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DOCTOR OF PHILOSOPHY

Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

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**University
of Dundee**

**ASSESSMENT OF THE IMPACT OF
LOADING PRESSURE ON
ENDOTHELIAL FUNCTION IN
DIABETIC FOOT**

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M.B. B.CH.

MSc (ORT)

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LIST OF ABBREVIATIONS

ACh	- Acetylcholine
ANOVA	- Analysis of Variance
AT-II	- Angiotensin-II
BMI	- Body Mass Index
CA	- Contact Area
DFS	- Diabetic Foot Syndrome
DL midfoot	- Dorsum Lateral midfoot
DMH1	- Dorsum 1st metatarsal head
DMH2	- Dorsum 2nd metatarsal head
DMH3	- Dorsum 3rd metatarsal head
DMH4	- Dorsum 4th metatarsal head
DMH5	- Dorsum 5th metatarsal head
DM midfoot	- Dorsum Medial midfoot
DPN	- Diabetic Peripheral Neuropathy
DT Lat	- Dorsum Toes Lateral Side
DT Med	- Dorsum Toes Medial Side
EDHF	- Endothelium-derived Hyperpolarization Factor
eNOS	- expressing Nitric Oxide Synthase
ET-1	- Endothelin1
gc	- guanylate cyclase
GRF	- Ground Reaction Force
HS	- Highly Significant
ICC	- Interclass Correlation Coefficient
IDF	- International Diabetes Federation
IMAR	- Institute of Motion Analysis and Research
IQR	- Interquartile range
IWGDF	- International Working Group on the Diabetic Foot
KPa	- Kilo Pascal
LDF	- Laser Doppler Flowmetry
LDPI	- Laser Doppler Perfusion Imaging
LDPM	- Laser Doppler Perfusion Monitoring
LSD	- Least Significant Difference
Max	- Maximum

MF - Maximum Force

Min - Minimum

MPP - Maximum Peak Pressure

N - Newton

NADPH - Nicotinamide Adenine Dinucleotide Phosphate

NHS - National Health Service

NO - Nitric Oxide

P - Probability

PG - Prostaglandins

PGH2 – Prostaglandins

PL heel - Plantar Lateral heel

PL midfoot - Plantar Lateral midfoot

PMH1 - Plantar 1st Metatarsal Head

PMH2 - Plantar 2nd Metatarsal Head

PMH3 - Plantar 3rd Metatarsal Head

PMH4 - Plantar 4th Metatarsal Head

PMH5 - Plantar 5th Metatarsal Head

PM heel - Plantar Medial heel

PM midfoot - Plantar Medial midfoot

PP - Peak Pressure

PT1 - Plantar Toes 1

PT2 - Plantar Toes 2

PT3 - Plantar Toes 3

PTI - Pressure Time Integral

PU - Perfusion Median Values

ReTIS - Rehabilitation Technology Information Service

S - Significant difference

SD - Standard Deviation

SDRN - Scottish Diabetes Research Network

SNP - Sodium Nitroprusside

SPSS - Statistical Package of Social Science software

TORT - Tayside Orthopaedic and Rehabilitation Technology Centre

VSM - Vascular Smooth Muscle

WHO - World Health Organization

DEDICATION

I thank Allah (Alhamdo Lilah) for this achievement and I dedicate this work to my beloved parents who always provide me with their endless, unconditional love and support. Thank you for your persistent belief in my abilities. I will not ever be able to thank you enough for everything you have done for me.

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DECLARATION

I hereby declare that this dissertation entitled “Assessment Of The Impact Of Loading Pressure On Endothelial Function In Diabetic Foot” has been prepared by me under the direct guidance of Prof Faisel Khan and Dr Graham Arnold as part of my study for the award of PhD Degree at the University of Dundee, Dundee, Scotland.

I have not submitted this dissertation previously for the award of any degree or diploma at any other institution.

14/10/2020

Rania Edris

2019–20 CORONAVIRUS PANDEMIC

A special note regarding the 2019–20 coronavirus pandemic, written on 16th April 2020.

In late 2019 and throughout 2020 severe disruption was caused to many aspects of life across the world. From approximately the end of February 2020, the work presented here was affected by this disruption.

ABSTRACT

Background: Despite increased awareness and efforts to improve prevention, foot problems account for the most serious and costly complication of diabetes. Amputations a major adverse outcome of diabetic foot can be mostly prevented as 85% of amputations are preceded by foot ulceration. Yet, it has been estimated that the lifetime risk of a patient with diabetes to develop foot ulcer is 15-25%. High pressure and endothelial dysfunction are two major contributing elements in the development of diabetic foot ulceration. Higher prevalence of non-plantar ulcers with the majority of ulcers located on the foot dorsum, in addition to lower healing rates compared with plantar ulcers have been reported in diabetic foot. However, earlier studies and guidelines have so far focused on plantar ulceration, plantar pressure measurements and different interventions for plantar pressure relief for ulcer prevention and treatment with no assessment of the dorsal surface.

Aim of the study: To investigate foot pressures experienced on both dorsal and plantar surfaces of the foot, and the impact of pressure application on endothelial function of the superficial skin blood vessels on both aspects of the foot in subjects with Type 2 diabetes mellitus in comparison to a group of control without diabetes.

Methods: In-shoe pressure experienced on dorsal and plantar surfaces of the foot were assessed using Pedar insole system in subjects' own shoes and orthopaedic shoes known to be prescribed for diabetic patients. In-shoe peak pressures (PP) were applied by a device designed to deliver a known pressure along with housing a laser Doppler flowmetry probe to assess blood flow changes. The effect of pressure on the skin blood flow response to iontophoresis of acetylcholine (ACh), an endothelium-dependent vasodilator and sodium nitroprusside (SNP), an endothelium-independent vasodilator, were assessed in a group of subjects with Type 2 diabetes and an age-matched control group of subjects with no diabetes.

Results: No significant differences were found between the two study groups in dorsal PP within the orthopaedic shoes ($p=0.409$) as well as in participants' own shoes ($p=0.389$). However, both study groups had a significantly higher dorsal PP ($p<0.001$) in their own shoes when compared with the orthopaedic shoes. No significant differences in planter PP were detected between groups in participants' own shoes ($p=0.384$), though, midfoot areas were significantly higher in diabetes groups and lateral areas under toes and metatarsal heads were significantly reduced. The orthopaedic shoes showed a significantly higher plantar PP ($p=0.013$) in the diabetes group compared to control. A significantly higher in-shoe plantar PP within participants' own shoes than orthopaedic shoes ($p<0.05$) was noted in both study groups. However, this significant difference was apparent in one foot area in the diabetes group. No significant correlations were detected between PP and changes in blood flow in response to the iontophoresis of ACh or SNP on dorsal and plantar surfaces in both study groups. Both study groups have shown a significant reduction ($p<0.001$) in blood flow response to the iontophoresis of ACh and SNP under own shoes PP as well as orthopaedic shoes PP than resting /no pressure condition on the dorsal and plantar surfaces of the foot. A significantly higher change in response ($p<0.05$) was recorded in the control group than diabetes in blood flow changes in response to iontophoresis of ACh under no pressure. The control group showed a significantly higher change in response ($p<0.001$) under the orthopaedic shoes dorsal PP than own shoes with ACh and SNP while diabetes group only recorded a significant change in response with SNP. The diabetes group had a significantly higher blood flux values on the plantar surface in response to ACh iontophoresis in resting /no pressure, under orthopaedic PP and own shoes PPs. However, no significant differences from the control group were detected in changes in response from baseline flux with the iontophoresis of ACh or SNP on the plantar surface under any of the pressure conditions.

Conclusion: Although orthopaedic footwear had significantly reduced total in-shoe PP, pressure assessment is essential to adjust shoe design in-order to better distribute dorsal as well as plantar pressures and achieve effective offloading required for ulcers prevention. Diabetes group showed an increased blood flow values on the plantar surface, which could have been caused by an early sympathetic neuropathy. Additionally, diabetes group had an impaired endothelium-dependent response which may predispose to foot ulceration and the development of vascular complications in this group. Though low PPs were recorded on the foot dorsum, a significant reduction in blood flux response was still present on applying dorsal PP. Also, the dorsal foot surface was more sensitive to changes related to endothelial dysfunction in patients with diabetes. Therefore, dorsal pressure measurement and pressure's impact investigation can provide valuable input in the assessment of diabetic foot and should be considered in the design and prescription of therapeutic footwear to reduce the risk of diabetic foot ulceration.

1.1 BACKGROUND

Diabetes mellitus is recognised as a syndrome; a collection of disorders that have hyperglycaemia and glucose intolerance as their hallmark, as a result of insulin deficiency, impaired effectiveness of the insulin's action, or a combination of both (Magliano *et al.*, 2015). The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels that significantly impair those patients' quality of life (American Diabetes Association, 2014).

No country, whether wealthy or poor, is immune from this global epidemic. In 2017 the worldwide prevalence of diabetes mellitus reached over 425 million people; that is 1 in 11 adults has diabetes. Two-thirds (327 million) are of working age (20-64 years). A further 352 million people have impaired glucose tolerance and are at a high risk of developing diabetes. International Diabetes Federation (IDF) estimates that 212 million people worldwide, close to half of the people with diabetes, are unaware of their disease with most of these cases being Type 2 diabetes. As many as one-third to one-half of Type 2 diabetes cases can go undiagnosed because sufferers may remain without symptoms for years until presenting with complications of hyperglycaemia (IDF Diabetes Atlas, 2017). In the UK, Type 2 diabetes accounts for 90.4% of all diabetes with approximately one in 22 being diagnosed with Type 2 diabetes (approximately 4.5% of the UK population) (Holman *et al.*, 2015). Despite being largely preventable, the number of people with Type 2 diabetes is growing rapidly worldwide (Kakkar, 2016). This is likely due to economic development, ageing populations, increasing urbanisation, dietary changes, reduced physical activity, and changes in other lifestyle patterns (Cho *et al.*, 2018). This rise in

prevalence is accompanied by a proportional increase in numbers of people with diabetes-related complications, including foot problems.

Foot complications occur in both Type 1 and Type 2 diabetes (Jeffcoate and Harding, 2003). The term ‘diabetic foot’ includes any foot pathology that results directly from diabetes or its long-term complications (Boulton, 2010). According to the World Health Organization (WHO), it is possible to encompass all foot complications in the term diabetic foot syndrome (DFS), that has been defined as ulceration of the foot (distally from the ankle and including the ankle) associated with neurological abnormalities, various degrees of peripheral vascular disease and infection (Katsilambros N *et al.*, 2007, Tuttolomondo *et al.*, 2015).

The major adverse outcomes of diabetic foot are foot ulcers and amputations. Studies suggest the global prevalence of diabetic foot ulceration is approximately 6.3% worldwide which is higher in males than females and more common in Type 2 Diabetic patients (Zhang *et al.*, 2017). It has been estimated that 15-25% of diabetics are at risk of developing a foot ulcer at some point during their lifetime with 9.1-26.1 million people developing diabetic foot ulcers annually (American Diabetes Association, 1999, Armstrong *et al.*, 2017). Moreover, 70% of foot ulcer patients will have recurrent lesions within five years after treatment (Ferreira *et al.*, 2004, Hooegeveen *et al.*, 2015).

It has been claimed that a lower limb is amputated every thirty seconds due to diabetes (Apelqvist, 2012). Studies show the rate of lower extremity amputation is 10-20 times higher in diabetic patients compared with non-diabetics (Moxey *et al.*, 2011). And it is estimated that up to 50-70% of all non-traumatic amputations throughout the world occur in diabetic patients (Abbott *et al.*, 1998, Hooegeveen *et al.*, 2015). An alarming proportion (50%) of those who have had a major amputation have died within two years following

their procedure (MacRury *et al.*, 2018). The majority of these amputations are considered preventable as 85% are preceded by foot ulceration (Forlee, 2010, Al Sayah *et al.*, 2015).

Therefore, several countries and organisations, such as WHO and the International Diabetes Federation, have worked toward setting a strategy with goals to reduce the rate of amputations by up to 50%. By identifying patients at risk of developing foot disease, taking the appropriate preventive measures, patient and staff education, multidisciplinary treatment of foot ulcers and close monitoring, it has been claimed that amputation rates can be reduced by 49–85% (Bakker *et al.*, 2012).

Despite increased awareness and efforts to improve prevention, diabetic foot accounts for the first cause for hospitalisation of diabetic patients with 4.7% of inpatients worldwide reported with diabetes-related foot disease (Lazzarini *et al.*, 2015). Thus, diabetic foot poses a heavy financial burden to healthcare systems all over the world. The UK National Health Service (NHS) expenditure on diabetic foot disease is equivalent to approximately £1 in every £175 spent by the NHS in England (Kerr *et al.*, 2014) and it is estimated that £60 million is spent on foot ulcers and amputations in Scotland alone (MacRury *et al.*, 2018). In addition to, the significant psychosocial effects it may have on patients' quality of life because of impaired mobility and substantial loss of productivity (Boulton *et al.*, 2005, Bakker *et al.*, 2012).

Many factors have been investigated as having a role in diabetic foot ulcer formation such as neuropathy, increased biomechanical stress, external trauma, impairment of the local vascular supply and endothelial dysfunction. Furthermore, the slower healing rate, which is often complicated by infection, leads to amputation as the final outcome. It is generally thought that a combination of several mechanisms has been involved in ulcer formation (Figure 1.1) (Schaper *et al.*, 2003, Pendsey, 2010, Korzon-Burakowska and Dziemidok, 2011, Bakker *et al.*, 2012).

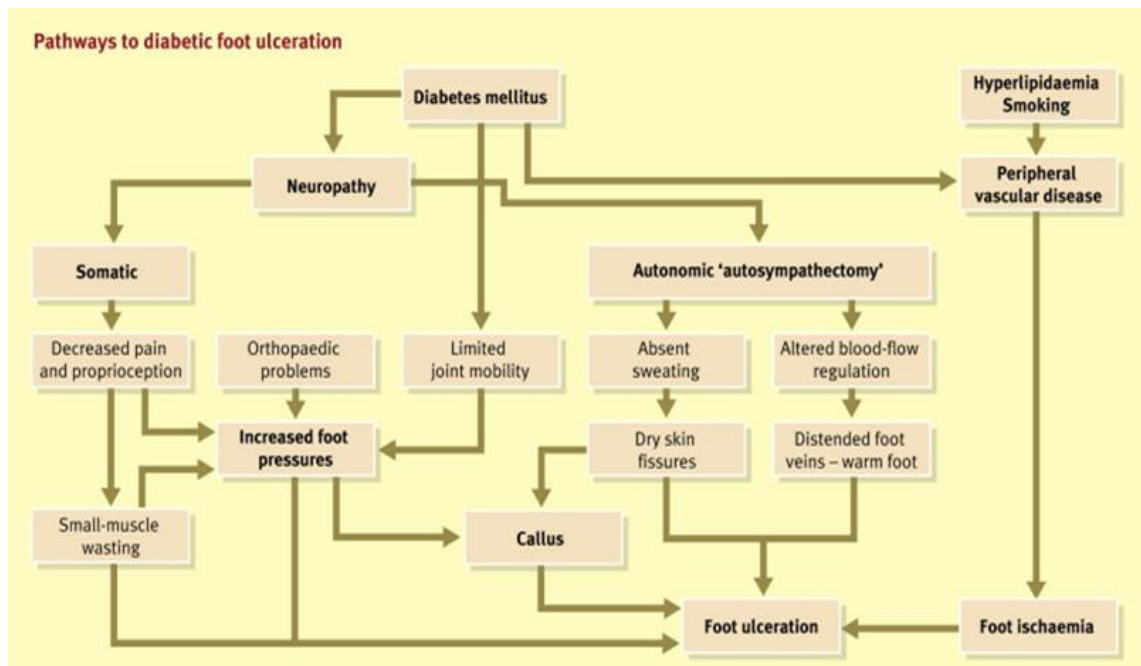


Figure 1.1 Mechanisms of Diabetic Foot ulceration
Adapted from Boulton 2010

Affecting up to 50% of diabetic patients, peripheral neuropathy is among the most common of all long-term complications of diabetes. Diabetic peripheral neuropathy plays a central role in ulcer pathogenesis and has been reported in >80% of ulcer affected patients (Abbott *et al.*, 1998, Korzon-Burakowska and Dziemidok, 2011). While impairment of local vasculature and endothelial dysfunction are other known complications of diabetes, regular trauma usually unperceived, may further damage the skin microcirculation including the endothelium, thus increasing the risk of ulceration (Newton *et al.*, 2005).

It was noted that 66% of patients with diabetic peripheral neuropathy wear shoes that are too narrow and yet ill-fitting footwear is well recognised as a cause of foot injury and ulceration (Veves *et al.*, 1992). Ninety-four percent of diabetic ulcers are known to occur under areas of increased pressure (Pataky *et al.*, 2000). Consequently, the reduction of plantar pressure has been suggested to play a key role in the treatment of plantar ulcers and thus, the prevention of amputations. This could be achieved by correctly fitting footwear, full contact insoles, orthopaedic footwear and foot orthoses which can be

effective in redistributing plantar pressure and as a result promote ulcer healing (Fiedler *et al.*, 2011).

Foot ulcers have previously been reported on both plantar and dorsal surfaces of diabetic foot, yet no research has been conducted to investigate the effect of pressure and endothelial dysfunction on the dorsum of the foot. Prompers *et al.* 2007, studied the prevalence of foot ulcers in diabetic patients and found that more than half of ulcers (52%) were non-plantar ulcers and the majority of all foot ulcers were located on the dorsal surface or in the interdigital spaces of toes (32%). Moreover, their results have indicated that ulcers in patients with peripheral arterial disease and infection are mainly non-plantar (65%) and are associated with more extensive tissue loss as they were also deeper and larger.

Despite the fact of relatively high rates of non-plantar ulcers and healing rates, which may be lower in dorsal ulcers compared with plantar ulcers (Eneroth *et al.*, 2004, Prompers *et al.*, 2007, Roth-Albin *et al.*, 2017), most earlier studies and guidelines have focused on plantar foot ulceration including plantar pressure measurements and different interventions for pressure relief as an ulcer treatment modality.

1.2 RATIONALE FOR THE STUDY

The current study proposes to investigate the effect of pressure application on endothelial function on both dorsal and plantar surfaces of the foot when comparing findings in subjects with Type 2 diabetes with an age-matched control group of subjects without diabetes.

Peak pressures exerted on both dorsal and plantar surfaces of the foot have been measured within participants' own comfortable shoes which they have chosen to bring on the assessment session as well as a therapeutic footwear that is commonly prescribed for at-risk patients with Diabetes Mellitus that will be referred to as "orthopaedic shoes". Endothelial function under the loading pressure has been non-invasively assessed using laser Doppler flowmetry (LDF). Blood perfusion changes under the loading pressure have been studied in response to the iontophoresis of two vasoactive agents, acetylcholine (ACh) an endothelium-dependent vasodilator and sodium nitroprusside (SNP) an endothelium-independent vasodilator. The premeasured loading pressures will be simulated using a pressure delivery equipment that was specifically developed to apply a given pressure to a local area whilst not damaging or interfering with the iontophoresis equipment or the laser Doppler probe which have been utilised as the endpoint for pressure delivery and monitoring of the skin blood flow changes.

This experimental work hypothesised that dorsal pressure measurement and its effect investigation will provide a more easily accessible tool for assessment of the diabetic foot (Newton *et al.*, 2005). Additionally, this work will add to the understanding of the mechanisms underlying the development of diabetic foot ulcer and help the future production and design of better therapeutic interventions to prevent diabetic foot ulceration and its subsequent burden.

1.3 AIM

The aim of the present study has been to investigate the foot pressure experienced on both dorsal and plantar surfaces of the foot, and the effect of pressure application on endothelial function of the superficial skin blood vessels on both aspects of the foot in subjects with Type 2 diabetes mellitus in comparison to the response in a group of control without diabetes.

1.4 OBJECTIVES

- Review relevant literature
- Determine peak walking in-shoe pressure on the dorsal as well as the plantar surface of the foot and examine differences in areas that experience high pressure between a group of Type 2 diabetic patients and a control group of subjects without diabetes
- Investigate the endothelial function of the superficial blood vessels of the foot by assessing blood flow changes in response to the iontophoresis of an endothelium-dependent vasodilator, acetylcholine (ACh) and endothelium-independent vasodilator, sodium nitroprusside (SNP) under peak walking pressures experienced in participants' own shoes and within a standard therapeutic footwear on the dorsal and plantar surfaces of the foot in both study groups
- Compare results of diabetic subjects with an age-matched control group of subjects with no diabetes

2.1 FOOT PRESSURE

2.1.1 Introduction

Pressure¹, a form of mechanical stress, is equal to the magnitude of the force applied to a specific surface area. Foot pressure represents the pressure field that acts between the foot and the external, supporting surface during everyday locomotor activities (Abdul Razak *et al.*, 2012). As the standard to reflect the balance of the human body, foot pressure is the one measurement which is of great interest in the clinical and research fields of kinematics (Park *et al.*, 2009, Huang *et al.*, 2013, Giacomozzi *et al.*, 2016). Foot pressure distribution is made by the anatomical and functional status or state of the foot/shoes and the ground surface while walking (Park *et al.*, 2009). Hence, foot pressure is a known critical variable in orthotic, prosthetic, and footwear design, especially with the known evidence linking high pressures to skin breakdown in patients with impaired sensation (Mueller, 1999, Pataky *et al.*, 2000, Patry *et al.*, 2013).

Foot pressure is affected by several factors including; the anatomical structure of the foot, body mass, gender, age, joints range of motion and different disease dependent modifications which alter foot function and gait, such as those in conditions like diabetes (Rosenbaum and Becker, 1997, Bosch *et al.*, 2009, Putti *et al.*, 2010, Charalambos *et al.*, 2015, McKay *et al.*, 2017, Sole *et al.*, 2017).

