.001	33.20	2.950	0.001	0.002
	Diabetic	33.56	1.641			29.66	2.621		

Appendix XIV

In-Shoe RH Analysis – Healthy Vs DM

In-Shoe RH Analysis – **Normal vs DM *(TOE)***

Table 1: In-Shoe RH Analysis – Normal vs DM (Toes)

		In-shoe Toe RH **Summer**				In-shoe Toe RH **Winter**			
Time **(/min)**	Location	Mean RH **(%)**	SD	P-value Non-Parametric test	P-value Parametric test	Mean RH **(%)**	SD	P-value Non-Parametric test	P-value Parametric test
0	Normal	72.68	8.419	0.031	0.026	68.65	8.658	0.162	0.099
	Diabetic	65.14	9.810			65.26	3.127		
1	Normal	75.30	7.841	0.205	0.126	71.08	8.438	0.520	0.564
	Diabetic	70.74	8.093			69.44	4.217		
2	Normal	76.00	8.362	0.205	0.219	73.16	8.860	0.341	0.463
	Diabetic	72.12	8.614			70.96	4.354		
3	Normal	76.67	8.773	0.302	0.295	73.46	9.486	0.483	0.354
	Diabetic	73.19	9.139			71.36	3.746		
4	Normal	77.11	8.951	0.334	0.315	74.27	9.579	0.418	0.247
	Diabetic	73.72	9.241			71.65	3.522		
5	Normal	77.87	9.205	0.317	0.330	74.45	10.203	0.602	0.272
	Diabetic	74.52	9.250			71.90	3.214		
6	Normal	79.08	9.433	0.218	0.242	74.51	11.058	0.733	0.408
	Diabetic	74.93	9.550			72.46	3.259		
7	Normal	79.80	9.732	0.194	0.222	74.91	10.761	0.794	0.354
	Diabetic	75.37	9.484			72.63	3.731		
8	Normal	80.66	9.891	0.142	0.189	74.92	11.219	0.958	0.444
	Diabetic	75.77	9.987			72.95	4.086		
9	Normal	81.23	10.136	0.172	0.199	74.64	12.272	0.931	0.665
	Diabetic	76.33	10.280			73.44	4.199		
10	Normal	81.86	10.492	0.182	0.224	75.38	12.232	0.843	0.630
	Diabetic	77.09	10.384			74.00	4.638		
11	Normal	82.49	10.777	0.191	0.230	76.05	12.577	0.773	0.614
	Diabetic	77.61	10.786			74.51	4.873		
12	Normal	82.85	10.879	0.216	0.230	76.20	12.278	0.986	0.611
	Diabetic	77.92	10.930			74.73	4.863		
13	Normal	83.42	11.087	0.191	0.240	76.53	13.129	0.872	0.624
	Diabetic	78.51	11.134			75.01	4.968		
14	Normal	83.38	11.109	0.257	0.274	76.41	14.067	0.741	0.990
	Diabetic	78.86	10.920			76.37	5.858		
15	Normal	83.64	10.949	0.244	0.262	77.20	14.518	0.639	0.951
	Diabetic	79.01	11.301			76.99	6.044		
16	Normal	84.19	11.050	0.205	0.205	77.78	14.808	0.733	0.858