Foot pressure provides valuable information about the mechanical behaviour and function of the human foot as well as the ankle, knee, hip and back in both static and dynamic load conditions. It also could illustrate an indication of potential musculoskeletal, neurological

¹ Pressure = Force/Area. Pascal is the International System (SI) unit of pressure.
Conversions: 1 psi = 6.9 KPa = 0.69 N/cm²

or other disorders that are reflected on the footprint (Ramirez-Bautista *et al.*, 2018). Therefore, foot pressure measurement, analysis have been used in various research studies to detect foot pathologies and evaluate many medical conditions and different therapeutic interventions (Alexander *et al.*, 1990, Abboud *et al.*, 2000, Lyons *et al.*, 2006, Ramanathan *et al.*, 2008, Park *et al.*, 2009).

In the currently available literature, several studies have identified and extensively examined plantar foot pressure characteristics during gait, the contact area of the plantar aspect of the foot and the forces produced (Alexander *et al.*, 1990, Natali *et al.*, 2010, Putti *et al.*, 2010, Charalambos *et al.*, 2015). However, very few studies have investigated the measurement of the pressure on the dorsal side of the foot (Jordan and Bartlett, 1995a, Olaso *et al.*, 2007, Hagen *et al.*, 2008, Rupérez *et al.*, 2009). This is likely because most foot pressure measurement systems are designed to evaluate the interaction between the foot plantar surface and the ground during static positions or different activities but not for measuring the pressure on the dorsal side of the foot (Herbaut *et al.*, 2016).

2.1.2 Methods of Foot Pressure Measurement

During functional activities such as walking, the human foot exerts a force, produced by body weight, upon the underlying surface, and in turn, an equal and opposite force is created that is known as ground reaction force (GRF) (Zammit *et al.*, 2010). This GRF is proportional to infinite discrete areas on the plantar surface of the foot when in contact with the ground and is described as plantar foot pressure (Abboud and Rowley, 1996). Due to the repetitive nature of that loading, any alterations of the biomechanical behaviour of the foot structure and/or soft tissue elements will have a greater impact on other body structures with multiple repetitions (Flynn, 2014).

To assess foot pressure, a discrete sensor or a matrix of multiple sensors is used to measure the force acting on each sensor while the foot is in contact with the supporting

surface. The magnitude of pressure is then determined by dividing the measured force by the known area of the sensor or sensors evoked while the foot was in contact with the supporting surface (Orlin and McPoil, 2000). Foot pressure can be measured under static and/or dynamic conditions, however, dynamic pressure measurement appears to be the more sensitive and reliable method for identifying “at-risk feet” (Abdul Razak *et al.*, 2012, Patry *et al.*, 2013, Zulkifli and Loh, 2020).

There are two main components of the loading experienced by the plantar surface of the foot: vertical and horizontal (shear). It has been confirmed that the shear component is vital in the determination of skin stresses and has an important role in the development of foot ulcers (Lord, 1981). Thus, it was thought, shear pressure may explain the variation noted between vertical peak pressure location and the location of foot ulceration (Yavuz *et al.*, 2007, Yavuz, 2014). Unfortunately, commercially available plantar pressure measurement devices only provide an indication of the vertical force acting on the foot ground interface, or with in-shoe systems the foot insole interface, but they do not measure the shear pressure that also impacts upon the function of the foot during activity (Flynn, 2014). The quantification of this lower magnitude horizontal components of pressure has proved to be technically challenging as shear is also dependent on frictional properties of the sensor surface (Fernando *et al.*, 2018).

Over the past century, many attempts have been made to develop a suitable technique to determine the distribution of pressure underneath the plantar surface of the foot. Early techniques were simple, innovative methods that provided investigators with semi-quantitative data (Lord, 1981). This was seen in detecting barefoot pressure distribution with the use of ink impressions produced using products such as Morton’s kinetograph then Harris & Beath™ mat (Abboud and Rowley, 1996). These initial investigations were only able to capture the shape of the foot and the impressions on its surface, in an attempt at the recognition of different foot pressure patterns (Rosenbaum and Becker, 1997). The

introduction of computer technologies has allowed quantitatively accurate and reproducible high-resolution measurements with high sampling rates and easily interpreted graphic displays. This has allowed pressure assessment systems to be commonly employed in research as well as clinical settings, providing data that helps in optimising patient assessments and treatment outcomes (Alexander *et al.*, 1990, Lyons *et al.*, 2006, Chevalier *et al.*, 2010). Currently, a range of systems are available to measure both static and dynamic foot pressure characteristics. These systems vary in their sensor technology, spatial resolution, pressure range, sampling rate, calibration and processing procedures (Giacomozzi, 2010).

Sensor technologies that are utilized within different foot pressure measurement devices include; capacitive sensors, resistive sensors, piezoelectric sensors, and piezoresistive sensors (Abdul Razak *et al.*, 2012, Fernando *et al.*, 2018, Zulkifli and Loh, 2020). In general, these pressure transducers have the ability to convert a mechanical event into an electrical signal that can be recorded and stored for further data analysis (Rosenbaum and Becker, 1997). The type of sensors used would be a great determinant of the measurement accuracy and precision (Giacomozzi, 2010, Fernando *et al.*, 2018). Each pressure measurement system can have a different number of sensors to provide an electrical signal output proportional to the measured foot pressure (Zulkifli and Loh, 2020).

Foot pressure measurement systems are commonly found in two formats: an in-shoe based or a platform-based system. Both barefoot (platform) and in-shoe measurements are of value and have been used by clinicians to assess foot pressure. For instance, the study of barefoot patterns is more applicable for the orthopaedic surgeon who wishes to evaluate foot surgery outcomes, whereas in-shoe prints would be appropriate to illustrate the redistribution of loading caused by wearing a particular design of shoe or insole (Lord, 1981). However, on comparison of both systems, it was noted that the system used has its effect on the measurement outcomes. It is therefore essential, in conjunction with the

standardisation of data collection conditions, the data obtained from these systems is not to be used interchangeably (Chevalier *et al.*, 2010).

Both systems, platform and the in-shoe have their own advantages and disadvantages and can provide different types of biomechanical information owing to the different interface studied, though the information contained can overlap. The decision of what type of system to use should not be based on the 'best' system but what the most appropriate system for the clinical or research prerequisites, the loading characteristics, and the outcomes of interest (Rosenbaum and Becker, 1997, Fernando *et al.*, 2018).

2.1.2.1 Platform Systems

Platform systems are constructed from a flat, rigid, floor embedded array of pressure sensing elements. These pressure sensors are arranged in a matrix configuration on a flat surface platform, that should set flush into a walkway, allowing the capture of foot pressure applied to its top surface in static posture or a single step of the gait cycle during simple dynamic activities such as walking, running, and jumping (Abdul Razak *et al.*, 2012, Zulkifli and Loh, 2020). Platform systems are manufacturer-specific, and comprise of different; sensor types, number of sensors per area (different resolutions), sampling rates, and ranges of detectable pressure (Giacomozzi, 2010, Hafer *et al.*, 2013, Telfer and Bigham, 2019). The installation of the platform requires a rigid flat surface area to avoid sensors breaking or bending due to an uneven surface. Since it is embedded into the floor, the platform is commonly installed in laboratories. The shorter platform (0.5 m) is usually used for the static position (postural analysis) while the longer platform (2 m) is mostly used for more dynamic movement (motion analysis). However, platform installation will always abide by the length constraints of indoor laboratories (Zulkifli and Loh, 2020).

Platforms classically measure plantar foot pressure in the barefoot state (Figures 2.1, 2.2). The barefoot assessment has the advantage of investigating the whole foot/ground contact

area and the inherent foot pressures experienced in healthy or impaired foot conditions, as in neuropathy or deformity, without the influence of footwear that can mask some crucial information regarding the loading of different anatomical structures of the foot (Fernando *et al.*, 2018).

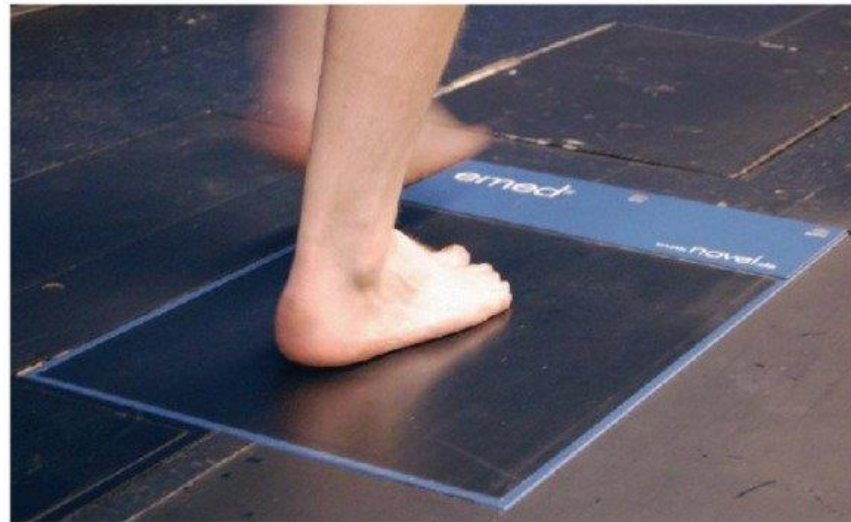


Figure 2.1 Barefoot plantar pressure measurement using Emed® platform
(Abdul Razak *et al.*, 2012)

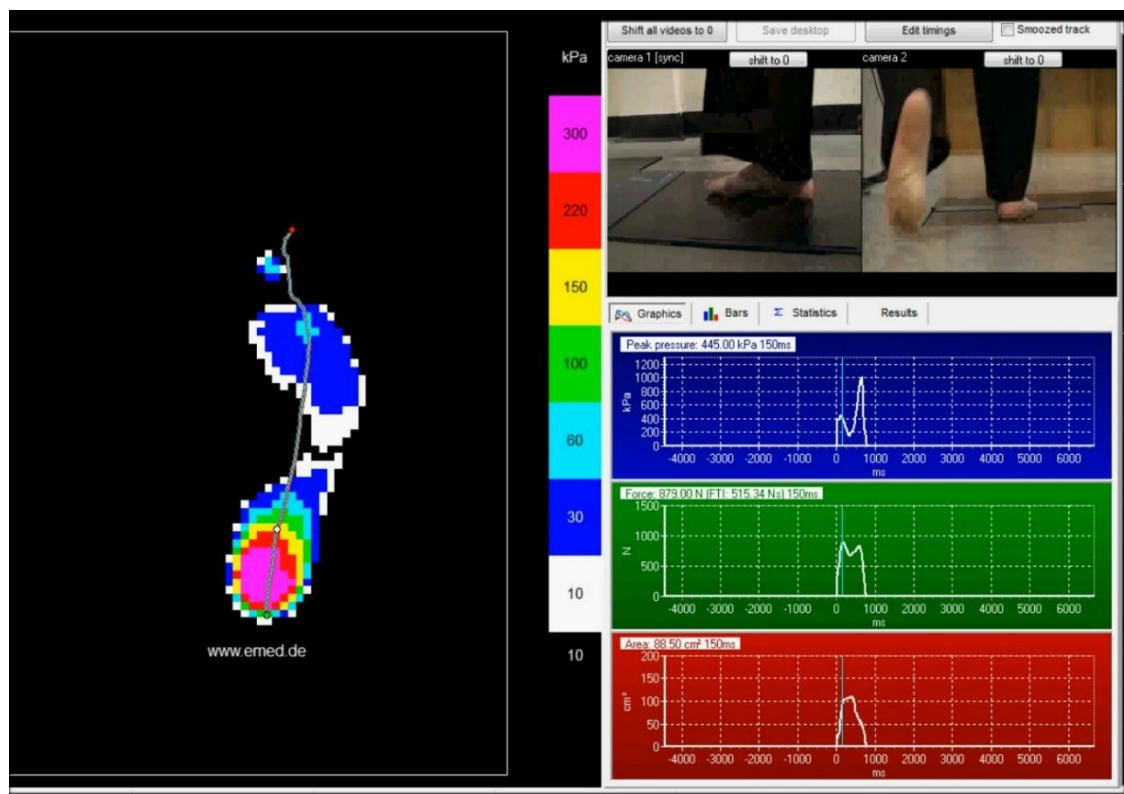


Figure 2.2 Pressure measurement using Emed® platform

Platforms include a greater number of sensors, thus a higher resolution. The pressure sensors are always positioned parallel to the supporting surface, which would provide a “true” vertical force measurement. Although subjects are able to walk naturally without wires or data boxes attached to them, the platform recording does not present the pressure changes under a continuous support surface. A larger number of steps are required for data collection and targeting of the force plate can be an issue that may result in alteration of the patient’s typical pressure pattern. This necessitates having a walkway with sufficient length to allow enough steps before reaching the platform and enough space behind it to prevent any intended slowing down during measurement. Furthermore, a ‘warm-up’ /practice period is required to familiarise the subject with the testing environment and determine the best starting position to reproducibly generate a normal walking pattern where contacting the platform does not generate any adjustment in stride length (Rosenbaum and Becker, 1997).

The midgait method is considered the ‘gold standard’ for the collection of plantar pressure using platform systems, where barefoot pressure from multiple repeated trials are collected during a steady state halfway along a relatively long walkway. Diabetic patients with poor vision and possibly neurological impairment may have difficulty striking the platform without a large number of attempts because of proprioception and coordination problems. This could lead to patient fatigue causing an abnormal pattern of pressure or increase the incidence of the platform targeting to ensure that the foot contacts the sensor surface. Moreover, patients with diabetes and neurological impairment could be placed at risk of plantar ulceration when collecting pressure data using this method, because of the increased repetitive stresses with a large number of attempts and numerous steps required for adequate data collection (Lord, 1981, Cavanagh and Ulbrecht, 1994, McPoil *et al.*, 1999, Orlin and McPoil, 2000, Flynn, 2014). This led to the development of different step-protocols involving fewer steps before contact is made with the pressure

platform. This includes the one-step, two-step, and three-step methods, relating to the number of steps a person must take before stepping onto a platform to capture their foot pressure. When compared, all are valid methods for obtaining barefoot plantar pressure in the diabetic neuropathic foot with the two-step method producing pressure values that are closer to those of the mid-gait technique (McPoil *et al.*, 1999, Bus and Lange, 2005). Irrespective of the protocol used, at least three to five gait trials are required to minimise variability in measurement to reliably assess foot pressure (Fernando *et al.*, 2018).

2.1.2.2 In-Shoe Systems

In-shoe pressure measurement systems offer great advantages over platform systems in the ability of data collection in the foot's normal functioning environment, experienced during daily activities. In-shoe pressure measurement devices have been used to investigate the interaction between the foot and the shoe, during static and dynamic activities where the influence of footwear and orthotics can be determined. The in-shoe system offers the key benefits of flexibility, mobility, simplicity, and lower cost, as opposed to platform systems, which are less portable and generally more expensive. One significant advantage of in-shoe measurement systems is that multiple steps can be easily collected. This feature allows robust relevant parameters to be obtained for the study of a wider variety of activities, with different gait tasks such as stair climbing or various sports activities, compared to the limited level locomotion study possible using platform systems (Urry, 1999, Abdul Razak *et al.*, 2012). Additionally, subjects can use their natural gait during testing which prevents the issues of platform targeting (Urry, 1999, Shu *et al.*, 2010, Melvin, 2014, Zulkifli and Loh, 2020).

The ability to collect multiple steps is very beneficial, due to the natural variability in human gait where no two steps are alike (Putti *et al.*, 2007). The ideal number of steps needed for reproducible and valid foot pressure data has been investigated in the

published literature (Owings and Grabiner, 2003, Najafi *et al.*, 2010). An average of 12 steps per foot has been found necessary to obtain valid and reliable in-shoe pressure data in neuropathic diabetic patients when wearing custom-made therapeutic footwear (Arts and Bus, 2011).

Many techniques have been utilised in measuring the pressure inside footwear. Both discrete transducers and matrix systems have been developed, with matrix systems (insoles) being preferable (Cavanagh *et al.*, 1992, Shu *et al.*, 2010). Using discrete sensors at anatomically pre-defined sites offer an inexpensive, simpler manufacture measurement technique since fewer sensors are required. However, it requires accurate locating of the sensors which has been an issue with this method due to imprecise positioning under the areas of interest or by sensors migrating during the investigation. Also, the sensors will act as a 'foreign body' within the shoe, changing the mechanical conditions at the foot-shoe interface. Matrix (Insole) in-shoe measurement systems consist of numerous pressure sensing elements arranged in rows and columns, aiming at covering the entire plantar surface of the foot. Unlike discrete systems this array pattern permits a larger area to be monitored at any given time, thereby limiting the amount of “dead space” between sensors, where loads applied remain unmeasured, with no need for precise localisation of sensors (Cavanagh *et al.*, 1992, Melvin, 2014).

In-shoe pressure measurement provides real-time information regarding foot pressures while wearing footwear. However, they can be technically challenging with sensors more susceptible to damage, cables may experience bending as they emerge from the shoe and the positioning of the wires/data box can alter the subject's natural gait. The material of the insole itself (e.g. a stiff insole) and the depth of the insole can alter the pressure and make it uncomfortable for the subject. Sensors should be suitably secured/inserted to avoid bending, slippage and ensure reliable results. Furthermore, the inside of the shoe can be described as a 'hostile environment' for sensors, with the trapped heat and sweat

of the foot inside the shoes. All of these factors have the potential to increase measurement error and device failure (Cavanagh *et al.*, 1992, Abdul Razak *et al.*, 2012, Flynn, 2014, Fernando *et al.*, 2018, Zulkifli and Loh, 2020).

2.1.3 Foot Pressure Outcomes

Using matrix systems, platforms or in-shoe, yield a considerable volume of information which needs to be reduced into useful subsets (masks) to facilitate data processing and specific variables extraction. Production of masks is considered a common approach, where pressure measurement data of the studied area is divided into discrete anatomical regions of interest by identifying the corresponding sensors. Defined masks are then analysed separately, and desired outcomes are extracted. Masks can be useful if the research is looking for identifying pressure behaviour/changes at specific sites. On the other hand, “masking” introduces artificial boundaries into the collected data. As the anatomical distinction between adjacent regions does not indicate functional distinction, though segmented data may be anatomically relevant, it may not be in functional terms (Pataky *et al.*, 2008a). Anatomical mapping also assumes that changes in location are pre-known which leads to potential loss of useful information. Studies have shown, some differences may exist at the pixel level but not at a foot region level (Pataky *et al.*, 2008b, Pataky and Maiwald, 2011). The use of statistical parametric mapping was proposed to solve the issue of the potential loss of data with the spatial mapping presumptions (Pataky and Goulermas, 2008, Pataky, 2010). This approach can produce continuous statistical maps based on individual sensor data rather than predetermined groups of sensors, with no prior assumptions regarding where the differences in pressures may occur (Melvin, 2014). However, the statistical parametric mapping should be implemented with caution, as different processing approaches within this method can lead to a variety of statistical results (Booth *et al.*, 2018).

Regardless of the analysis approach, a wide range of variables can be extracted from the foot pressure measurements data. One of the most commonly reported variables is Maximum Peak Pressure (MPP), which corresponds to the highest pressure at a specific sensor, or in a specific mask (anatomical region), at any point during the gait cycle, while mean pressure is calculated as the average pressure at a specific sensor, or in a specific mask, over the entire gait cycle. Another important variable is Pressure Time Integral (PTI) which is defined as the time integral of the peak pressure measured in any sensor within the specified region during one-foot step. This is calculated as the area under the peak pressure versus time curve of a particular region (Waaijman and Bus, 2012). Other variables include force, contact area, force-time integral, mean area, and contact time (Melvin, 2014).

Peak pressure and the pressure-time integral are the most commonly used outcomes in studies investigating foot pressure behaviour. Yet, it is still not well understood which is superior as a predictor for ulceration or ulcer healing as in individuals with diabetes and diabetic peripheral neuropathy (Bus and Waaijman, 2013, Fernando *et al.*, 2018). MPP has historically been more popular as a representative of the magnitude of pressure with the assumption that high loads are the cause for tissue damage leading to ulceration (Ledoux *et al.*, 2013). As PTI incorporates the magnitude as well as the time of exposure of pressure, it may be a more accurate indicator of mechanical loading than either parameter, pressure or time, individually. PTI, signifying the cumulative effect of pressure over time, explains the application of lower pressures over a longer time period may also cause tissue damage (Sauseng *et al.*, 1999, Hsi *et al.*, 2002, Melvin, 2014). These could be of significance in evaluating lower pressure values as those of dorsal pressure. Hence, the two measurements provide different types of information, it may be appropriate to measure both outcomes to fully comprehend the influence of pressure (Fernando *et al.*, 2018).

2.1.4 Foot Pressure Abnormalities in Diabetes Mellitus

Foot pressure measurements in patients with diabetes have been widely investigated in the literature, where high loads have been reported at the sites of ulceration (Stokes *et al.*, 1975, Veves *et al.*, 1992, Frykberg *et al.*, 1998, Pham *et al.*, 2000, Fernando *et al.*, 2014).

An abnormal redistribution of loading was generally noted in patients with diabetes compared to nondiabetic control. This included a reduction in the load carried by toes, especially in case of ulceration, and shift of loading either, to the lateral side of the foot (Stokes *et al.*, 1975), medial side to be transmitted through the metatarsal heads (Ctercteko *et al.*, 1981, Plank *et al.*, 2000) or neither both, though a transfer of peak pressures from the great toe to the first metatarsal head was seen in diabetic patients with neuropathy (Veves *et al.*, 1991). Another observation was the lower frequency of existence of peak pressures under the heel in diabetic patients with and without neuropathy compared to a control group. This finding, in diabetic patients without neuropathy, may suggest early changes where the pressure starts rising under the forefoot but still within normal limits (Veves *et al.*, 1991, Lyons *et al.*, 2006).

These abnormal patterns of pressure distribution and eventually raised pressure were highly associated with diabetic neuropathy (Frykberg *et al.*, 1998, Bus *et al.*, 2005, Fernando *et al.*, 2014). Although a transfer of pressure from the heel and the toes to metatarsal heads leads to an increased forefoot pressure in patients with diabetic neuropathy (Rich and Veves, 2000), raised midfoot and hindfoot pressures were also reported (Bacarin *et al.*, 2009), with an increased forefoot/hindfoot ratio in severe diabetic neuropathic foot (Caselli *et al.*, 2002).

2.1.5 Factors Influence Foot Pressure in Diabetic Foot

The elevated plantar pressure found in association with diabetes is multifactorial. Although a correlation exists between neuropathy and high plantar pressure, other factors including vascular deficit, limitation in joint mobility and foot disorders have also been considered.

2.1.5.1 Neuropathy

Diabetic peripheral neuropathy (DPN) is one of the most common complications of diabetes mellitus with a prevalence ranging from 13 to 68% among patients with diabetes (Van Dieren *et al.*, 2010, Fernando *et al.*, 2013). It is believed that approximately 50% of patients with diabetes will develop DPN within 10 to 15 years of diabetes diagnosis (Cavanagh *et al.*, 1993) and this risk increases with the longer duration of the disease and poor glycaemic control (Alam *et al.*, 2017).

DPN is typically described as a chronic, symmetrical, length-dependent polyneuropathy which is attributed to metabolic and microvascular alterations associated with diabetes (Tesfaye *et al.*, 2010). Peripheral neuropathy in patients with diabetes may be sensory, motor and/or autonomic.

Sensory neuropathy results in a decrease or loss of the protective sensations that impedes the identification of repetitive or isolated trauma to the foot which may occur during simple daily activities such as walking or within narrow ill-fitting footwear (Veves *et al.*, 1992, Mueller *et al.*, 2008). Also, proprioception impairment will result in the inability to make adjustments to different surfaces/loads experienced during walking and therefore affecting balance (Alam *et al.*, 2017). A decrease in walking speed and stride length and prolonged stance phase were also reported (Fernando *et al.*, 2013) which eventually results in a prolonged mechanical loading, leading to skin breakdown and ulceration.

Motor neuropathy leads to muscle dysfunction and degeneration, limited joint mobility and subsequent deformities that lead to altered foot pressures (Abboud et al., 2000, Rao et al., 2007, Rao et al., 2010, Guiotto et al., 2013). Autonomic neuropathy results in diminished sweating and skin dryness. Dehydrated skin loses its elastic properties and therefore, its ability to adapt to the foot movement, plus causing the skin tending to break easily. It also predisposes to callus formation under areas of increased pressure, which in turn, further alter foot pressures (Murray et al., 1996, Abouaesha et al., 2001). All this together will cause an altered pressure pattern in the neuropathic foot, inability to properly distribute high pressures, leading to the maintenance of high pressures and damaging the already altered soft tissue and subsequently skin breakdown (Martinez Santos, 2016).

Although elevated plantar pressures and neuropathy are found to frequently coexist in association with diabetes (Hazari *et al.*, 2016), a direct causal relationship is thought to be speculative, as increased and abnormal plantar pressures have been reported in some diabetic patients with no signs or symptoms of neuropathy, independently associated with ulceration (Frykberg *et al.*, 1998), and lower peak plantar pressures have been reported in diabetic patients with neuropathy than patients without neuropathy whilst performing different daily-life activities (Guldmond *et al.*, 2007). Furthermore, the progression of diabetic neuropathy was not found to influence plantar pressure distribution (Bacarin et al., 2009). Thus changes in foot pressure distribution in diabetes may be related to other factors that result in abnormal foot function and structural foot pathology, not only to neuropathy (Bevans, 1992, Lazaro-Martinez *et al.*, 2011, Flynn, 2014).

2.1.5.2 Soft Tissue Changes

Soft tissue changes in diabetic foot have been frequently correlated with elevated plantar pressure (Murray et al., 1996, Bevans and Bowker, 1999, Abouaesha et al., 2001, Rao et al., 2006, Wrobel and Najafi, 2010, Searle et al., 2017). Patients with diabetes have been

found to develop atrophy/relocation of the heel and forefoot fat pads and stiffer plantar tissues than non-diabetic subjects (Bus *et al.*, 2004, Cheung *et al.*, 2006, Pai and Ledoux, 2010, Ledoux *et al.*, 2016, Naemi *et al.*, 2016).

The plantar soft tissue interface acts as an efficient shock absorber, to dampen the effects of impact forces during gait. Structural changes due to pathological conditions such as diabetes can result in altered mechanical properties of the foot soft tissues that impair its cushioning effect and reduce its capacity to uniformly distribute loads. Additionally, the repetitive excessive loading that patients do not recognise, due to the commonly associated neuropathy, can also make changes to the mechanical behaviour of soft tissues. Reduced flexibility means that these tissues are less able to distribute pressure via deformation which makes them more vulnerable to trauma and significantly increases plantar pressure (Abouaasha *et al.*, 2001, Sun *et al.*, 2011, Martinez Santos, 2016, Naemi *et al.*, 2016).

Hyperkeratosis (callus formation) is a common presentation under high-pressure areas. It allows the skin to better resist repetitive traumas, though, itself can act as an extrinsic source of stress to further elevate the plantar pressure on its location that regular removal of callus was found to be associated with a significant drop in foot pressure (Young *et al.*, 1992, Pitei *et al.*, 1999). A variety of diabetic factors and complications may contribute to the development of callus and it is considered a significant risk factor in diabetic foot (Murray *et al.*, 1996, Hamatani *et al.*, 2016, Yazdanpanah *et al.*, 2018). Yet, it is contradictory whether patients with diabetes may produce more callosities than those without diabetes or if it is only an association to the pre-existing risk factors (Bevans and Bowker, 1999, Flynn, 2014, Arosi *et al.*, 2016).

Another soft tissue change that can alter foot biomechanics and influence the foot loading in diabetic patients is diminished joint mobility. The limited joint mobility in diabetic foot

has been linked to neuropathy as well as, the longstanding hyperglycaemia with the accumulation of advanced glycosylation end products (AGEs) that may change the structural properties of different collagen-containing tissues of the foot including; tendons, ligaments and joints capsules (Wrobel and Najafi, 2010, Abate et al., 2013, Gerrits et al., 2015). These structural changes were noted in the increased thickness of diabetic foot tendons (Bolton et al., 2005, Giacomozzi et al., 2005). That subsequently leads to decreased elasticity and tensile strength, which result in joint instability causing subluxations as seen in Charcot joint or overall stiffness of the foot with the added effect of joint capsule contracture (Kim, 2013). The increased rigidity of diabetic foot joints is associated with elevated peak plantar pressure and pressure time integral, especially in the forefoot area. This raised pressure and prolonged loading time will add to the risk of ulceration (Fernando *et al.*, 1991, Zimny *et al.*, 2004, Rao *et al.*, 2010, Guiotto *et al.*, 2013). Limited ankle joint dorsiflexion was the most reported in relation to elevated plantar pressure even independent of neuropathy (Searle *et al.*, 2017) so that its routine screening was suggested as a tool to identify diabetic patients at increased risk of elevated plantar pressures and therefore diabetic foot complications (Searle *et al.*, 2018). The progression of limited joint mobility with the presence of neuropathy, muscular dysfunction and atrophy leads to fixed deformities and high loading at the developed bony prominences, causing callus formation or the worst scenario, skin breakdown.

2.1.6 Foot Pressures a Risk Factor in Diabetic Foot Ulcers

Although the development of diabetic foot ulcers is multi-factorial, elevated plantar pressures have been frequently reported as a significant risk factor and predictive of foot ulceration in patients with diabetes (Veves *et al.*, 1992, Frykberg *et al.*, 1998, Pham *et al.*, 2000, Ledoux *et al.*, 2013, Patry *et al.*, 2013, Yazdanpanah *et al.*, 2018). However, alteration in plantar pressure often coexists with other ulceration risk factors such as

neuropathy, peripheral arterial disease, muscular dysfunction, foot deformities and previous foot ulcers (Abboud *et al.*, 2000, Plank *et al.*, 2000, Bus *et al.*, 2005, Guiotto *et al.*, 2013).

Foot pressures were measured in static and dynamic conditions, though, dynamic pressure measurement appears to be the more sensitive and reliable method for evaluating the at-risk foot (Kim, 2013, Patry *et al.*, 2013). Owings *et al.*, (2009) proposed that examining in-shoe pressure is essential when considering foot ulcer risk in diabetic patients as they noted, barefoot peak pressure is a poor predictor of peak in-shoe pressure. Barefoot pressure was able to predict only 35% of the variance of in-shoe peak pressure (Owings *et al.*, 2009).

There have been several attempts to establish a pressure threshold that predicts the risk for development of foot ulceration in diabetic patients (Armstrong *et al.*, 1998, Frykberg *et al.*, 1998, Lavery *et al.*, 1998, Owings *et al.*, 2009, Waldecker, 2012). Although many threshold values have been proposed, a peak pressure threshold that possesses a high enough sensitivity and specificity for ulceration risk has not been definitively established, and the only certainty is that the higher the peak pressure, the higher the risk of diabetic foot ulceration (Armstrong *et al.*, 1998, Plank *et al.*, 2000, Waldecker, 2012). It is likely that each region of the foot has a different ulceration threshold pressure, depending upon its basic structure and tissue viability (Cavanagh and Ulbrecht, 1994, Plank *et al.*, 2000, Bennetts *et al.*, 2013). Patry *et al.*, (2013) had anticipated that tissue repair threshold may not be the same as the tissue breakdown threshold in patients with DPN, with healed sites more prone to subsequent re-ulceration due to their lower tissue breakdown pressure threshold (Patry *et al.*, 2013). Moreover, diabetic foot ulceration is a multifactorial pathology. It can be influenced by other factors such as peripheral vascular disease, neuropathy, duration of diabetes, glycaemic control, level of activity and lifestyle (Patry *et al.*, 2013, Fawzy *et al.*, 2014). The altered pressure represents only one factor within

the process, besides foot pressure itself can be affected by several elements (Wrobel and Najafi, 2010). Other factors to consider are the large variations in systems and ways of measuring, which make it difficult to compare between different studies or come to a consensus regarding the best system and the best way of obtaining a sensible and reproducible measurement (Patry *et al.*, 2013, Martinez Santos, 2016).

Along with the association between increased peak pressures and diabetic foot ulceration, there is also the cumulative effect of lower loads experienced over an extended period of time. For instance, the exposure of a moderately high pressure for a relatively long duration at a vulnerable site could be more damaging than a very high pressure introduced for a short duration (Plank *et al.*, 2000). Therefore, lower walking speed and prolonged stance phase noted in diabetic patients (Brach *et al.*, 2008, Fernando *et al.*, 2013) will contribute to the rise in the cumulative stress of foot tissues due to the subsequent increased contact time. Another escalator for cumulative stress could be the repetitive minor trauma that may occur during different daily activities such as walking. Yet, subjects with diabetes and a history of previous ulcers may be more susceptible to plantar tissue injury, even at relatively low levels of cumulative tissue stress (Maluf and Mueller, 2003). Here, Pressure-time integral (PTI) may be more valuable than peak pressure in estimating this cumulative effect of pressure over time at a specific area of the foot (Melai *et al.*, 2011, Bus and Waaijman, 2013). PTI showed a significant increase in diabetic patients groups (Waldecker, 2012) and a higher sensitivity to changes related to footwear. Therefore, some authors recommended PTI routine investigation in the evaluation of diabetic footwear (Hsi *et al.*, 2002, Sacco *et al.*, 2009).

Diabetic foot ulcers due to abnormal loading have been reported on both plantar and dorsal aspects of the diabetic foot (Haji Zaine *et al.*, 2014, Kalburgi *et al.*, 2017, Ousey *et al.*, 2018). Nevertheless, it has been found that more than half of diabetic foot ulcers

(52%) were non-plantar (Figure 2.3) and the most frequent ulcer site was the dorsal or interdigital area of the toes (32%) (Prompers *et al.*, 2007).

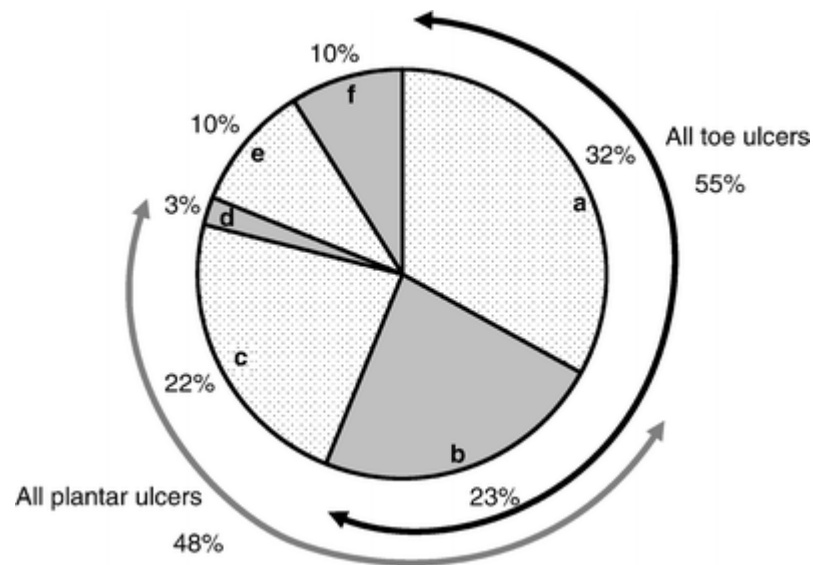


Figure 2.3 Location of diabetic foot ulcers

a) Dorsal/interdigital toes, b) Plantar toes, c) Plantar forefoot/midfoot, d) Plantar hindfoot, e) heel, f) Dorsal/lateral aspect foot (Prompers *et al.*, 2007)

Guidelines recommend routine inspection of both foot sides for any skin changes, in particular at the sites of common foot deformities that increase pressure, predisposing to skin breakdown on both aspects of the foot. For instance, the claw toe deformity, which combines metatarsophalangeal joints' hyperextension with interphalangeal flexion or distal phalangeal extension (hammertoe). This buckling phenomenon causes an increased pressure on the digits' dorsal surface, as well as on the plantar metatarsal heads. Another frequently seen foot deformity is Hallux Valgus, "Bunion". The overlapping toes deformity can lead to pressure ulceration between the digits, on the dorsal or plantar surfaces of displaced digits, and over the medial first metatarsophalangeal joint (Figure 2.4) (Lavery *et al.*, 1998, Boulton *et al.*, 2008).

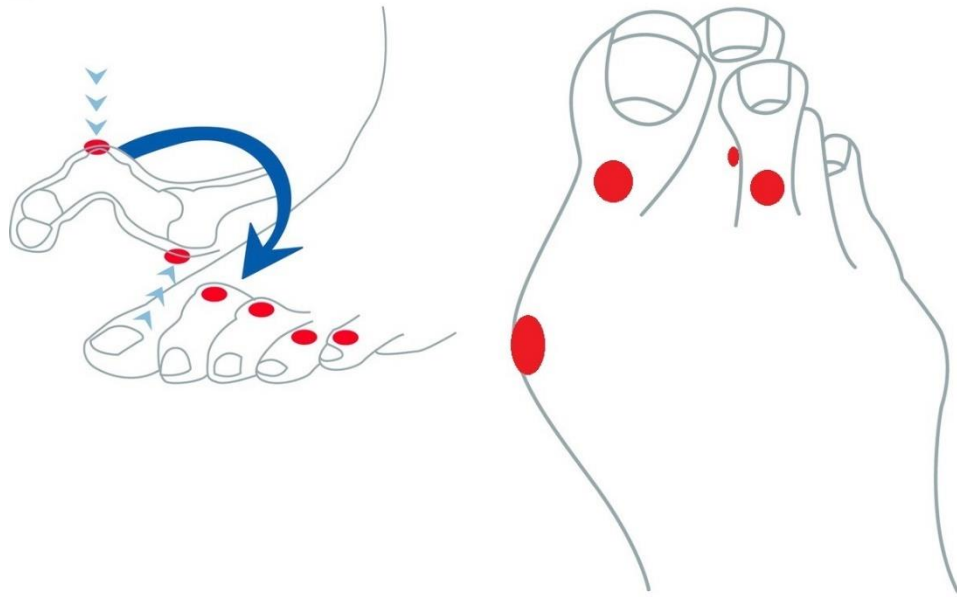


Figure 2.4 Foot deformities sites as frequent locations for diabetic foot ulcers (Boulton *et al.*, 2008)

Dorsal ulcers are believed to be usually caused by ill-fitting footwear (Sabapathy and Madhu, 2016), tight-fitting non-adjustable straps that tend to prevent the footwear from slipping off especially in neuropathic patients (Premkumar *et al.*, 2017) or in some cultures the frequent adoption of a specific prayer position (Chadwick *et al.*, 2013). Ill-fitting footwear may increase the risk of tissue damage at some foot sites as the lesser toes, to have more dorsal ulcers (up to 91%) than plantar (Peters *et al.*, 2007), that was also found to be the least likely to heal as the infection spreads easily along the loose dorsal tissue planes and a large percentage of these lesser toe ulcers ended with an amputation (Lavery *et al.*, 1998, Peters *et al.*, 2007, Aragón-Sánchez *et al.*, 2012, Sabapathy and Madhu, 2016).

Yet, there are very few published studies on the assessment or management of dorsal or non-plantar ulcers (Caravaggi *et al.*, 2003, Prompers *et al.*, 2007). Most of the literature suggests the dorsal ulcers are due to friction with the footwear and should be managed simply through offloading as plantar ulcers and the prescription of customised footwear

with spacious toe boxes to accommodate deformities and ensuring the foot does not move within the footwear to avoid re-injury (Peters *et al.*, 2007, Sabapathy and Madhu, 2016). Due to, the general belief that dorsal ulcers are caused by friction, which corresponds to the shear component of pressure, compounded by the lack of commercial devices that can measure shear forces, little research has been conducted to assess the role of pressure in diabetic dorsal foot ulcers (Shankhdhar *et al.*, 2016).

With the aim of this study to assess both surfaces of the foot, we started our work by exploring different available modalities that could be used in the measurement of the dorsal pressure. Literature regarding existing techniques is discussed in the next chapter with details for the study protocol development.

2.2 ENDOTHELIUM

2.2.1 Introduction

The endothelium comprises the mono-cellular layer lines tunica intima, the innermost layer of all blood vessels' wall. It represents a diffuse organ of over 700 g in the adult human, and consists of approximately 10 trillion (10^{13}) cells, contributing close to 1.5% of the total body mass (Bakker *et al.*, 2008, Triggle *et al.*, 2012). The endothelium serves as an interface/barrier between the circulating blood components and other tissues. However, it is no longer considered a simple inert physical barrier, but it is believed to act as a complex organ with paracrine and autocrine functions that, plays a crucial role in the vascular as well as, the overall tissue homeostasis (Hadi and Suwaidi, 2007).

Although endothelial cells share a common origin, some local differences exist in the endothelium of various vascular beds reflecting differing in the specialised functions of the hosting organs. In general, the endothelium facilitates a range of functions through a complicated system of chemical mediators that exerts effects on both the adjacent

vascular smooth muscle (VSM), located in the median layer of the blood vessels' wall (tunica media) and the blood constituents in the blood vessels' lumen.

The endothelium has been known to respond to different stimuli including mechanical forces, oxidative and metabolic stresses, inflammation, hypoxia, and many other stresses while adapting different functions accordingly. These include; the production of vasoactive substances (vasodilators or vasoconstrictors) to regulate blood flow, actively regulates the delivery of nutrients and other macromolecules into the surrounding tissue, production of fibrinolysis or coagulation regulators to ensure the fluidity of blood and avoidance of bleeding, modulation of VSM including proliferation, migration and/or changes in the phenotypic characteristics, cascading pro-inflammatory or anti-inflammatory changes, generation of oxidising and anti-oxidising agents and many others (Mombouli and Vanhoutte, 1999, Hadi and Suwaidi, 2007, Bakker *et al.*, 2008).

2.2.2 Endothelial Functions

One of the key characteristics of the endothelium is the sensing of different chemical/hormonal and physical stimuli, in particular the mechanical forces related to shear stress with the flowing blood. This enables it to counteract, regulating the vascular tone through the production of various flow-dependent vaso-regulatory mediators in order to maintain tissues' blood flow in response to local metabolic requirements, blood pressure or stress conditions. These chemical mediators included endothelium-derived relaxing factors (Vasodilators) e.g. Nitric Oxide (NO), Prostaglandins (in particular PGI₂ or Prostacyclin and PGE₂) and Endothelium-derived Hyperpolarization Factor (EDHF), as well as endothelium-derived contracting factors (Vasoconstrictors) e.g. Endothelin-1 (ET-1), Angiotensin-II (AT-II) and Prostaglandins (PGH₂/Thromboxane A₂) (Verma and Anderson, 2002, Hadi and Suwaidi, 2007).

One of the first recognised mediators was an endothelium-derived relaxing factor, which was subsequently shown to be Nitric Oxide (NO) (Furchgott and Zawadzki, 1980, Ignarro *et al.*, 1987, Palmer *et al.*, 1987). NO is well known as the single most important molecule for vascular homeostasis and nearly all stimuli that produce vasodilatation do their effect through it. NO is produced through the conversion of the amino acid, L-arginine to L-citrulline in the presence of molecular oxygen and cofactors, e.g. reduced Nicotinamide Adenine Dinucleotide Phosphate (Dihydro-nicotinamide adenine dinucleotide phosphate) (NADPH) and tetrahydrobiopterin (BH₄), by the enzyme, NO-synthase (NOS). NOS type III or eNOS, constitutively expressed by the endothelial cells is enhanced after binding Ca²⁺/calmodulin and generates NO that rapidly diffuses to the VSM where it activates the enzyme guanylate cyclase. This results in the formation of cyclic guanine monophosphate (cGMP), activating a cGMP dependent protein kinase, which leads to an increased extrusion of Ca²⁺ in VSM cells and evoking relaxation thus vasodilation. Circulating agonists, such as Acetyl Choline (ACh), Bradykinin, Histamine and Serotonin, stimulate endothelial cells to dissociate NOS, permitting its activation and binding to Ca²⁺/calmodulin thus increase NO production. It was noted that physical stimuli such as shear stress caused by the increase in velocity of blood flow can stimulate the activation of endothelial NOS through a Ca²⁺/calmodulin independent pathway. The end result of both means is endothelium-dependent vasodilation which is proportional to the amount of NO released by the endothelium. Nitrates given in any way as in the management of angina, are NO donors. Circulatory NO, directly releasing cGMP in VSM, triggering a relaxation response which is known as Endothelium-independent vasodilatation (Figure 2.5) (Mombouli and Vanhoutte, 1999, Esper *et al.*, 2006, Dhananjayan *et al.*, 2016, Rafnsson, 2018).