	Diabetic	78.86	11.668			77.13	6.733		
17	Normal	84.52	11.273	0.194	0.217	77.70	14.963	0.689	1.000
	Diabetic	79.29	11.412			77.69	6.701		
18	Normal	85.08	11.536	0.161	0.212	79.11	14.753	0.639	0.795
	Diabetic	79.68	11.512			78.16	6.940		
19	Normal	85.18	10.959	0.205	0.226	79.44	15.033	0.741	0.697
	Diabetic	80.13	11.585			78.01	6.768		
20	Normal	85.35	11.235	0.182	0.209	79.51	15.183	0.639	0.942
	Diabetic	79.96	11.980			79.14	7.587		
21	Normal	85.20	11.483	0.257	0.264	79.86	15.551	0.689	0.769
	Diabetic	80.32	12.255			78.73	7.167		
22	Normal	85.20	11.280	0.230	0.209	80.33	16.812	0.658	0.790
	Diabetic	80.41	12.281			79.20	7.288		
23	Normal	85.61	11.408	0.218	0.264	79.17	15.779	0.358	0.900
	Diabetic	80.54	12.756			79.83	7.492		
24	Normal	85.90	11.325	0.302	0.267	80.14	16.678	0.565	0.956
	Diabetic	80.91	13.189			79.81	7.496		
25	Normal	85.56	11.131	0.351	0.250	80.15	16.836	0.424	0.882
	Diabetic	81.11	13.285			81.03	8.504		
26	Normal	85.83	11.123	0.404	0.259	80.50	16.510	0.492	0.881
	Diabetic	81.33	13.487			81.37	8.749		
27	Normal	85.60	11.388	0.482	0.308	80.56	16.596	0.516	0.969
	Diabetic	81.37	14.025			80.79	8.149		
28	Normal	85.34	12.220	0.386	0.306	80.34	16.984	0.397	0.838
	Diabetic	81.47	13.822			81.58	8.855		
29	Normal	85.91	11.757	0.442	0.368	80.41	16.467	0.403	0.829
	Diabetic	81.77	13.827			81.68	9.119		
30	Normal	85.80	11.803	0.462	0.405	80.41	16.898	0.492	0.900
	Diabetic	81.95	14.058			81.16	8.701		
31	Normal	86.22	11.671	0.368	0.358	79.96	17.688	0.342	0.760
	Diabetic	82.02	13.919			81.86	9.012		
32	Normal	86.25	11.578	0.244	0.310	80.04	18.193	0.446	0.777
	Diabetic	81.60	14.102			81.37	8.919		
33	Normal	86.34	11.710	0.317	0.289	81.79	18.030	0.536	0.945
	Diabetic	81.47	13.918			82.22	9.263		
34	Normal	86.55	11.691	0.462	0.311	80.30	17.199	0.355	0.773
	Diabetic	81.80	14.744			82.07	9.254		
35	Normal	86.79	11.653	0.116	0.111	81.27	18.490	0.701	0.971
	Diabetic	79.24	13.378			81.51	9.086		
36	Normal	86.92	11.771	0.286	0.238	81.28	17.959	0.643	0.934
	Diabetic	81.31	15.085			81.81	9.597		
37	Normal	86.96	11.393	0.142	0.096	81.65	18.303	0.715	0.979
	Diabetic	79.40	13.683			81.53	9.032		
38	Normal	87.19	11.481	0.125	0.105	81.82	18.133	0.741	0.774
	Diabetic	79.84	13.504			80.49	9.108		

In-Shoe RH Analysis – **Normal vs DM *(Arch)***

Table 2: In-Shoe RH Analysis – Normal vs DM (Arch)									
		In-shoe Arch RH **Summer**				In-shoe Arch RH **Winter**			
Time **(/min)**	Location	Mean RH **(%)**	SD	P-value Non-Parametric test	P-value Parametric test	Mean RH **(%)**	SD	P-value Non-Parametric test	P-value Parametric test
0	Normal	71.41	8.787	0.613	0.593	68.30	7.881	0.568	0.692
	Diabetic	69.52	11.334			69.43	7.067		
1	Normal	71.31	8.888	0.568	0.685	69.06	8.450	0.683	0.665
	Diabetic	69.41	13.418			70.45	9.186		
2	Normal	71.40	9.235	0.625	0.790	68.91	8.851	0.482	0.529
	Diabetic	70.11	13.915			71.00	9.200		
3	Normal	71.34	9.637	0.749	0.827	69.03	9.419	0.732	0.658
	Diabetic	70.21	14.960			70.58	9.403		
4	Normal	71.53	9.556	0.732	0.940	68.93	9.574	0.683	0.618
	Diabetic	71.14	15.110			70.70	9.528		
5	Normal	72.09	9.691	0.782	0.868	68.99	9.610	0.858	0.749
	Diabetic	71.20	15.585			70.12	9.494		
6	Normal	72.48	10.137	0.775	0.882	69.34	10.107	0.749	0.741
	Diabetic	71.67	15.751			70.58	9.798		
7	Normal	72.85	10.300	0.749	0.826	69.17	10.200	0.708	0.725
	Diabetic	71.64	15.827			70.50	10.137		
8	Normal	73.17	10.474	0.724	0.816	68.87	10.132	0.568	0.621
	Diabetic	71.86	16.180			70.73	10.138		
9	Normal	73.39	10.686	0.724	0.828	68.96	10.280	0.568	0.608
	Diabetic	72.16	16.302			70.90	9.861		
10	Normal	73.42	11.102	0.749	0.787	68.95	10.370	0.568	0.588
	Diabetic	72.14	16.392			71.04	10.334		
11	Normal	73.70	10.763	0.757	0.870	68.52	10.666	0.546	0.526
	Diabetic	72.73	17.201			71.00	9.978		
12	Normal	74.07	10.818	0.732	0.855	68.56	10.755	0.404	0.436
	Diabetic	73.00	17.048			71.64	10.176		
13	Normal	74.40	10.869	0.782	0.866	69.16	11.068	0.546	0.549
	Diabetic	73.41	16.872			71.59	10.419		
14	Normal	74.29	11.019	0.832	0.930	69.56	11.163	0.482	0.554
	Diabetic	73.77	17.022			71.97	10.367		
15	Normal	74.40	11.295	1.000	0.992	69.87	11.616	0.546	0.593
	Diabetic	74.44	16.774			72.13	10.517		
16	Normal	74.27	11.768	0.858	0.964	69.59	12.007	0.546	0.540
	Diabetic	74.05	17.114			72.26	10.722		
17	Normal	74.72	11.330	0.987	0.968	69.90	11.847	0.442	0.514
	Diabetic	74.52	17.108			72.71	10.814		
18	Normal	74.86	11.604	0.832	0.993	70.35	12.662	0.442	0.531
	Diabetic	74.91	16.807			73.21	10.988		
19	Normal	75.00	11.556	0.961	0.987	70.59	12.787	0.503	0.514
	Diabetic	75.08	17.129			73.59	10.932		
20	Normal	75.21	11.659	0.961	0.990	70.70	12.988	0.546	0.514
	Diabetic	75.15	17.210			73.74	11.017		
21	Normal	75.02	11.899	0.807	0.960	70.63	13.458	0.590	0.530
	Diabetic	75.27	17.601			73.64	11.083		
22	Normal	75.03	11.995	0.832	0.960	70.70	13.201	0.613	0.488
	Diabetic	75.28	17.599			73.99	11.431		