In addition to the potent vasodilator effect of NO, it mediates many other protective functions. It inhibits the expression of pro-atherogenic and pro-inflammatory cytokines,

chemokines and leukocyte adhesion molecules. Thereby limiting vascular recruitment of leukocytes, reducing vascular permeability, tissue oxidation, tissue inflammation, activation of thrombogenic factors, and platelets aggregation. It also inhibits VSM cells growth, proliferation and migration, an early sign of atherosclerosis. Hence, NO is considered an essential anti-atherogenic factor and the decrease in its production is thought to play a crucial role in vascular diseases such as atherosclerosis, hypertension and peripheral arterial disease (Nyström, 2005, Esper *et al.*, 2006). Thereby, the assessment of vasodilator properties resulting from NO has become the most widely used end-point for assessment of endothelial function as it reflects the function of various properties of the endothelium (Matsuzawa and Lerman, 2014).

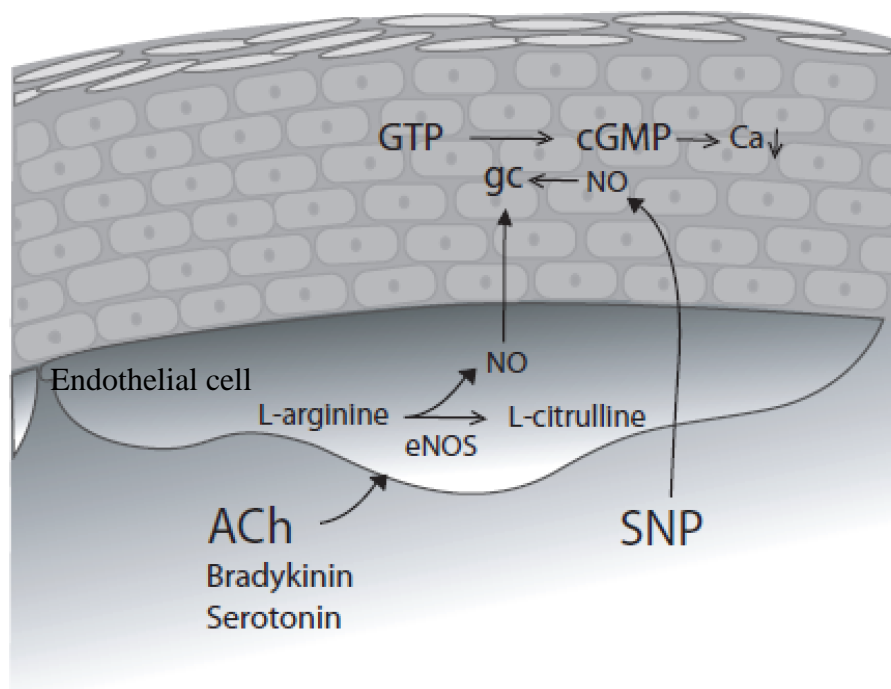


Figure 2.5 The nitric oxide pathway in the vasculature

Endothelial cells constitutively expressing nitric oxide synthase (eNOS) generate nitric oxide (NO) using L-arginine as a substrate together with certain cofactors NO then rapidly diffuses into vascular smooth muscle cells and binds to guanylate cyclase (gc). This event results in the formation of cyclic GMP (cGMP), activating a cGMP dependent protein kinase, which leads to an increased extrusion of Ca^{2+} from the cytosol inhibiting the contractile machinery and thereby evoking vasodilation. Production of NO can be further induced by e.g. ACh or by shear stress, which causes flow-mediated vasodilation. Nitrates, frequently used clinically in the management of angina, function as direct NO donors (here exemplified by SNP) thereby causing vasorelaxation (Nyström, 2005)

2.2.3 Assessment of Endothelial Functions

Over the past few decades, the discovery of the endothelium's crucial role in the regulation of vascular functions and the recognition of endothelial dysfunction as a key pathological condition in most, if not all cardiovascular adverse events, has led to a tremendous interest in endothelial research and ways of assessing its function as a predictor of cardiovascular health status, a potential therapeutic target and an essential marker in investigating the effects of different therapeutic interventions (Al-Qaisi *et al.*, 2008, Jezovnik, 2011). Several techniques have been used for exploring various aspects of endothelial pathobiology comprising, invasive and non-invasive methods, tests within coronary or peripheral arteries, and assessment of response to pharmacological agents and/or to hemodynamic provocation tests. However, there remains a considerable debate regarding the most appropriate way of assessing endothelial function. The ideal test should be safe, non-invasive, easy to perform, reproducible, repeatable, inexpensive, and standardised between laboratories. Moreover, the clinical use will prerequisite results to reflect the dynamic biology of the endothelium throughout the natural history of the cardiovascular disease. It must also define subclinical disease processes, as well as provide prognostic information for risk stratification in the later clinical phase of the disease. No single test currently fulfils these requirements and comprehensively assesses endothelial function, let alone the reported limited reproducibility and high inter-subject variability of the available methods (Gori, 2018, Small *et al.*, 2019). Thus, the introduction of a technique specifically aimed at measuring endothelial function as a routine clinical tool in daily practice has not been established, nor has any method been recommended in clinical guidelines for planning primary or secondary prevention of vascular diseases (Deanfield *et al.*, 2005, Deanfield *et al.*, 2007, Flammer *et al.*, 2012, Higashi, 2015, Gori, 2018, Small *et al.*, 2019).

Endothelial function can be assessed by examining the endothelium's capacity to perform its various physiologic functions, including regulation of vasomotor tone, expression of adhesion molecules and maintenance of an anti-thrombotic microenvironment (Matsuzawa and Lerman, 2014). The work of Ludmer, et al., 1986 was the first attempt to demonstrate the presence of endothelial dysfunction in atherosclerotic arteries. A paradoxical coronary artery vasoconstriction was induced by intracoronary infusion of acetylcholine in patients with atherosclerotic coronary arteries and endothelial function was measured. Changes in the blood vessel diameter were assessed by quantitative coronary angiography and changes in the coronary blood flow were examined by Doppler flow wire. This research drew attention to the functional manifestations of atherosclerosis, exaggerated vasoconstriction, as a consequence of poorly functioning endothelium (Ludmer *et al.*, 1986, Flammer *et al.*, 2012, Higashi, 2015). Although these early tests directly assess coronary circulation and are predictive of cardiovascular events, their invasive nature limits their use to patients requiring a coronary angiography for clinical indications and makes them very challenging if serial follow-up measurements are required (Deanfield *et al.*, 2007, Small *et al.*, 2019). Later, less invasive techniques were developed using the forearm circulation. Whereas peripheral techniques to assess endothelial function offer a more accessible, non- or less invasive surrogate approaches, certain phenomena cannot be explained by systemic endothelial dysfunction; it is likely that local factors (e.g. flow patterns) and local vascular bed function/dysregulation may also contribute to disease state as that observed at some branch's points (El-Tamimi *et al.*, 1994, Deanfield *et al.*, 2005, Flammer *et al.*, 2012).

All available approaches have their advantages and disadvantages which have been extensively explored in the literature (Alam *et al.*, 2005, Deanfield *et al.*, 2005, Deanfield *et al.*, 2007, Flammer *et al.*, 2012, Flynn *et al.*, 2012, Matsuzawa and Lerman, 2014, Higashi, 2015). Yet, the basic principle remains similar: healthy arteries, coronary or

brachial, dilate in response to reactive hyperaemia (flow-mediated vasodilatation, known as the ‘gold standard technique’) or after intra-arterial infusion of pharmacological stimuli including endothelium-dependent vasodilators such as ACh, bradykinin or serotonin, via the release of NO and/or other endothelium-derived vasoactive substances. Such endothelium-dependent vasodilatation is reduced or absent in response to disease states as in atherosclerosis. Additionally, exogenous NO donators (e.g. Glycerol-trinitrate, SNP) can be applied to differentiate endothelium-dependent vasodilatation from endothelium-independent responses. An impaired endothelium-independent function is associated with structural vascular changes and malformations in VSM cells, rather than changes in the endothelium (Flammer *et al.*, 2012, Harbin *et al.*, 2018).

Endothelial dysfunction is known to be a diffuse, systemic condition hence, the peripheral endothelial function (microvascular and macrovascular) have been able to correlate well with endothelial function in the coronary arteries (Anderson *et al.*, 1995, Takase *et al.*, 1998, Khan *et al.*, 2008) and have demonstrated to be a significant independent predictor of future cardiovascular events (Matsuzawa and Lerman, 2014). The accessibility of the skin makes it an appropriate site for the peripheral assessment of endothelial function. Skin microvascular function has been demonstrated to be an independent determinant of cardiovascular diseases in patients with type 2 diabetes (Yamamoto-Suganuma and Aso, 2009).

Therefore, the assessment of skin microvascular function could provide insights into the mechanism of the underlying disease changes, offer a prognostic marker and help in evaluating the effect of drugs in cardiovascular diseases (Roustit and Cracowski, 2013). Hence, investigating the skin vasculature response to loading foot pressures could add to the understanding of the mechanism leading to foot ulceration in diabetic patients (Flynn, 2014).

Subcutaneous microvasculature function can be assessed in response to stimuli such as pharmacological agents, arterial occlusion or thermal alterations to reflect changes that occur in other important central vascular beds (Khan *et al.*, 2008, Gutterman *et al.*, 2016).

Many methods are available to test this peripheral endothelial function while providing a direct or indirect indication of the changes in the subcutaneous microvascular perfusion. Laser Doppler flowmetry with iontophoresis to the skin on the dorsal as well as the plantar surfaces of the foot was the chosen technique to be utilised in the current research study.

2.2.3.1 Laser Doppler Flowmetry (LDF) and Iontophoresis

Laser Doppler Flowmetry (LDF) is a non-invasive method of measuring microvascular blood flow in the tissues and the real-time changes in perfusion under various conditions or during provocation testing. The technique is based on measuring the Doppler Effect or better known as “Doppler shift”. It comprises detecting the wavelength changes in the reflected beam of laser light which scatters upon hitting moving blood cells. The magnitude and frequency of such wavelength changes correspond to the number and velocity of blood cells. A laser Doppler instrument output often gives “flux”, the signal used for flow measurement. Flux is an indirect (relative) index of perfusion. It is the product of velocity and concentration of moving blood cells within the measured volume, known to be proportional to the real blood flow and expressed as arbitrary perfusion units (PU) or millivolts (1PU = 10 mV). Utilising standardised protocols and controlling external environmental factors including temperature, movement and/or reflection artefacts and removal of topical agents, are essential for the reliability and reproducibility of the technique. Yet, there are some limitations that motivate the ongoing research in both the instrumentation and theoretical aspects of the technique. Major limitations include; the influence of the tissues’ optical properties on the perfusion signal, motion artefact noise, lack of quantitative units for perfusion, lack of knowledge of the depth of

measurement and the biological zero signal (perfusion measured at no flow condition) (Cracowski *et al.*, 2006, Rajan *et al.*, 2009, Small *et al.*, 2019).

There are two techniques available in practice, laser Doppler perfusion monitoring (LDPM) and laser Doppler perfusion imaging (LDPI).

Laser Doppler perfusion monitor (usually referred to as LDF) is the one-point measurement method, which involves the placement of a laser probe in direct contact with the skin surface. The single-point fibre-optic probe is connected to a delivery fibre from the source semiconductor laser diode, as well as a collection fibre for detection and processing of the backscattered signal from tissues (Figure 2.6). The measurement depth and sampling volume depend on the wavelength and the fibre separation used. In normal skin with a standard fibre separation probe and the often-used wavelength is 780 nm, the measuring depth is 0.5–1.5 mm into the dermis and the measurement volume is approximately 1mm^3 . It can detect dermal blood flow without the influence from the flow in the underlying skeletal muscles from a single point or vessel at any time with a high sampling frequency (often 32 Hz) (Low *et al.*, 2020). Therefore, it provides continuous, real-time flow information and any variations in response to different tested conditions or given stimuli.

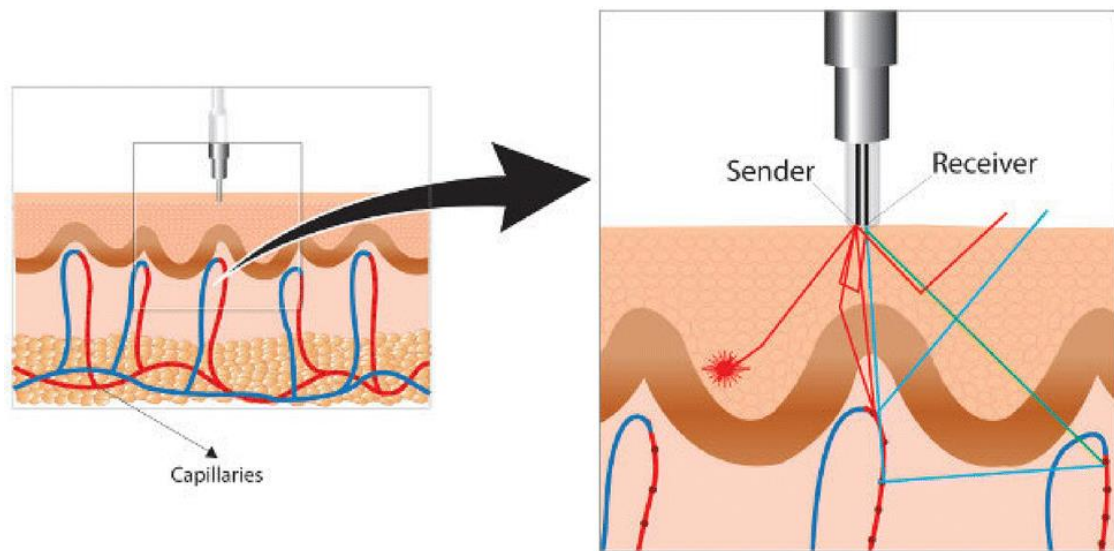


Figure 2.6 Laser Doppler Flowmetry assessment of skin blood flow

A beam of laser light is emitted from a fibre-optic probe (Sender), light reflected on hitting moving blood cells undergoes a change in wavelength (Doppler shift) while light hitting static objects is unchanged, the information is picked up by a returning fibre (receiver), converted into an electronic signal (Low *et al.*, 2020)

As LDPM assesses blood flow over a small volume, it offers the advantage of constant measurement of blood flow at the specified examined location which could be detrimental in monitoring the reactive flow and the study of the dynamics of the dilator response. However, this affects the reproducibility of the procedure, mainly due to the spatial variability resulting from the inherently inhomogeneous microvasculature related to the skin anatomy. Reproducibility has been improved by using “integrated probes”. These probes are composed of multiple collecting fibres to cover a larger skin area thus increasing the spatial resolution. Fibres are positioned in a ring around a central light delivery fibre and averaging the signal from different scattering volumes, decreasing spatial variability (Turner *et al.*, 2008, Rajan *et al.*, 2009, Roustit and Cracowski, 2013). The integrated probe also helps to solve the problem of fibre-based noise from movement artefacts, where light delivery and detection are on the same probe (Rajan *et al.*, 2009).

LDPM does not provide any visual information for morphological or density assessment of the tested vascular bed like those produced with LDPI. This limits its use to quantitative

flow studies rather than qualitative morphological assessments (Deegan and Wang, 2019).

Laser Doppler perfusion imager (LDPI) provides 2D images, mapping the skin blood flow into coloured pixels which represent the scanned perfusion values. The laser beam is illuminated from a specific distance above the skin surface, reflected by a computer-driven mirror, to progressively scan the area of interest while detecting a fraction of the reflected backscattered light from tissues (Roustit and Cracowski, 2012). Figure 2.7 shows the setup of LDPI system.

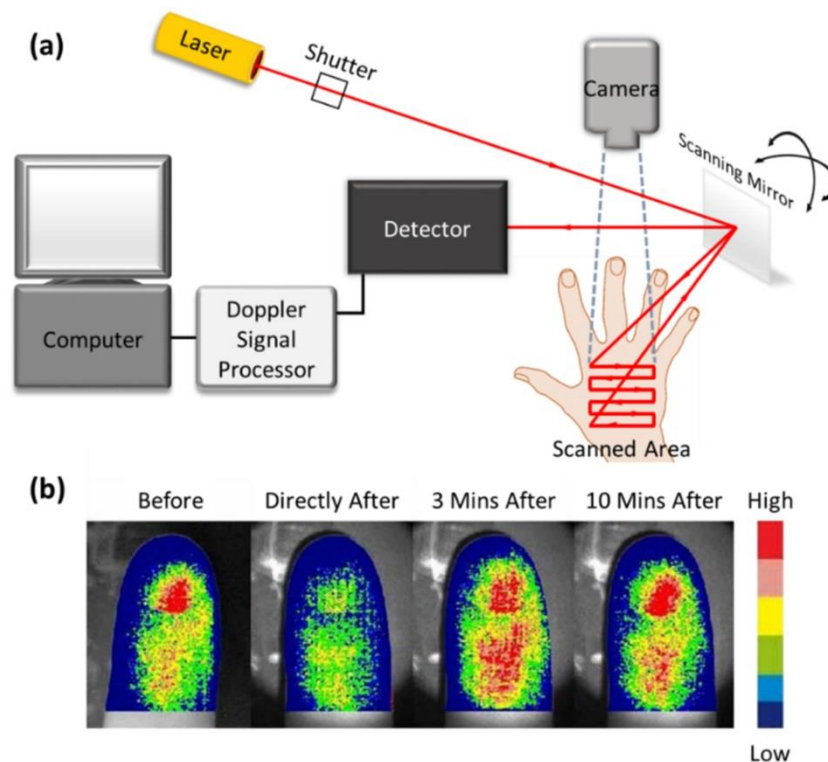


Figure 2.7 LDPI

(a) A schematic diagram showing the typical arrangement of LDPI setup. **(b)** LDPI of an index finger before, directly after, 3 min after, and 10 min after immersion in iced water. The 6-level colour scale represents relative low–high tissue perfusion (Deegan and Wang, 2019)

LDPI offers a non-contact assessment of the skin perfusion which may be desirable in clinical situations as for wound assessment. Also, it maps the perfusion from a larger surface area (up to 50 cm × 50 cm is possible) and can give the average perfusion of a heterogeneous tissue in a single measurement. Thus, reduces the spatial variability caused by vascular in-homogeneities and improves results reproducibility. However, LDPI is much slower than LDPM. It gives a “snapshot” of the perfusion at a given point of time, producing a series of images or “scans”. A few minutes may be required to capture one image, making rapid variations in the skin blood flow over the larger areas more difficult to record. Additionally, tissue motion and physical movements of the subjects produce more artefacts in a non-contact measurement technique such as LDPI than with monitoring using the LDF probe fixed on the skin (Rajan *et al.*, 2009).

As the LDPI technique cannot measure blood flow continuously, its output signal has a lower temporal resolution compared to LDF and can no longer be considered a real-time imaging modality. This problem can be partially resolved by reducing the area to be scanned and/or increasing the scan speed which may result in producing slightly less detailed images. To attempt to address this issue some recent imagers have used a multi-channel laser Doppler line. Many studies are not concerned with the dynamics of the cutaneous response that prerequisite the single point continuous flow assessments, but instead they focus more on the maximum response at a given stimulus/time point and the larger perfusion maps which can be adequately acquired by LDPI (Turner *et al.*, 2008, Rajan *et al.*, 2009, Roustit and Cracowski, 2012, Roustit and Cracowski, 2013, Deegan and Wang, 2019).

A single point LDF was chosen in the current study, as it required the contact feature of the LDF probe in delivering the premeasured in-shoe foot pressure while assessing the changes in the skin perfusion.

A major limitation of laser Doppler flowmetry is that no exact measure of blood flow can be extracted. However, a linear relationship between the laser Doppler signal and the microvascular blood flow has been established. Therefore, the relative laser Doppler signal or flux is mostly used to assess the microvascular reactivity, by challenging the examined vascular bed with various functional tasks. Among the tests used in combination with laser Doppler, the most common are iontophoresis of vasoactive drugs, post-occlusive reactive hyperaemia, and thermal provocations (Cracowski *et al.*, 2006, Roustit and Cracowski, 2012).

Iontophoresis in conjunction with LDPM and LDPI have been utilised in the literature. It has been frequently used to investigate and evaluate microvascular perfusion changes in response to local administration of vasoactive drugs across the skin in many pathological conditions, such as diabetes mellitus and peripheral arterial disease. Iontophoresis has been widely used as a non-invasive, convenient and simple pharmacological tool for transdermal drug administration (Roustit and Cracowski, 2012). Its principle mechanism is based on the transfer of charged molecules across the skin using a direct low-intensity electric current (Figure 2.8). The molecules of the drug to be delivered are either positively or negatively charged in a solution and will migrate across the skin under the influence of the applied monopolar current according to the rule that like charges repel each other (Turner *et al.*, 2008).

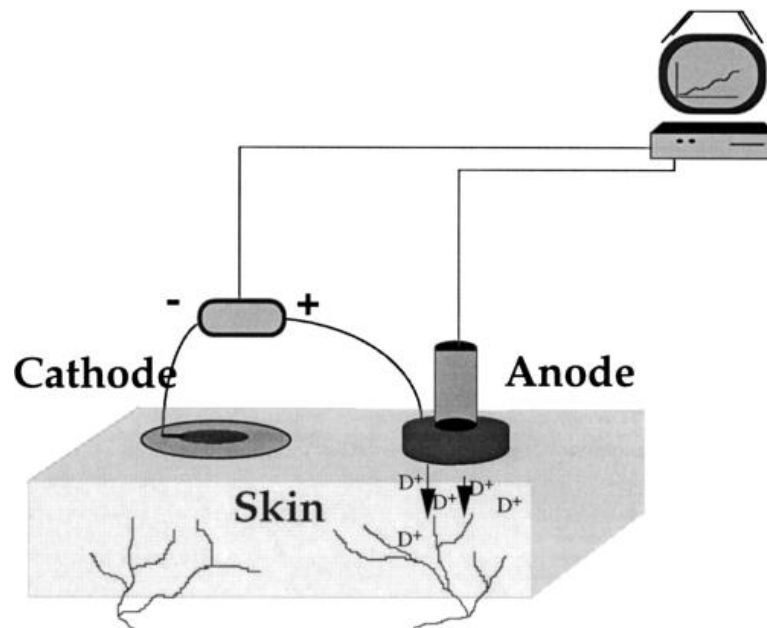


Figure 2.8 Schematic diagram for the delivery of positive drug ions (D^+) such as ACh. For negatively charged ions, such as SNP, the current to the chamber is reversed. Current is delivered by an iontophoretic device connected to a computer that controls iontophoretic settings and collects the data (Noon *et al.*, 1998)

The rate and quantity of the drug delivered depend on the concentration and the pH of the solution, the magnitude of the applied current and its duration and the nature of the skin surface. The main iontophoresis applications with Laser Doppler, involve a time-controlled delivery of a vasoactive drug, mostly ACh or SNP (although other substances have also been used e.g. bradykinin), onto a patch of the subject's skin, while the blood flow response is recorded by laser Doppler, to assess microvascular endothelium-dependent and independent vasodilation, respectively. The use of LDPI allows perfusion measurement over the entire distribution of the administered drug, whereas laser Doppler flowmetry restricts blood flow measurements to single points in the distribution. A typical set up for laser Doppler iontophoresis is shown in Figure 2.9.

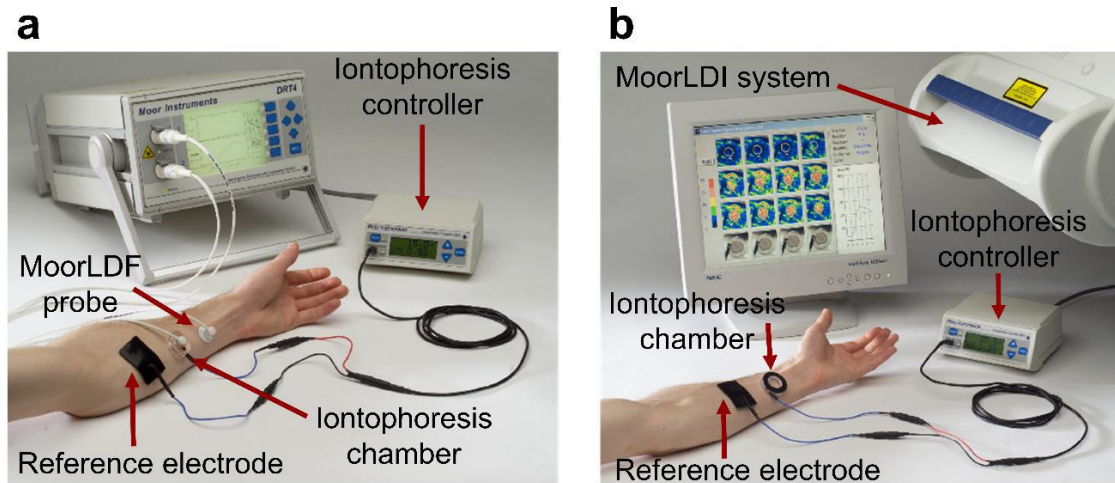


Figure 2.9 Laser Doppler iontophoresis equipment

(a) Example of the combination of the iontophoresis equipment with the Moor LDF probe
(b) Example of the combination of the iontophoresis equipment with the Moor LDPI system
 (Smirni, 2018)

The Iontophoresis equipment comprises a reference electrode and a ring-shaped chamber which is fitted with an internal electrode and will be filled with the vasoactive drug. The two electrodes are connected to a controller with the polarity of the chamber (active) electrode having the same charge of the vasoactive drug (e.g., chamber positive for ACh and negative for SNP). The circuit is completed by attaching the chamber and the reference electrode on the skin of the testing subject, and finally filling the chamber with the solution of the vasoactive agent. Under the influence of the applied current, the charges of the chamber electrode will repel the charges of the same polarity drug inducing the transfer of the molecule into the skin (Smirni, 2018).

There are different approaches for the electric current application in iontophoresis, depending on the substance used and the protocol chosen. Either, a protocol with a continuous application of current, or, more often, intermittent (interval) application of constant or increasing current is utilised (Lenasi, 2011). However, in any procedure, the electric current used is very weak (less than $100\mu\text{A}$), hence it is usually a painless procedure. Additionally, iontophoresis offers a non-invasive delivery of vasoactive drugs without the trauma associated with intradermal injection (microdialysis) that may

influence the skin blood flow. The quantity of the drug to be delivered is also too small to have any systemic effects, and avoids the first-pass metabolism, although, mild allergic reactions and skin irritation have been reported (Alam *et al.*, 2005, Turner *et al.*, 2008, Lenasi, 2011).

Several methodological issues need to be considered when using LDF with iontophoresis. For instance, all factors that could be a source of variability when assessing cutaneous microvascular reactivity should be kept at a minimum and must be controlled whenever possible, to remain constant throughout the procedure. Such factors include; subject-related elements, such as age group, the subject position (supine is the best posture to maintain throughout the measurement process in addition to subjects lying completely still to avoid movement artefacts), prior physical activity and mental stress, previous consumption of food, beverages containing caffeine or alcohol, smoking (subjects should refrain from tobacco, caffeine and food ingestion for a period of at least 2 hours prior to assessments), diseases and any vasoactive drug intake, menstrual cycle and the use of oral contraceptives, and the time of day when measurements are taken (temporal variations affiliated to; circadian rhythm, inter-day variability, seasonal variations). Also, environmental-related factors may influence the LDF signal such as room temperature (preferably set at 22-24°C), air humidity and movement of the adjacent air. Altogether, prerequisite subjects to reset for an appropriate acclimatisation period (at least 20 minutes) before any measurements are taken (Lenasi, 2011, Roustit and Cracowski, 2012, Roustit and Cracowski, 2013). Skin resistance, which varies significantly between individuals, is another vital factor that influences the efficiency of the procedure. Skin resistance varies between different skin sites and depends on other elements such as skin hydration status, thickness and nature (glabrous or non-glabrous) (Ramsay *et al.*, 2002). To minimise skin resistance, a general good practice is to clean the area where the iontophoresis electrodes are applied with an alcohol swab and gently rub the skin using

an adhesive tape to strip off the epidermis, clearing away any lipids or dead skin/keratinocytes (Turner *et al.*, 2008, Lenasi, 2011). Visible veins and hairy regions should be avoided and if any shaving is required, this has to be done at least 24 hours prior to data collection to avoid any associated skin flare response (Low *et al.*, 2020).

Another issue allied to the iontophoresis technique, is the confounding effect of current-induced vasodilation, referred to as ‘galvanic response’. The exact mechanism of this current-induced hyperaemia remains debatable. Proposed explanations include the induction of an axon reflex, competition between ions of the active substance and the vehicle used to dilute it (Turner *et al.*, 2008, Roustit and Cracowski, 2013). Topical anaesthesia before iontophoresis can minimise this nonspecific current-induced vasodilation but does not completely abolish the response and can impact upon the results. Thus, the addition of a control site is recommended to allow a quantitative correction of this issue. The magnitude of the current-induced-vasodilation was found to be dependent on the vehicle used. A range of preparations has been used, including deionized water, tap water, sodium chloride, mannitol solutions, as well as different cellulose gels. The use of deionized water as a vehicle limits the adjunction of competing ions, therefore enhancing the iontophoretic transport. Deionised water causes excessive current-induced vasodilation when used on its own, however, when a vasoactive drug such as ACh or SNP is added, the electrical characteristics of the resulting solution become different and the resistance of water was reduced (Khan *et al.*, 2004).

2.2.3.2 Assessment of Endothelial Functions in Diabetes Mellitus

The endothelium has been found to be susceptible to develop pathological changes in both Type 1 and Type 2 Diabetes Mellitus (Bertoluci *et al.*, 2015, Dhananjayan *et al.*, 2016). No single definition would cover the possible pathological changes in the endothelial function that is known as “Endothelial dysfunction”. This condition of

endothelial dysfunction is characterised by an alteration in the endothelium regulating functions, resulting in impaired vasodilation and a pro-inflammatory and pro-thrombotic status that favours the development of atherosclerosis and vascular complications associated with several cardiovascular and metabolic diseases including diabetes. In addition to endothelial dysfunction being known as a key initial event in the development of atherosclerosis, it is also one of the earliest signs of insulin resistance that appears to precede overt hyperglycaemia as in patients with Type 2 Diabetes, suggesting a cause-effect relationship (Balletshofer *et al.*, 2003). Signs of endothelial dysfunction were evident in diabetes, irrespective of the presence or absence of complications (Dinh and Veves, 2005). Furthermore, endothelial dysfunction was found to precede the development of diabetes in individuals with impaired glucose tolerance (Caballero *et al.*, 1999, Vehkavaara *et al.*, 1999) as well as, determined in healthy nondiabetic subjects who have a first-degree relative with Type 2 Diabetes (Balletshofer *et al.*, 2000). Therefore, endothelial dysfunction may represent a potential link between diabetes and atherosclerosis that would contribute to further understanding of the progression of vascular complications and end-organ damage in diabetes and provide an early target for preventing diabetic vascular diseases. In fact, there is evidence that treatment with metformin can assist in decreasing cardiovascular risk in patients with Type 2 Diabetes through ameliorating endothelial dysfunction (De Jager *et al.*, 2014, Stehouwer *et al.*, 2015).

However, a large variety of contributing mechanisms, which are still incompletely understood, have been proposed for the development of endothelial dysfunction in diabetes. Nevertheless, the high prevalence of other associated cardiovascular risk factors with diabetes, such as dyslipidaemia, hypertension, obesity, or a combination of these factors are also well known to negatively impact upon endothelial function (De Vriese *et al.*, 2000, Hadi and Suwaidi, 2007, Tabit *et al.*, 2010).

Reduced production and/or bioavailability of NO is considered one of the fundamental/initial elements of endothelial dysfunction, hence, assessment of endothelium-dependent vasodilatation, in particular, using the iontophoresis technique, offering a reproducible and accessible investigation, is commonly used to study endothelial functional changes in diabetes. Furthermore, advances in non-invasive techniques such as LDF which can reliably quantify skin microvascular blood flow and evaluate endothelial reactivity, have made it possible to study these endothelial functional changes in diabetes that contribute to the endothelium's lack of exerting the appropriate response to stress and injury, leading to various diabetic vascular complications including diabetic foot. It is generally believed that both structural and functional microvascular pathological changes contribute to the risk of tissue breakdown and poor wound healing in diabetic foot ulceration (Dinh and Veves, 2005, Chao and Cheing, 2009). Additionally, endothelial dysfunction was found to be an independent predictor of peripheral diabetic neuropathy, a key contributor to diabetic foot disease (Roustit *et al.*, 2016).

3.1 DORSAL FOOT PRESSURE MEASUREMENTS

Little research has been conducted to investigate Dorsal Foot Pressure and its impact on high-risk feet such as diabetic foot. Much research has been carried out into plantar pressure measurements, abnormalities and effect on tissue viability and blood flow, particularly when investigating pathologies such as diabetes mellitus.

Further studies would be beneficial in understanding the reason for the high prevalence of non-plantar ulcers and lower healing rates of foot wounds located on the dorsal surface (Eneroth *et al.*, 2004). Furthermore, a better understanding of dorsal foot pressure and its implications will help to inform future footwear design to achieve better comfort, injury prevention and performance improvement (Greenhalgh *et al.*, 2012, Rupérez *et al.*, 2012, Mei *et al.*, 2014).

It is generally agreed that there is a minimal pressure exerted on the dorsal surface of the foot (Walker *et al.*, 2015), hence, the lack of commercial devices available for dorsal foot pressure measurement. Therefore, in order to investigate the dorsal foot pressure, the current study began by exploring different means that could be used to measure the dorsal foot pressure and subject suitability for the study group. Available devices found will be discussed in the following section.

3.1.1 Smart Socks

Although a range of Smart Socks is available, they were mostly in exploratory prototypes at the time this research project commenced. Textile-based systems such as Smart Socks use embedded textile pressure sensors weaved or knitted in a specific pattern, making it possible to create a pressure-sensitive fabric with the desired number of pressure-sensing

sites. This approach has been used previously to develop sock-based systems for temporal gait analysis, and plantar foot pressure distribution/control in medical and sports applications (Preece *et al.*, 2011, Perrier *et al.*, 2014, Oks *et al.*, 2017, Mokhlespour Esfahani and Nussbaum, 2018).

An example of these smart socks included Sensoria® Fitness Smart Sock (Figure 3.1) (Esposito *et al.*, 2015). Each sock is infused with three proprietary textile sensors under the plantar area to detect foot pressure and conductive fibres relay data collected by these sensors to an anklet. When connected to the sock, the anklet communicates continuously with a mobile app through Bluetooth Smart. Another product that was proposed from the same manufacturer to use in the current study was the Sensoria® Developer Kit. This kit allows the development of a customised sensor strip. The sensor strip is essentially a strip of fabric containing the textile pressure sensors that enables placement of sensors to the desired location including the dorsal surface of the foot (Sensoria®).



Figure 3.1 Sensoria® Fitness Smart Sock (Sensoria®)

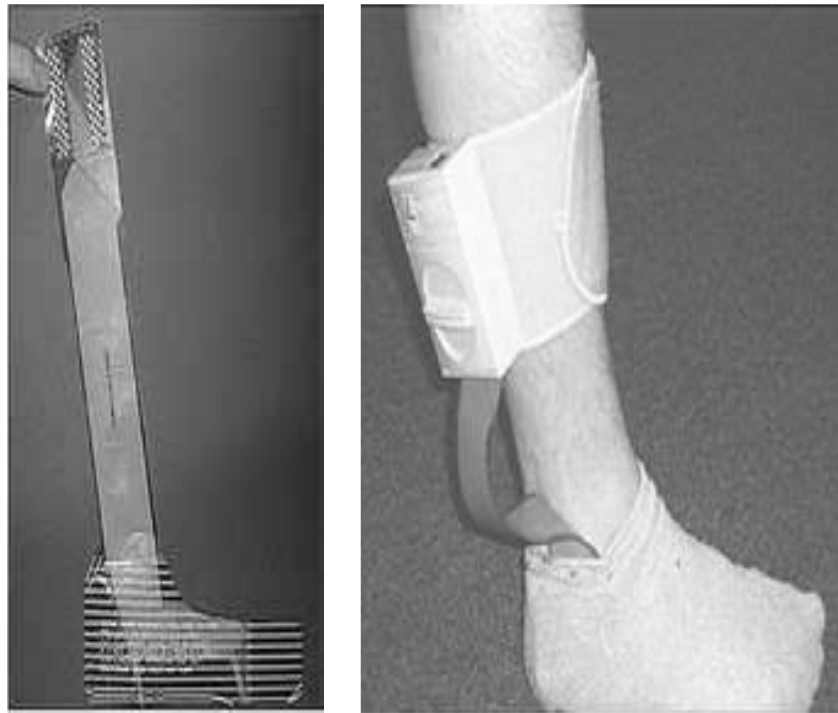
Further research evaluating this smart garment technology was published after the current study began (Rosenberg *et al.*, 2016, Raviglione *et al.*, 2017, Yeung *et al.*, 2019).

Other available Smart socks were also designed with pressure sensors embedded into the plantar surface only e.g. Taxisense Smart Sock “Taxisocks” (Taxisocks™, Perrier *et al.*, 2014) and DAid® Pressure Sock System (Oks *et al.*, 2016, Oks *et al.*, 2017). The Taxisense smart socks were later modified to measure dorsal foot pressure and correlate it with footwear comfort (Herbaut *et al.*, 2016).

Another two smart sock prototypes were found under investigation, aiming at recording pressure distribution around the whole foot whilst walking. These were the Alpha-Fit GmbH - Smart Sock (Alphafit, Koydemir and Ozcan, 2018), and the SmartSox project by a research group at the University of Arizona (Najafi *et al.*, 2017).

3.1.2 In-Shoe Pressure Measurement Inserts

In-shoe pressure measurement insoles/inserts were the only method used by the very few studies found in the literature that examined the characteristics of dorsal foot pressure. Greenhalgh *et al.* 2012 adapted an F Scan in-shoe pressure insole to measure pressure across the lateral side of the dorsum of participants’ feet. Although the insoles were designed to measure in-shoe plantar pressure, these sensors can be trimmed to the desired shape to measure pressure over any desired area. The insert was cut and inserted into the participant’s sock on the lateral side of their right foot’s dorsal surface with a small piece of a double-sided adhesive tape to hold the sensor in place (Figure 3.2) (Greenhalgh *et al.*, 2012).



**Figure 3.2 Adapted pressure sensor inserted into a sock
(Greenhalgh et al., 2012)**

The F Scan[®] in-shoe pressure measurement system uses micro-thin insoles (0.1 mm) with multi-laminate construction. The F Scan[®] insoles record pressure with ranges of 50-75 psi/345-517 KPa (sensitive) to 125 psi/862 KPa (standard). They offer a high spatial resolution with 960 resistive technology sensing elements spaced at 5 mm intervals (Tekscan Inc). This design allows the insole to be cut to fit any individual shoe size. Unfortunately, the advantage of these insoles being so thin, makes them prone to wrinkling both when being inserted within the shoe and while walking; this may lead to track failure and faulty data collection. The system also suffers from calibration issues with the sensitivity of the insoles declining as much as 20% with multiple uses (Abboud and Rowley, 1996, Urry, 1999, Nicolopoulos *et al.*, 2000).

Another TekscanTM system used in detecting dorsal foot pressure is FlexiForce[®] sensors (Olaso et al., 2007, Rupérez et al., 2012, Takesue et al., 2019). Similar discrete pressure sensors, FSA (Vista Medical, Winnipeg, Manitoba, Canada) were also used by Cheng and Hong, 2010 to quantify the subjective perception of fit of running shoes (Figure 3.3) (Cheng and Hong, 2010).



**Figure 3.3 Anterior view of FSA pressure sensors attachment
(Cheng and Hong, 2010)**

These thinner flexible piezoresistive pressure sensors can be customised according to the prerequisite tested area (Tekscan, Abdul Razak *et al.*, 2012). However, it was noted that the response of this type of sensors is sensitive to folding which causes measurement errors and therefore may lead to potential bias (Herbaut *et al.*, 2016).

The most reliable method used in the literature is Pedar (Novel®) In-shoe pressure measurement system. First attempts involved placing a custom-designed rectangular capacitance sensor pad on the dorsal side of the foot and correlating pressure distribution with perceived comfort (Jordan and Bartlett, 1995a, Jordan and Bartlett, 1995b, Jordan *et al.*, 1997). The dorsal pad could not be used in conjunction with the plantar insole therefore data were collected from the insole and dorsal pad separately. A significant correlation was noted between pressure and comfort, the lower the dorsal pressure, the better the perceived comfort.

Pedar in-shoe plantar pressure measurement insoles have been used for the determination of plantar and dorsal pressure in patients who had undergone rotationplasty. This surgical procedure alters the anatomical position of the foot so that the foot is rotated to a vertical posterior-facing position. The insoles were worn inside the sock during walking with the prosthesis (Figure 3.4) (Hillmann *et al.*, 2000).

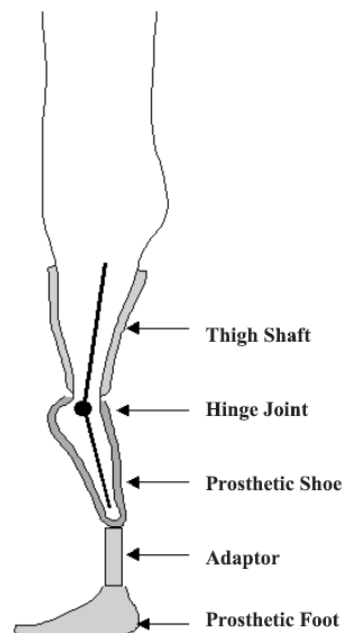


Figure 3.4 Schematic of the rotated foot and the prosthesis
The Pedar insoles were placed inside the customised prosthetic shoe
(Hillmann *et al.*, 2000)

The measurements were able to describe the main areas of loading for the plantar as well as the dorsal aspect of the foot within the shaft of the prosthesis (Figure 3.5). Although pressure distribution characteristics on the dorsal aspect were recorded, due to the altered position and function of the foot, the data could not be generalised to participants who had not undergone this surgical intervention (Greenhalgh *et al.*, 2012).

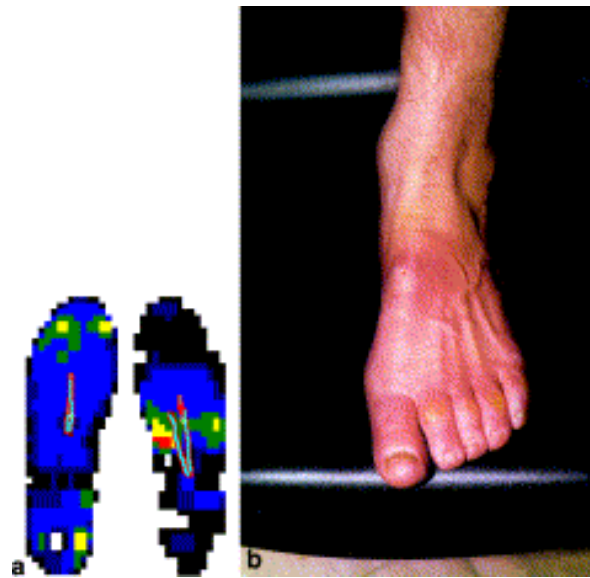


Figure 3.5 Average plantar (left) and dorsal loading (right) patterns in a rotationplasty patient

The photo of the rotated foot of the same patient indicating a callosity on the medial dorsum of the foot over the tarsometatarsal joint that corresponds to the pressure peak on the dorsal loading pattern (Hillmann *et al.*, 2000)

Hagen *et al.* (2008, 2010) used Pedar dorsal insoles to investigate the effects of different shoe-lacing patterns on dorsal pressure distribution and the perception of comfort and stability during running. The insoles were inserted into a specifically sewn pocket attached to the inside of the shoe's tongue. Planter loading was recorded using piezoelectric force platforms (Kistler 9281 B). Different shoe lacing patterns may affect the dorsal pressure; however, the loosest lacing may not be the most comfortable and a certain amount of lacing tightness is necessary to feel comfortable in running shoes (Hagen *et al.*, 2008). The study concluded that knowledge of the location of the peak

dorsal pressures would be useful for new tongue constructions and lacing systems to improve comfort in running shoes (Hagen *et al.*, 2008, Hagen *et al.*, 2010).