23	Normal	75.37	12.366	0.807	0.890	71.14	13.591	0.590	0.504
	Diabetic	76.08	17.762			74.38	11.188		
24	Normal	75.83	12.568	0.853	0.988	71.10	13.460	0.546	0.452
	Diabetic	75.76	17.815			74.75	11.558		
25	Normal	76.37	12.310	0.853	0.963	71.37	13.735	0.590	0.502
	Diabetic	76.14	17.483			74.66	11.302		
26	Normal	75.80	12.705	0.807	0.876	71.60	13.968	0.613	0.525
	Diabetic	76.61	17.575			74.77	11.480		
27	Normal	76.54	12.695	0.909	0.936	71.61	14.123	0.613	0.500
	Diabetic	76.11	18.104			74.99	11.390		
28	Normal	76.91	12.791	0.853	0.949	71.28	14.587	0.462	0.405
	Diabetic	76.57	18.129			75.60	11.693		
29	Normal	77.19	12.906	0.960	0.903	70.94	14.459	0.286	0.311
	Diabetic	76.54	17.544			76.22	12.232		
30	Normal	77.19	12.941	0.880	0.891	71.33	14.975	0.462	0.400
	Diabetic	76.46	17.578			75.81	12.104		
31	Normal	78.29	12.016	0.958	0.742	71.50	15.061	0.462	0.364
	Diabetic	76.61	17.318			76.38	12.143		
32	Normal	78.47	12.058	0.876	0.656	71.38	15.045	0.503	0.368
	Diabetic	76.18	17.585			76.17	11.510		
33	Normal	77.96	12.319	0.960	0.818	71.03	14.764	0.442	0.306
	Diabetic	76.76	17.726			76.41	11.770		
34	Normal	77.27	13.483	0.909	0.926	70.92	14.886	0.302	0.289
	Diabetic	76.77	17.702			76.54	11.833		
35	Normal	78.52	12.687	0.931	0.725	70.85	14.735	0.351	0.293
	Diabetic	76.62	18.339			76.39	11.836		
36	Normal	78.30	12.380	0.987	0.728	71.19	15.188	0.404	0.396
	Diabetic	76.44	18.751			75.75	11.805		
37	Normal	78.96	12.275	0.741	0.443	71.06	15.469	0.519	0.436
	Diabetic	74.93	17.910			75.52	12.073		
38	Normal	77.84	13.162	0.880	0.621	70.93	15.635	0.566	0.471
	Diabetic	75.12	18.539			75.07	11.826		