Mei *et al.*, 2014 also used the Novel[®] in-shoe measurement system to study the difference of plantar pressure and upper pressure among three types of sports shoes. A Pedar plantar pressure measurement insole and four pressure sensor chips were utilised to obtain plantar and dorsal pressure. Novel Pedar pressure sensor chips were positioned on the dorsal side of the foot at the medial first metatarsophalangeal joint, lateral fifth metatarsophalangeal, the contact position between just under the medial condyle and medial-upper and the contact position between just under the lateral condyle and lateral-upper (Figure 3.6) (Mei *et al.*, 2014).



Figure 3.6 Position of Novel insole (black) and sensor chips (blue)
(Mei *et al.*, 2014)

Although the plantar pressure distribution among the three pairs of shoes compared in the study was not significantly different, a great difference of upper pressure existed within different sports shoes (Mei *et al.*, 2014).

3.1.2.1 Pedar In-Shoe Pressure Measurement System

The Novel[®] Pedar system is considered one of the most popular and reliable in-shoe pressure measurement devices that can monitor local loads between the foot and the shoe (Murphy *et al.*, 2005, Hurkmans *et al.*, 2006, Putti *et al.*, 2007, Gurney *et al.*, 2008, Ramanathan *et al.*, 2010). It has been shown to have lower variance across sensors when compared to F Scan (Quesada *et al.*, 1997) and the best repeatability for plantar pressure collection during dynamic activities (Martinez Santos, 2016).

Thus, for the current research project, the Pedar dorsal pads and plantar insoles were chosen to be used for pressure data collection within the tested footwear.

The Pedar insoles have a matrix of sensors arranged in rows and columns to cover the entire area of the foot studied during walking. The highly conforming, elastic sensor insoles are made of capacitive sensors. These sensors measure the change in capacity related to a change in distance between two conducting wires which varies with different loading. These insoles are connected by cables to a body-mounted transmitter box which transfers the final pressure data to the computer workstation via a wireless connection using a Bluetooth[®] telemetry. Data can then be observed in real-time on the computer screen. The Pedar-x[®] system has the capacity to record in-shoe pressure at a sampling frequency of up to 100Hz (Novel Gmbh).

With the aid of the Novel TruBlu[®] calibration device (Figure 3.7), all sensors of the Pedar insoles are individually calibrated using known air pressures. The computer-assisted procedure uses a bladder and air cylinder to load the insole to a chosen pressure evenly over the insole surface. Calibration guarantees accurate and reproducible data (Novel Gmbh).



Figure 3.7 Novel TruBlu® calibration device

Pedar dorsal pads (Figure 3.8) are available in two sizes: VD (equal to 38/39 European shoe size) and XD (equal to 42/43 European shoe size). Pedar insoles have sensor thickness of 1.9 mm and house 85–99 capacitive sensors. Individual sensors, measure an area that approximately equates to 1cm^2 per sensor, and can be calibrated to a pressure range of 15 - 600 KPa (Novel GmbH).



Figure 3.8 Pedar dorsal pads
Come in 2 sizes VD (left); equal to 38/39 European Shoe Sizes
and XD (Right); equal to 42/43 European Shoe Sizes

3.2 PILOT STUDY

3.2.1 Introduction and Background

Many factors have been investigated in the literature as having a role in diabetic foot ulcer formation. The current research experiment aims at simultaneous studying two of the major ulcer contributors namely, loading pressure and microvascular abnormalities, investigating the impact of pressure application on peripheral blood flow in diabetic foot. In particular, the changes in the endothelial response under applied pressure on both dorsal and plantar surfaces of the foot in patients with Type 2 Diabetes and a matched control group of non-diabetic individuals. The study planned to utilise a custom-made pressure delivery equipment, which was able to apply the premeasured dorsal and plantar in-shoe pressures on the study groups' feet while recording blood flow measurement. This device was designed to deliver a known pressure while housing a Laser Doppler Flowmetry probe that acts as the endpoint of pressure delivery and assesses the skin blood flow changes in response to the iontophoresis of an endothelium-dependent vasodilator, acetylcholine, and endothelium-independent vasodilator, sodium nitroprusside. The pressure delivery equipment was developed and validated to investigate the impact of replicated barefoot walking plantar pressure on endothelial function of the superficial blood vessels supplying the plantar aspect of the forefoot in subjects with diabetes mellitus (Flynn, 2014).

The premeasured pressure was delivered via a spring housed within the device's metal tube allowing for an unimpeded movement. A measuring gauge was attached to indicate the pressure delivered and this pressure could be set and adjusted via a control dial (Figure 3.9). The spring selected for pressure delivery had a known rate of 4 N/mm. Full specifications are shown in Table 3.1 (Lee Springs).

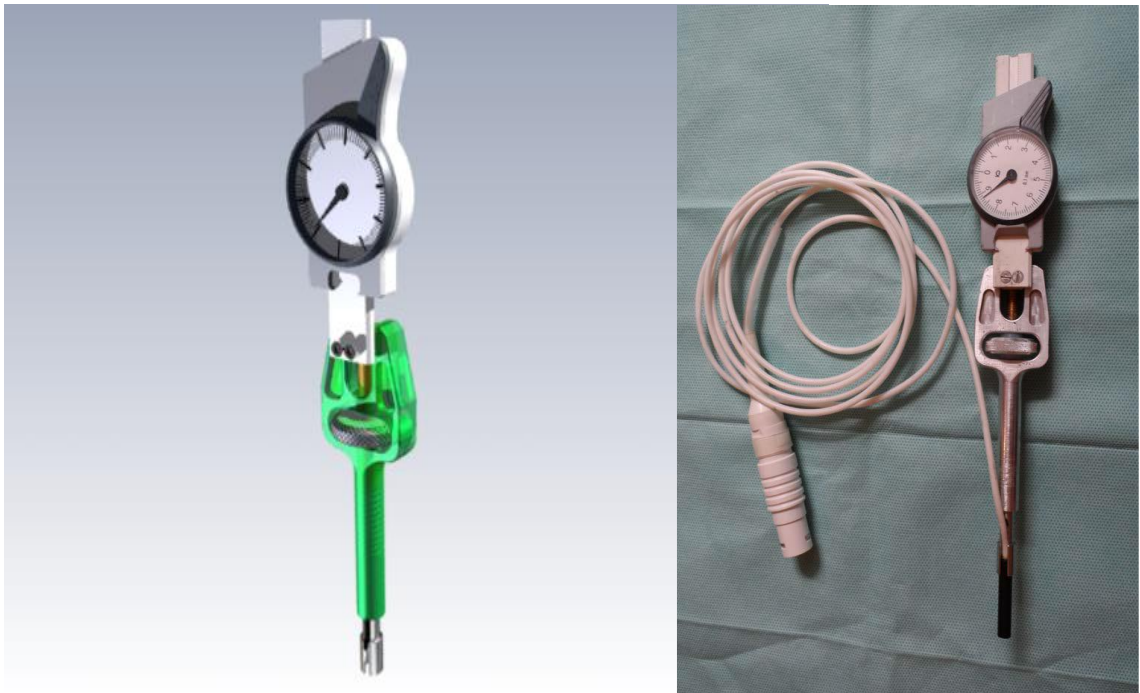


Figure 3.9 The pressure delivery system diagram
The Laser Doppler Flowmetry probe attached to the pressure delivery equipment (left)
(Flynn, 2014)

Table 3.1 Compression Spring (used on the plantar surface) Specifications
(Lee Springs)

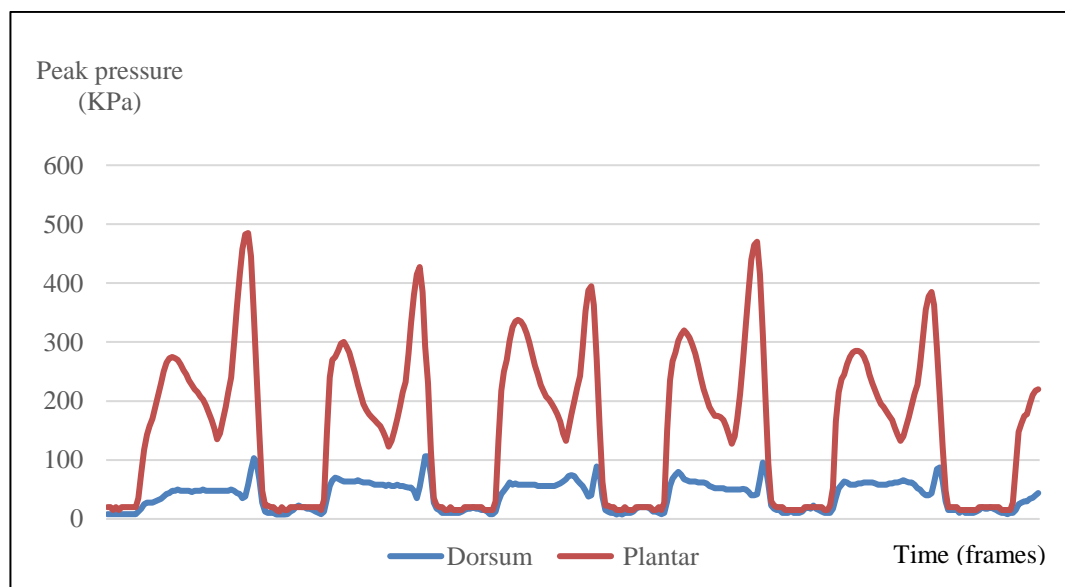
Part Number	LC 029BB 11M
Outside Diameter	4.775 mm
Hole Diameter	5.156 mm
Wire Diameter	0.736 mm
Load At Solid Length	47.282 N
Free Length	22.224 mm
Rate	4.00 N/mm
Solid Length	10.261 mm
Rod Diameter	3.098 mm
Number of Coils	11.5
Total Coils	13.5
Finish	ZINC PLATE AND BAKE PER ASTM B633

The Laser Doppler Flowmetry probe that was used as the surface contact for pressure delivery, had a radius of 3 mm, thus a surface area of 28.274 mm². Utilising the principle of pressure equals force/area, the pressure delivery system was capable of delivering a pressure ranging between 141KPa to 1343KPa (Table 3.2).

**Table 3.2 Pressure values correspond to the displayed pressure delivery device dial readings
(When Spring Rate = 4N/mm, LDF Probe surface area =28.274 mm²)**

Pressure Device Dial reading	Force Rate N/mm	Pressure N/mm2	Pressure K Pa
0.5	2	0.07	70.74
1	4	0.14	141.47
1.5	6	0.21	212.21
2	8	0.28	282.95
2.5	10	0.35	353.68
3	12	0.42	424.42
3.5	14	0.50	495.15
4	16	0.57	565.89
4.5	18	0.64	636.63
5	20	0.71	707.36
5.5	22	0.78	778.10
6	24	0.85	848.84
6.5	26	0.92	919.57
7	28	0.99	990.31
7.5	30	1.06	1061.05
8	32	1.13	1131.78
8.5	34	1.20	1202.52
9	36	1.27	1273.25
9.5	38	1.34	1343.99

These ranges fit well with the pressure to be collected on the plantar aspect of the foot. However dorsal pressure noted in the literature (Jordan and Bartlett, 1995a, Mei *et al.*, 2014) and those detected in our laboratory while testing the Pedar dorsal pads had much lower values, of approximately 100KPa (Figure 3.10), and therefore could not be delivered by the same spring used for plantar surface testing.



**Figure 3.10 Provisional peak pressure data
shows the difference in values between dorsal and plantar peak pressures**

This imposed the need to conduct a pilot study to acquire an average range for the peak dorsal pressure values, in order to determine the correct spring to be used within the pressure delivery equipment to investigate the pressure effect upon the dorsal surface of the foot.

Another factor that had been highlighted while formulating the measurement procedures, was the anticipated effect of the difference in shoe type, shape and manufacturing material on the dorsal pressure measurement. Significant differences have already been recorded in peak dorsal pressure between different types of sports shoes (Mei *et al.*, 2014). Similarly, different pressure distribution patterns were displayed when the Pedar dorsal pad was tested in our laboratory using different types of shoes that were all belonging to the same subject (Figure 3.11).



Figure 3.11 Testing Pedar Dorsal pad in different types of shoes of the same subject

These findings necessitated the search for a standardised shoe to be used for this project. A standard pair of shoes with a clear upper surface (Figure 3.12), used in the Institute of Motion Analysis and Research (IMAR) laboratory to teach referred subjects how to choose their best shoe fit, was tested first. It was hoped that the clear upper surface would

guarantee the standard placement of the dorsal pad. However, the very stiff shoe material made it challenging to use, especially with the need for repeated walking trials. The fear of inducing foot injury to the known high-risk feet of diabetic participants excluded these shoes from being used in the current project.



Figure 3.12 Standard shoes with a clear upper surface

The best choice found, was one of the orthopaedic footwears that is frequently prescribed for patients with high-risk feet at Tayside Orthopaedic and Rehabilitation Technology (TORT) Centre's orthotic clinic. The Chaneco Diabetic shoes, Calais (for men) and Venice (for ladies), are frequently provided for diabetic patients referred to the orthotic clinic. These shoes are available off-the-shelf or with some orthotic modification as custom moulded insoles. They can be supplied either with lace or straps (Figure 3.13), however, according to the orthotist recommendation, patients have a preference for laces, and therefore, the lace type shoes were used for the current research.



Figure 3.13 Orthopaedic shoes supplied with laces (right) and straps (left)

After finding the most suitable shoes, the study began to explore the best method of holding the dorsal pad in place when recording the in-shoe pressure measurement during walking. Methods used in the literature include placing the measuring sensors inside participants' socks (Greenhalgh *et al.*, 2012) or into a sewn pocket attached to the inside of the shoe's tongue (Hagen *et al.*, 2010). Chaneco has provided one shoe sample with a strap attached to the shoe tongue to hold the Pedar dorsal pad while conducting the measurement procedure (Figure 3.14).



Figure 3.14 Sample shoe with a strap attached to the shoe tongue

Dorsal pressure measurement with the dorsal pad inserted inside socks compared with placing it into the strap stitched to the bottom of the shoe tongue showed the socks to be more reliable. Socks allowed good adjustment of the measuring pad on the foot asperities. Thus, dorsal pressure recorded in socks was more representative of the real experienced

pressure than that with the strap holding the measuring pad at one end which may have modified the pressure distribution pattern as seen in Figure 3.15. Also, the tested subject felt more comfortable with the pad inserted in socks procedure. Consequently, the study proceeded using socks.

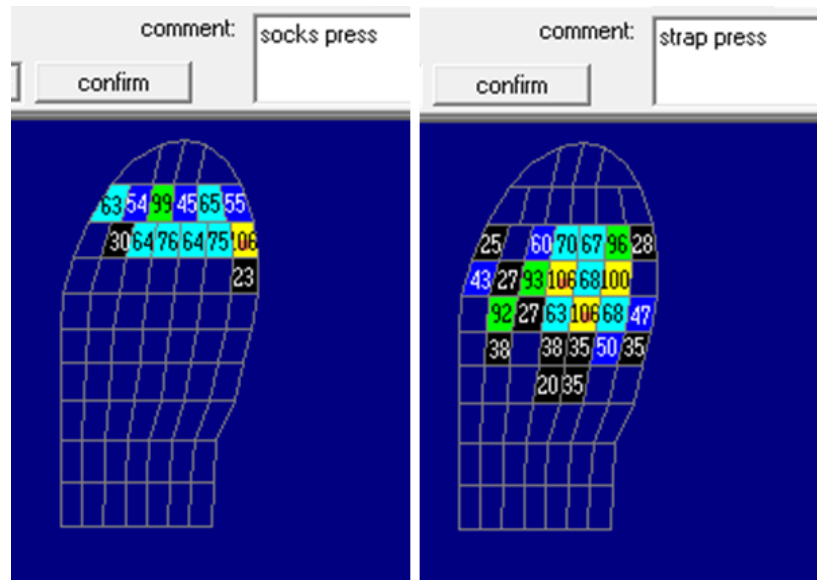


Figure 3.15 Dorsal pressure distribution while inserting Pedar Dorsal pad in socks (right) vs shoe tongue strap (left)

All these factors uncovered while designing the study protocol necessitated the need for conducting a pilot study to achieve the following objectives:

- Quantify a range for peak pressure values on the dorsal surface of the foot in a group of non-diabetic volunteers using the Pedar dorsal pad. This will help to determine the rate for the spring to be used in the pressure delivery equipment to investigate the pressure effect on the dorsal surface of the foot
- Test the equipment and conduct a provisional comparison between dorsal and plantar pressure values recorded in participants' own comfortable shoes and then when wearing size-matched tested orthopaedic shoes

- Investigate the time of occurrence of the dorsal peak pressure during the gait cycle. It was hypothesised that simultaneous in-shoe plantar pressure measurement can help in timing of dorsal peak pressure by correlating it with the well-known time of plantar peak pressure in the gait cycle
- Test the iontophoresis protocol to be used in endothelial function assessment on the dorsal surface of the foot and assesses its repeatability to be used on both dorsal and plantar surfaces of the foot

3.2.2 Participants

Initially, ethical approval was sought and granted from the University of Dundee Research Ethics Committee (Appendix 1). A volunteer recruitment poster for the study was advertised in Ninewells Hospital, University of Dundee notice boards and circulated via the University e-Newsletter (Appendix 2). Non-diabetic adult volunteers (males and females), age range 18 to 75 with no known underlying lower limb vascular or neurological conditions were invited to participate in the study. Exclusion criteria included any foot deformity, amputation or underlying pathology of the spine/lower limbs which results in an inability to walk unaided. All subjects were provided with a written information sheet (Appendix 3) and informed consent (Appendix 4) to be signed prior to any participation.

Thirteen non-diabetic adult volunteers were recruited from the university staff and student populations (Table 3.3). The study group consisted of 7 females (53.8%) and 6 males (46.2%). Age at the time of testing ranged from 22 to 62 years old with mean age \pm SD of 32 ± 10.8 years. Body mass index (BMI) ranged from 20.7 to 46.9 kg/m² with mean BMI \pm SD of 27.8 ± 7.9 kg/m². UK shoe sizes of participants ranged from 4 to 10 with the mean \pm SD being 7 ± 2 .

Table 3.3 Demographic data distribution of the pilot study

Demographic data (n=13)		Range (Mean \pm SD)
Sex	Female	7 (53.8%)
	Male	6 (46.2%)
Age (years)		22-62[32.31 \pm 10.85]
Height (m)		1.51-1.82[1.66 \pm 0.09]
Weight (kg)		49-120[76.08 \pm 21.04]
BMI [Weight/(Height)²]		20.66-46.88[27.84 \pm 7.92]
UK Shoe size		4-10[6.69 \pm 2.36]

3.2.3 Pressure Data Collection

Pedar dorsal pads and plantar insoles were calibrated using the Novel TruBlu[®] calibration device (Figure 3.7). This calibration device applies a homogeneous air pressure on all sensors through incremental steps of pressure. An individual calibration curve for each sensor is then calculated and used during data acquisition.

The dorsal pads were calibrated at lower values (up to 200KPa) to suit the anticipated low dorsal foot pressure while the plantar insoles were calibrated to record pressures up to 600KPa, which is the recognised range of plantar pressure. For a simultaneous recording of dorsal and plantar pressure of the foot, each dorsal pad was coupled with one of the plantar insoles. Due to the result difference in calibration between the two measuring insoles, coupling was not possible with the Pedar data acquisition software. Therefore, Novel Pliance[®] mobile measurements box and data acquisition software were used as these were able to pair the dorsal pad with the plantar insole while having different calibration ranges. Yet, the dorsal pressure values were represented to correspond to the higher calibration range of the plantar insoles (as if the dorsal pad were calibrated at 600KPa, not 200KPa). The correct dorsal pressure values were later obtained by dividing the extracted peak pressure values by 3.

A Pedar dorsal pad, labelled left, was inserted into a standardised sock to be secured onto the dorsal surface of the participant's right foot and connected to the left cable of the

Pliance® mobile measurements box. A matched shoe size, right-sided Pedar plantar insole was placed inside the right side of the tested shoes and connected to the right cable of the Pliance® mobile measurements box (Figure 3.16). Any excess length of cable was fastened around the participant's lower limb using Velcro tabs to avoid cables getting in the way while walking. The transmitter (fastened around the waist) wirelessly transferred recorded data to a PC via a Bluetooth dongle.



**Figure 3.16 In-shoe measurement system connection
(right) Placement into participants' shoes (left)**

Pressure differences were recorded on the dorsal surface in the standing position, so zero check is recommended before testing (Jordan and Bartlett, 1995a). Therefore, after placement, subjects were asked to raise their right foot into a horizontal non-weight bearing position on a chair with a loosened shoelace to offload both the dorsal pad and the plantar insole before any recording was made (Figure 3.17). Subjects were then asked to tie their shoelace the way they usually do to feel comfortable (either the shoes they were wearing, if applicable or the orthopaedic shoes provided).



Figure 3.17 Offloading the dorsal pad and plantar insole before pressure recording

Participants, wearing each type of footwear, were asked to do six walking trials at their own self-selected speed along a 6-meter-long walkway. Simultaneous in-shoe dorsal and plantar pressures of the right foot were recorded (Figure 3.18) with the participants wearing first their own comfortable shoes followed by wearing the size-matched orthopaedic shoes provided.

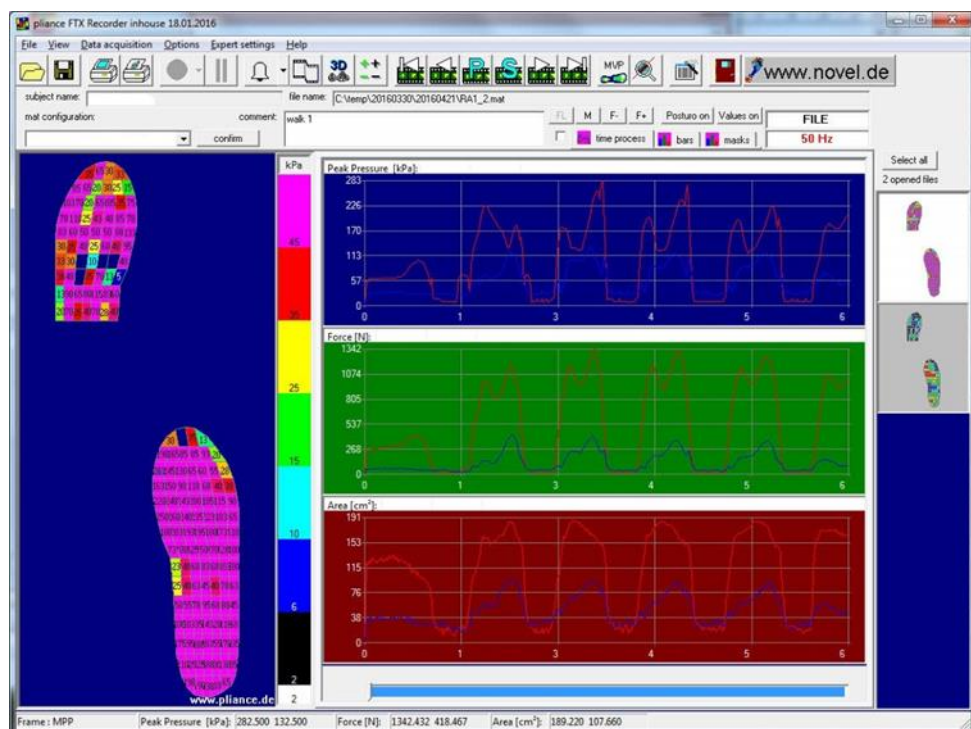


Figure 3.18 In-shoe pressure recorded on dorsal and plantar surfaces

The orthopaedic shoes were provided with two extra insoles to offer more customisation of the shoe's depth. One of these extra insoles was removed for comparison with the full-insole orthopaedic shoes and participants' own shoes.

Data were trimmed to have at least three good steps in each trial for processing. Foot Masks were then defined; one for the whole plantar surface and another for the dorsal surface. Peak pressure, defined as the highest pressure in any sensor across a given mask, were exported into Microsoft Excel spreadsheet to compare the three tested shoe conditions on both plantar and dorsal surfaces of participants' feet. The data were then imported to a Statistical Package of Social Science (SPSS) software for statistical analysis.

3.2.4 Pilot Study Results

Statistical comparison was conducted using the General Linear Model to estimate the Peak Pressure Means across successful steps in all trials for each subject in each tested condition. Analysis of variances and Post Hoc test: Least Significant Difference (LSD) were used to determine the significance of differences between the three tested conditions within for both dorsal and plantar surfaces.

Pairwise Comparisons of Estimated Marginal Means showed a significant difference in in-shoe peak pressure between participants' shoes and the orthopaedic shoes on the dorsal surface ($p < 0.001$). However, no significant difference was detected on the plantar surface ($p > 0.05$). Furthermore, removing one of the extra insoles supplied with the orthopaedic shoes revealed a significant increase in plantar pressure ($p < 0.05$) when compared to full insole orthopaedic shoes. No significant differences were noted on the dorsal surface within the orthopaedic shoes on removing the extra insole.

Table 3.4 The three tested shoe conditions peak plantar pressure and peak dorsal pressure (KPa). Own shoes, Orthopaedic shoes and Orthopaedic shoes-1 insole

Whole foot In-shoe pressure		Own shoes	Ortho Shoes	Ortho Shoes-1
Peak Plantar Pressure (KPa)	Mean \pm SD	269.20 \pm 49.38	267.37 \pm 46.50	283.27 \pm 61.40
	Range	170-385	162.5-397.5	165-452.5
Peak Dorsal Pressure (KPa)	Mean \pm SD	75.18 \pm 28.40	55.66 \pm 21.97	54.38 \pm 20.21
	Range	33.33-147.5	27.5-123.33	27.5-100

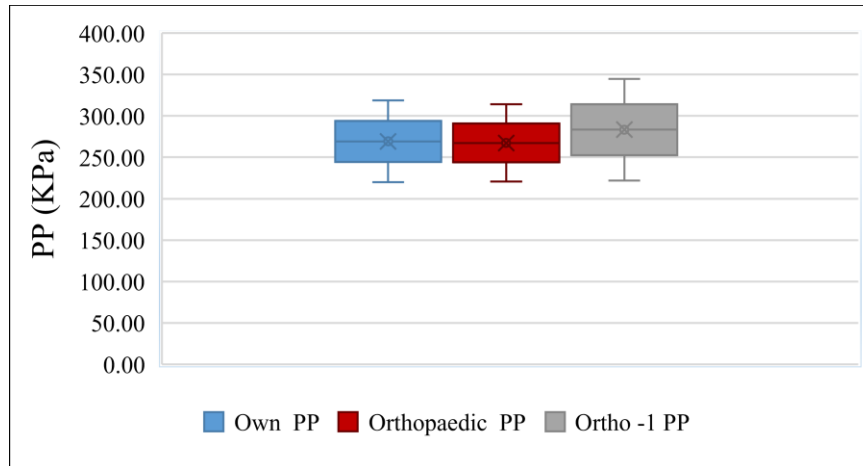


Figure 3.19 Plantar Peak Pressure in own shoes, orthopaedic shoes and orthopaedic shoes-1 insole conditions

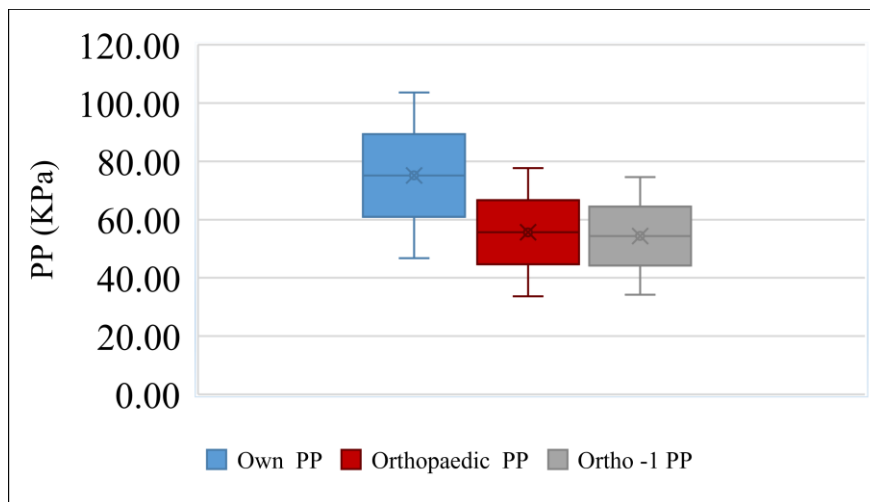


Figure 3.20 Dorsal Peak Pressure in own shoes, orthopaedic shoes and orthopaedic shoes-1 insole conditions

These results show how the design of the orthopaedic shoes with a deeper box, effectively offloaded the dorsal surface of the foot. The noted significant reduction in pressure will ultimately lower the risk of skin trauma and the development of pressure ulcers on the dorsum of the foot (Pinzur *et al.*, 2005). However, the insignificant difference between

participants' own shoes and the orthopaedic shoes recorded on the plantar surface and the significant increase in plantar pressure when removing one of the insoles in the orthopaedic shoes, confirms the difficulty of predicting the effect of therapeutic footwear as found by other studies in the literature (Ashry *et al.*, 1997, Praet and Louwerens, 2003). Therefore, in-shoe plantar pressure measurement remains an essential tool for the evaluation of at-risk feet prior to therapeutic footwear prescription and/or any insole adjustment implementation (Waaijman *et al.*, 2012, Bus *et al.*, 2016a, Bus *et al.*, 2016b).

The results of this pilot study show the reliability of this in-shoe pressure measurement system in detecting dorsal pressure simultaneously with the plantar pressure and was therefore used in the final experimentation setting. Furthermore, dorsal pressure variation across different testing conditions demonstrates the capability of the dorsal pressure assessment to be used as a guidance tool to effectively improve the design and evaluation of footwear, especially in high-risk feet as in diabetes. This will eventually provide a better offloading approach that may reduce the risk of pressure-related diabetic foot ulcers.

Although this pilot study simultaneously recorded in-shoe plantar and dorsal pressures, it was not possible, at this point in the research, to produce the time extraction software. There was an issue with defining the events of the gait cycle and detecting when exactly dorsal peak pressure occurs. The pattern of dorsal peak pressure varied extensively across participants and multiple peaks in a sawtooth pattern were sometimes observed. Later, in-house software was developed, which was able to define the gait cycle by the first plantar peak pressure that was believed to correspond to heel strike, as the starting event of each gait cycle. The plantar peak pressure data were correlated with the dorsal peak pressure and dorsal maximum force as it showed more homogenous and clear peaks on the produced graphs. This software was used for processing the final experiment data.

3.2.5 Setting the Pressure Delivery System

As discussed earlier, the pressure delivery equipment developed and validated by Flynn (2014) was used (Figure 3.9) in the final procedure to study the effect of loading pressure on the endothelial response in the feet of patients with Type 2 Diabetes and a matched control group of non-diabetic individuals. One of the main objectives of the pilot study was to obtain a range of values for dorsal peak pressure to guide in identifying the appropriate spring for the pressure delivery equipment, to be used in delivering the premeasured pressure on the dorsum of the foot while recording the changes in blood flow via the enclosed LDF probe. The pilot study data indicated dorsal peak pressure across all subjects within different testing conditions, to range between 27.5KPa and 147.5KPa (Table 3.4).

The outcome of the investigation of available springs showed that it would be more practical to use two springs. A spring with a rate of 0.39 N/mm (full specifications are shown in Table 3.5) to be used with lower pressure values and the other with a rate of 0.50 N/mm (full specifications are shown in Table 3.6) for higher pressure values. This made it easier to tune the control dial on the pressure delivery equipment across different anticipated pressures recorded on the dorsum of participants' feet.

**Table 3.5 0.39 N/mm Compression Spring Specifications
(Lee Springs)**

Part Number	LC 016AB 12S
Outside Diameter	3.759 mm
Hole Diameter	3.962 mm
Wire Diameter	0.406 mm
Load At Solid Length	7.036 N
Free Length	25.400 mm
Rate	0.39 N/mm
Solid Length	7.543 mm
Rod Diameter	2.743 mm
Number of Coils	15.8
Total Coils	17.8
Finish	PASSIVATE PER ASTM A967

**Table 3.6 0.5 N/mm Compression Spring Specifications
(Lee Springs)**

Part Number	LC 016AB 11M
Outside Diameter	3.759 mm
Hole Diameter	3.962 mm
Wire Diameter	0.406 mm
Load At Solid Length	8.451 N
Free Length	23.825 mm
Rate	0.50 N/mm
Solid Length	7.112 mm
Rod Diameter	2.743 mm
Number of Coils	14.7
Total Coils	16.7
Finish	ZINC PLATE AND BAKE PER ASTM B633

These springs are capable of delivering pressure values demonstrated in, respectively Table 3.7 and Table 3.8. It was noted that the 0.39 N/mm spring was the one mostly used except in two cases where the 0.50 N/mm spring was used instead because the dorsal pressure recorded was very high and difficult to accommodate within the equipment control dial.

**Table 3.7 Pressure values correspond to the displayed pressure delivery device dial readings
(When Spring Rate = 0.39 N/mm, LDF Probe surface area = 28.274 mm²)**

Pressure Device Dial reading	Force	Pressure N/mm ²	Pressure K Pa
0.5	0.195	0.007	6.90
1	0.39	0.014	13.79
1.5	0.585	0.021	20.69
2	0.78	0.028	27.59
2.5	0.975	0.034	34.48
3	1.17	0.041	41.38
3.5	1.365	0.048	48.28
4	1.56	0.055	55.17
4.5	1.755	0.062	62.07
5	1.95	0.069	68.97
5.5	2.145	0.076	75.86
6	2.34	0.083	82.76
6.5	2.535	0.090	89.66
7	2.73	0.097	96.56
7.5	2.925	0.103	103.45
8	3.12	0.110	110.35
8.5	3.315	0.117	117.25
9	3.51	0.124	124.14
9.5	3.705	0.131	131.04

**Table 3.8 Pressure values correspond to the displayed pressure delivery device dial readings
(When Spring Rate = 0.5 N/mm, LDF Probe surface area = 28.274 mm²)**

Pressure Device Dial reading	Force	Pressure N/mm ²	Pressure K Pa
0.5	0.25	0.009	8.84
1	0.5	0.018	17.68
1.5	0.75	0.027	26.53
2	1	0.035	35.37
2.5	1.25	0.044	44.21
3	1.5	0.053	53.05
3.5	1.75	0.062	61.89
4	2	0.071	70.74
4.5	2.25	0.080	79.58
5	2.5	0.088	88.42
5.5	2.75	0.097	97.26
6	3	0.106	106.10
6.5	3.25	0.115	114.95
7	3.5	0.124	123.79
7.5	3.75	0.133	132.63
8	4	0.141	141.47
8.5	4.25	0.150	150.31
9	4.5	0.159	159.16
9.5	4.75	0.168	168.00

To test the pressure delivery equipment, a set of known forces was applied by placing weights of known values, on the top of the device while in an upright position and checking the dial readings. Accurate readings were registered, and a linear relationship was observed between forces applied and readings on the device scale, indicating good repeatability of the equipment.

In the final experiment, a rigid post-surgical boot was used to hold the limb securely and also clamp the pressure delivery equipment housing the LDF probe while recording the blood flow changes and delivering pressure (Figure 3.21). This setup was established to prevent any movement during the procedure, that may produce artefacts in the LDF reading and/or alter the pressure applied as adjusted on the device dial. The customised boot had a fleece lining for subject comfort and a metal plate attached to the foot backplate, which is adjustable in two directions, height and depth (Figure 3.22). This allows the position of the pressure delivery device to be altered to ensure that the LDF probe was held in the correct position against the skin surface. This system design worked well when applying pressure while recording blood flow changes on the plantar surface

of the foot (Flynn, 2014). However, to test the dorsum of the foot an extra part was required to hold the pressure delivery equipment opposite the other side of the foot.



Figure 3.21 The pressures delivery system measuring blood flow using LDF while delivering pressure on the plantar surface of the foot (Flynn, 2014)



Figure 3.22 The post-surgical boot with the adjustable baseplate (Flynn, 2014)

Testing the equipment on the dorsum of the foot began with adding an extra bar to be attached to the metal plate attached to the boot's foot backplate (Figure 3.23). However, the topography on the dorsum of the foot was more dome-shaped than the relatively flat surface found on the plantar of the foot. The rigid structure of this setup failed to attain a flush position of the LDF probe against the skin. Applying pressure by the pointed edges of the probe rather than the whole surface area can change the pressure values intended to be delivered. This may cause subjects to experience more pain or discomfort which will lead to mental stimulation and affect the blood flow in the area under pressure. Moreover, with the anticipated lengthy procedure, there will be a potential increase in the risk of skin breakdown due to the pressure application on such a small surface area of the probe edges.



Figure 3.23 Original setting for investigating the plantar surface of the foot (left), when adding a bar for testing the dorsum of the foot (right)

On searching for a more flexible means for holding the equipment on the dorsum of the foot, it was decided to use the Snake Shape Arm with the Magnetic Base Holder shown in Figure 3.24. The connecting arm bends freely like a snake, providing infinite flexibility. It can also be locked rigidly in almost any position by a controlling lever situated at its base. It is fitted to an ON/OFF switchable magnetic base with a 40kg (400N) pull that holds it solidly on ferrous metal surfaces, either vertically or upside down (RS PRO). This provided a flexible clamp that enabled easy adjustment of the LDF probe orientation on the dorsum of the foot with secured fixation.



Figure 3.24 the Snake Shape Arm with the Magnetic Base Holder

The magnetic base was mounted and allowed to move along a metal bar fixed to the bed on which participants lay during data collection. All parts were covered by foam sheets to prevent any injury to subjects while positioning their feet (Figure 3.25). To ensure the subject's foot would not move downwards via the pressure applied on the dorsum of the foot, the adapted boot was firmly fixed to the end of the bed. This helped to maintain the

correct and stable positioning of the equipment as well as the subject's foot during the data gathering procedure.



Figure 3.25 Setting equipment for the data collection on the dorsum of the foot

3.3 IONTOPHORESIS PROCEDURE

3.3.1 Equipment

Iontophoresis combined with the single point LDF was the technique chosen to assess endothelial function on both foot surfaces in the study groups. The ability to monitor changes in the blood flow whilst in contact with the skin, allows the LDF probe to act as a component of the pressure delivery system to transfer the premeasured pressure as well as assess blood perfusion changes in response to iontophoresis. This setup was tested, and

the probe was found durable enough to deliver the pressures measured on the plantar surface of the foot (Flynn, 2014).

The instrumentation used in the current study was moorVMS-LDF2 Dual channel Laser Doppler Perfusion and Temperature Monitor combined with battery-powered MIC2™ Iontophoresis Controller. Data were recorded and processed using moorVMS-PC recording and analysis software (Moor® Instruments Ltd.). The LDF probe transmits a low power laser light (Maximum output power 2.5mW) of the temperature-stabilised output laser diode at 785nm. For safety, both participant and operator wore protective laser goggles when the laser was on.

The VP2T, straight LDF probe with fibre separation of 0.5mm, body 30mm in length and a diameter of 6mm was used. The optical properties of probes may change over time thus, the probe was calibrated prior to use and on a regular basis (every 6 months) or if a warning calibration message was displayed. The calibration began by checking and cleaning the optical surfaces of the probe tip and probe connector with a soft non-abrasive cloth provided with the instrument. The calibration solution (the motility flux standard) uses a thermal (Brownian) motion of polystyrene microspheres in water to produce the reference signals required (Flynn, 2014). As it is temperature dependent, the flux standard solution needs to be at a stable temperature. This was achieved by leaving the vial of the motility standard liquid in the temperature-controlled room (between 20°C and 24°C) where the calibration was conducted, for 30 minutes prior to use in order to reach room temperature. The container was next shaken gently for 10 seconds and left to rest for 2 minutes and placed in the middle of the base of the assembled calibration stand (Figure 3.26). The probe was connected and calibrated with the channel of the system to be used.



Figure 3.26 Calibration of the LDF probe
Assembled calibration stand holding the standard solution and clamping the LDF probe

The probe was secured by the clamp on the calibration stand, with the fibre optic lead unsupported, and the probe tip pointing downwards in the centre of the calibration solution without any contact with the container. Following the running of a successful calibration, the software displays a ‘Calibration successful’ message.

MIC-ION1R-P1, a direct ion chamber, was utilised for iontophoresis (Figure 3.27). The chamber was constructed from Perspex with an internal platinum wire electrode running around its inner surface. It has a central aperture or drug chamber of 9.5 mm, and an overall diameter of 36 mm. The drug chamber can accommodate the LDF probe and allows some space for the drug solution around the probe during the pressure delivery procedure. The chamber has two small upper holes connected by a drill which allows the flow of solutions and top up when required. This ion chamber offered the best solution retention and contact in the vertical position that was anticipated for the iontophoresis procedure on both foot surfaces. It was also strong enough to endure the pressure applied through the delivery system (Flynn, 2014).



Figure 3.27 MIC-ION1R-P1 chamber (Moor® Instruments Ltd.)

The vasoactive agents chosen for iontophoresis were acetylcholine (ACh) as an endothelium-dependent vasodilator and sodium nitroprusside (SNP) as an endothelium-independent vasodilator for comparison. The vehicle for dissolving both vasoactive agents was deionised water. Because iontophoresis was conducted with the chamber fixed in a vertical position and the use of the pressure delivery equipment prevented inserting a cover to stop leakage and spillage, an inert thickening agent was added to help to maintain sufficient vasodilator solution in contact with the skin for iontophoresis. The solution, comprising 2% Methylcellulose and 2% of the vasoactive agent in deionised water, was reasonably viscous and did not impact the drug delivery (Flynn, 2014).

Methylcellulose is a water-soluble derivative of pine pulp. It is colourless, odourless, non-ionic and stable at room temperature. It is used as a thickener, binder, emulsifier and/or stabiliser in a variety of pharmaceutical products (Sigma Chemicals Ltd). Some authors have noted that using 2% Methylcellulose as a vehicle may eliminate the current-induced vasodilation known as the galvanic response (Noon *et al.*, 1998) while others could not confirm this finding (Ferrell *et al.*, 2002, Turner *et al.*, 2008). Preparing Methylcellulose into a viscous solution needs some care as it tends to form a lumpy solution if improperly

dispersed when dissolved in water. The most convenient method recommended by the manufacture is to heat 1/3 of the required volume of water to at least 80°C then add the methylcellulose powder to the hot water with agitation until the particles are thoroughly wetted and evenly dispersed. The remainder of the water is then added as cold water and the solution is cooled to 0-5 °C for 20-40 min. Once it reaches the temperature at which it becomes water-soluble, the powder begins to hydrate and the viscosity increases. Agitation was continued for at least 30 minutes after the proper temperature was reached. However, the end solution had lots of micro-bubbles that needed to be cleared before it can be used in iontophoresis. The solution was placed overnight in a Vacuum chamber (Figure 3.28) to get rid of these bubbles prior to any use.



Figure 3.28 Vacuum Chamber & Pump Kit (BACOENG)

3.3.2 Iontophoresis Protocol

3.3.2.1 Introduction

The last objective of the pilot work was to choose and test the iontophoresis protocol to be used in the final experiment to assess endothelial function on the dorsal as well as the

plantar surfaces of the foot. The plan was to use the same protocol tested and used by Flynn, 2014, however, on reproduction of the protocol on the dorsal surface of the foot, the sought response was not attained. Different protocols routinely used in our laboratory were explored with both ACh and SNP. Testing was carried out on 3 male and 3 female student volunteers in a temperature-controlled room of $24^{\circ} \pm 1^{\circ}\text{C}$ under the same conditions as would be followed in the final study; i.e. avoiding food ingestion and caffeine-based drinks for 2 hours prior to measurement and at least a 10 minutes period of acclimatisation to the testing environment (as recommended in the laboratory Working Practice Document For The Assessment Of Vascular Function Using Laser Doppler Imaging And Iontophoresis). The protocol that ensured a sufficient, sustained vascular response to a plateau that did not return to baseline by the end of measurement was chosen and tested for repeatability.

3.3.2.2 Testing the Iontophoresis Protocol Repeatability

Volunteers from the pressure pilot study were asked to participate in testing the repeatability of the iontophoresis protocol. Nine adults (6 males and 3 females) were recruited. They were non-diabetic, non-smoker, with no underlying lower limb vascular or neurological conditions, no history of cardiovascular disease and taking no medication. Testing was carried out on two separate sessions, at least two days apart. Each assessment was held in the same controlled environment following 10-20 minutes acclimatisation period, at the same time of day following at least two hours abstention from ingesting food and caffeine.

The volunteers were initially acclimatised in the temperature-controlled room of $24^{\circ} \pm 1^{\circ}\text{C}$ for a period of 10-20 minutes where details of the procedure were explained, and information sheets provided before consent forms were signed. Subjects were asked to adopt a supine position with their feet at heart level and ensure no movement for

the duration of the measurement session. Due to the laboratory setting, the right foot was the selected side for testing. Prior to any application, the tested skin sites on the dorsum of participants' right foot was prepared by stripping the epidermal surface with an adhesive tape then gently cleaning the area with an alcohol swab and deionized water. The site was left to dry before the iontophoresis drug chamber was attached using double-sided adhesive tape.

It was ensured that the polarity of the chamber electrode had the same charge as the vasoactive drug. A reference electrode with the reverse charge was attached to a conductive hydrogel pad to be stuck onto the anterior surface of the participants' legs. The solution of 2% of the vasoactive drug and 2% Methylcellulose in deionised water was dispensed into the chamber using a sterile syringe, ensuring no air bubbles were trapped between the LDF probe and the skin.

The tested protocol began by delivering a $0\mu\text{A}$ current for a baseline reference period of 60 seconds (Label 1). This was followed by 14 spells of iontophoresis, 60 seconds each (Label 2-15), using a $100\mu\text{A}$ current. Finally, drug-free perfusion with $0\mu\text{A}$ current was recorded for 120 seconds (Label 16). Statistical data were extracted from the recording software into a Microsoft Excel sheet.

There were some movement artefacts during the early trials, therefore, noise filtration was implemented and median perfusion values for each iontophoresis period were extracted. The Peak of Maximum Response for each measurement was calculated from the average of all maximum perfusion values of the 16 iontophoresis periods and was found to have a mean $\pm\text{SD}$ of 132.37 ± 50.31 PU. Mean perfusion values in each period were examined to check for differences with median values which were primarily sought to avoid false extreme values due to artefacts.

Interclass Correlation Coefficient (ICC) with absolute agreement and average measure was utilised to assess the repeatability of data. Using median perfusion values (Table 3.9), the ICC was 0.945, 95% confidence interval (CI) between 0.840-0.996, ANOVA $F=155.496$, $p<0.0001$. This indicated good repeatability of the protocol.

On retesting for protocol repeatability using the mean values of perfusion (Table 3.10), the same good repeatability was noted. The ICC was 0.938, 95% C.I. (0.859-0.998), F value= 165.827, $p<0.0001$. That confirmed the repeatability of the protocol and the decision was taken to use this protocol in the final study on both dorsal and plantar surfaces of the foot.

Table 3.9 Mean and Standard Deviation (SD) for perfusion Median values (PU) during each iontophoresis periods

Iontophoresis periods	Current	Mean	SD
Label 1	0 μ A	16.24	17.60
Label 2	100 μ A	22.06	22.92
Label 3	100 μ A	42.97	37.87
Label 4	100 μ A	64.63	39.25
Label 5	100 μ A	80.22	36.23
Label 6	100 μ A	90.66	41.16
Label 7	100 μ A	94.70	43.79
Label 8	100 μ A	97.41	43.56
Label 9	100 μ A	97.64	39.50
Label 10	100 μ A	99.99	38.79
Label 11	100 μ A	97.29	39.53
Label 12	100 μ A	99.66	38.75
Label 13	100 μ A	100.02	39.38
Label 14	100 μ A	100.39	40.08
Label 15	100 μ A	102.42	41.73
Peak of Max response		132.37	50.31
ICC	0.945 (C.I. 0.840-0.996)		
ANOVA	$F=155.496$, $P<0.001^{**}$		

Probability (P-value) * $P<0.05$ was considered significant. ** $P<0.001$ was considered as highly significant. $P>0.05$ was considered insignificant.

Table 3.10 Mean and SD for Mean of perfusion values (PU) during each iontophoresis periods

Iontophoresis periods	Current	Mean	SD
Label 1	0 μ A	16.72	17.99
Label 2	100 μ A	23.26	23.20
Label 3	100 μ A	44.77	38.26
Label 4	100 μ A	66.02	39.49
Label 5	100 μ A	81.89	36.44
Label 6	100 μ A	92.22	41.01
Label 7	100 μ A	96.07	43.61
Label 8	100 μ A	98.86	43.37
Label 9	100 μ A	99.41	39.95
Label 10	100 μ A	101.43	39.14
Label 11	100 μ A	99.22	39.10
Label 12	100 μ A	101.49	39.09
Label 13	100 μ A	101.78	39.69
Label 14	100 μ A	102.16	40.47
Label 15	100 μ A	104.09	42.21
ICC	0.938 (C.I. 0.859-0.998)		
ANOVA	F=165.827, P<0.001**		

Probability (P value) *P <0.05 was considered significant. **P <0.001 was considered as highly significant. P >0.05 was considered insignificant.

3.4 APPLICATION OF THE STUDY PROTOCOL

All procedures were conducted at the Institute of Motion Analysis and Research (IMAR), Tayside Orthopaedic and Rehabilitation Technology (TORT) Centre, University Department of Orthopaedic and Trauma Surgery, Ninewells Hospital and Medical School, University of Dundee. Ethical approval was sought and granted from London-Hampstead Research Ethics Committee with Research sponsor from NHS Tayside-University of Dundee (Appendix 5, 6). Conducting the pilot work and ending with a 17-minute iontophoresis protocol to be repeated 6 times on each foot surface, revealed that, completing the procedure in one session would be too long for participants. Diabetic patients, in particular, might be distressed by the long fasting period associated with the study procedures. Thus, a substantial amendment was submitted to complete the study over two sessions, each of 3 hours duration, and further approval for this amendment was granted (Appendix 7, 8).

3.4.1 Participants

The sample size was determined according to the following equations, where the power of analysis aimed at 80% or 0.8 (Armitage *et al.*, 2002).

Estimate sample size n

$$n > 2 \left[\frac{(Z_{2\alpha} + Z_{2\beta})\sigma}{\delta} \right]^2 \quad (1)$$

Where α represents the probability of occurrences of type I error which = 0.05, β represents the probability of occurrences of type II error which = 0.2 and σ is the standard deviation (SD). $2\alpha = 0.05$, $1-\beta=0.8$, $\delta=\text{AveX1}-\text{AveX2}$, and $Z_{2\alpha}$ and $Z_{2\beta}$ are fixed, e.g. in normal distribution equal to 1.96 and 0.842, respectively. Thus,

$$n > 2 \left[\frac{(2.802)\sigma}{\delta} \right]^2 \quad (2)$$

As, $p=0.05$, when Null hypothesis (H_0) is rejected, the Type 1 Error is 0.05 (α). If H_0 is accepted, how much is the Type 2 Error (β)? Take β as 0.2, power = $1-\beta = 0.8$ or 80%. The study used σ as 80 KPa, based on the maximum standard deviation of Peak Pressure from Putti et al. 2007 (Putti et al., 2007). We used $\delta = 100$ KPa which in clinical practice could be considered as significant when examining normal foot in-shoe pressure. Based on the above, $n = 10$, thus, the minimum number of subjects to be recruited in order to reach 80% power of analysis should be 10 or more. We were looking to recruit 30 participants in each group (study and control). However due to difficulties in recruitment of the study age group and the lengthy protocol we were content with the number recruited which were more than the prerequisite numbers.

Recruitment posters (Appendix 9) were in Ninewells Hospital, University of Dundee notice boards and circulated via the University e-Newsletter, NHS Tayside Volunteering

Services emails. Caldicott Guardian Approval was applied for and granted (Appendix 10) to access the Rehabilitation Technology Information Service (ReTIS) database at TORT Centre, in order to recruit patients from those attending orthotic clinics at the centre. However, most patients had foot deformities or amputation which would impact upon foot pressure measurement and were thus, excluded from the study.

Volunteers with diabetes suitable for inclusion in the study were identified from diabetic patients registered on the Scottish Diabetes Research Network (SDRN). This is an electronic database of diabetic patients who are willing to take part in diabetes research and have agreed to be contacted regarding research for which they may be a good match. This research register uses the latest clinical data on each patient's records to identify suitable patients for studies, thus increasing the recruitment rate and decreasing the screen failure rate. Database Search criteria were: Resident in Tayside, Type 2 Diabetes, No active ulceration, No foot amputation, Palpable peripheral pulsation, No vascular intervention (CABG or Carotid Endarterectomy event), Present protective sensations. A list was issued, and invitation letters were posted with return slips included (Appendix 11, 12). Subjects who returned their slips with the agreement to participate were contacted via telephone and further details provided. If individuals showed interest, the information sheet and an invitation letter to attend on their available dates were issued.

The issued patients' list included 969 subjects, 283 who had chosen letter as the mean of contact were approached and invitation letters were posted. Forty-five subjects returned slips with No to participate, 90 returned with willing to take part or interested in knowing further details about the study. Thirty subjects living in Dundee were randomly selected to be phoned in-order to explore their potential involvement. Six subjects found the procedure too lengthy, two were taking part in other research and two had foot deformity which excluded them from participation. Twenty subjects accepted to take part on an

agreed date, two did not attend, three had their pressure data collected but were unable to complete the iontophoresis procedure and fifteen had the entire protocol completed.

The noted age for participants with diabetes was above 50 years old, which is the common presentation age for patients with Type 2 Diabetes. Consequently, the study aimed at recruiting non-diabetic volunteers from the matching age group, which was difficult due to the prerequisite commitment to our lengthy protocol. Volunteers with no history of diabetes, from both sexes, in the age range from 50 to 75 years old with no known underlying lower limb vascular or neurological conditions were invited to participate in the study. Exclusion criteria included foot deformity, amputation or underlying pathology of the spine/lower limbs which results in an inability to walk unaided. It should be noted that subjects were asked about their medical history, in particular, diabetes mellitus and vascular problems, however, no testing of blood glucose was carried out.

Twenty-two non-diabetic volunteers were invited to participate; however, two subjects were unable to lie still to complete the iontophoresis protocol.

Both groups' subjects were provided with a written information sheet, a full explanation of the study and informed consent to be signed prior to any participation (Appendix 13, 14, 15).

The subjects in the diabetes group were advised to review their participation with their GP/diabetician, the needed fasting period (approximately 5 hours) and any diet and diabetes medications changes/arrangement they should follow before and after the study session prior to any visit. Additionally, blood sugar was checked at the end of the session using Accu-Chek Performa Blood Glucose Meter, to ensure diabetic patients are safe to send home after the lengthy procedure. If a low blood sugar was detected a sugary beverage was provided.

Prior to the procedure, a short interview was conducted with each volunteer. Details such as age, sex, occupation, smoking history, alcohol intake, exercise routine, general health, medications history and mode of management for diabetes, any comorbidity, past history of any foot/back surgery or vascular intervention were obtained. Assessment of the lower limbs was then carried out by checking skin appearance and any foot deformity. Weight and height were recorded and Body Mass Index (BMI) was calculated using the equation $BMI = \text{weight (kg)} / \text{height}^2 \text{ (m)}$.

3.4.2 In-Shoe Foot Pressure Measurement

Initially, in-shoe dorsal and plantar walking foot pressures were recorded from the participants' right foot when they were wearing their own comfortable shoes and then when they were wearing the size-matched orthopaedic shoes we provided (Chaneco Diabetic shoes). Tests in own shoes were performed within the participants' usual footwear they have chosen to bring to the measurement session. This included sports shoes, walking boots, slip-on shoes and Oxford-style shoes. This would give an accurate representation of participants' foot pressure within their usual footwear chosen for their regular daily activities.

A Pedar dorsal pad, labelled left (for coupling), was inserted into a standardised sock to be secured onto the dorsal surface of the participant's right foot and connected to the left cable of the Pliance[®] mobile measurements box. A shoe size-matched, right-sided Pedar Plantar insole was placed inside the right side of the tested shoes and connected to the right cable of the Pliance[®] mobile measurements box. The same procedures used in the pilot study were followed, and these are explained in section 3.2.3 of this chapter. On the session day, the average of Peak Pressure (the highest pressure in any sensor across a given mask/ trial) across the 6 walking trials was calculated in each tested shoes for both dorsal and plantar surfaces of the foot using on-monitor data. These Peak Pressure values

were used later for the Pressure delivery equipment during the assessment of endothelial function under the applied pressure. Collected pressure data were later extracted for analysis using Novel Automask, Novel Group Editor, and Novel Group Mask Evaluation software. For the plantar surface of the foot, the IMAR Pedar Mask was used. This mask was developed in IMAR laboratory for analysis of in-shoe pressure measured by Pedar Insoles. The software is set to create areas (masks) automatically based upon a predetermined algorithm. The algorithm recognises the footprint dimensions and defines the areas as percentages. It expresses the boundary between midfoot and heel as 73% of the foot length and between midfoot and forefoot as 45% of the foot length. A mask was developed for the dorsum of the foot guided by the IMAR Pedar Mask. The heel areas were cut off and toes areas were smaller (one row less than plantar) and divided into two masks instead of three. This was more appropriate with the smaller area covered by the dorsal pad in contrast to the larger plantar insole.

The foot was divided into 21 anatomical areas (masks). Masks from M01 to M09 corresponded to areas on the dorsal surface and M10 to M21 to the plantar surface of the foot (Figure 3.29). The masks' names and areas they represented on the footprint are shown in Table 3.11. The 21-area mask was applied to subjects' data files for extraction accordingly. However, due to the difference in the start and end points between the dorsal and plantar masks, the software was unable to resize masks with changes in the foot size and the subsequent differences in the insole used. Therefore, masks had to be created individually according to each subject's shoe size.

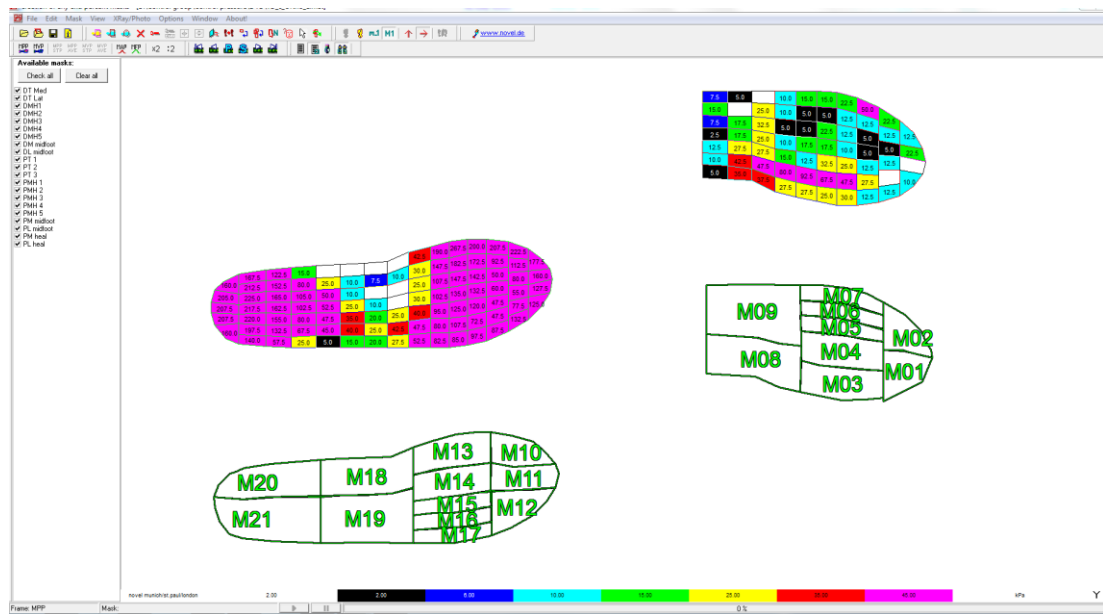


Figure 3.29 Footprint masks

Table 3.11 Masks names and anatomical areas covered

Mask Number	Mask Name	Anatomical Areas
M01	DT Med	Dorsum Toes Medial Side
M02	DT Lat	Dorsum Toes Lateral Side
M03	DMH 1	Dorsum 1 st metatarsal head
M04	DMH 2	Dorsum 2 nd metatarsal head
M05	DMH 3	Dorsum 3 rd metatarsal head
M06	DMH 4	Dorsum 4 th metatarsal head
M07	DMH 5	Dorsum 5 th metatarsal head
M08	DM midfoot	Dorsum Medial midfoot
M09	DL midfoot	Dorsum Lateral midfoot
M10	PT 1	Plantar Toes 1 (greater toe)
M11	PT 2	Plantar Toes 2 (second toe)
M12	PT 3	Plantar Toes 3 (3rd-5th toes)
M13	PMH 1	Plantar 1 st Metatarsal Head
M14	PMH 2	Plantar 2 nd Metatarsal Head
M15	PMH 3	Plantar 3 rd Metatarsal Head
M16	PMH 4	Plantar 4 th Metatarsal Head
M17	PMH 5	Plantar 5 th Metatarsal Head
M18	PM midfoot	Plantar Medial midfoot
M19	PL midfoot	Plantar Lateral midfoot
M20	PM heel	Plantar Medial heel
M21	PL heel	Plantar Lateral heel

Group Editor software allows the combination of foot pressure data for analysis. Two groups were created using Group Editor software: patients and control groups. Group Mask Evaluation software was then used for group evaluation and IMAR software for Novel Data Extraction was used to extract data in a spreadsheet suitable for statistical analysis. From parameters available to be extracted by the Novel software, those considered to be the most clinically relevant and frequently discussed in the literature were chosen. These included Peak Pressure (PP), Pressure-Time Integral (PTI), Contact Area (CA) and Maximum Force (MF). All extracted data were uploaded into SPSS for statistical analysis.

In-house software was developed to extract the time of PP on the dorsum of the foot during the gait cycle. The software interface is shown in Figure 3.30.

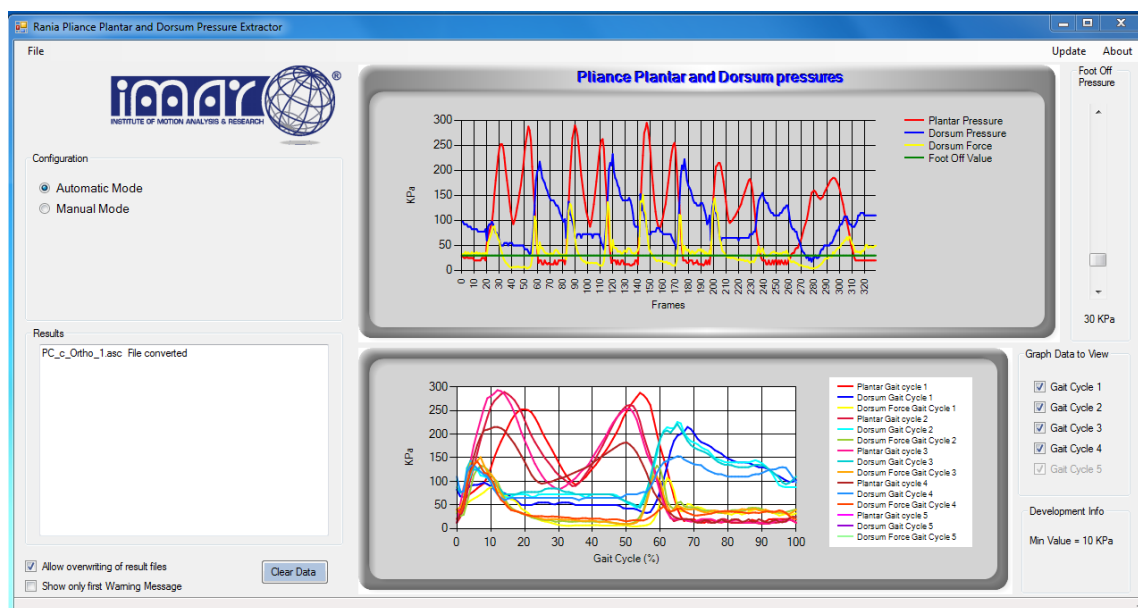


Figure 3.30 PP time extraction software

Based on earlier testing of the Pedar dorsal pad and the work done by Jordan and Bartlett (1995a), dorsal pressure may show some exertion in the swing phase as well the stance phases of the gait cycle (Jordan and Bartlett, 1995a). Jordan and Bartlett (1995a) reported two force activities, a low force activity exerted just before the foot contacts the ground

and a higher force just prior to the foot leaving the ground. They anticipated that these force peaks coincided with dorsiflexion of the foot during the gait cycle. This observation would endorse the significance of dorsal pressure assessment, as also being present during the non-contact phase of the gait cycle, in contrast to the plantar pressure that is only recorded during the stance phase. The simultaneous measurement of plantar and dorsal pressures enabled the current study to determine the time of the dorsal PP in relation to the well-recognised plantar PP. The software defined the beginning of the gait cycle by the first peak plantar pressure which was believed to correspond to the heel strike event of the gait cycle. The peak plantar pressure data were correlated with the peak dorsal pressure and the peak dorsal force. Maximum dorsal force showed more homogenous and clear peaks on the produced graphs than the dorsal PP, hence was included in the analysis.

3.4.3 Assessment of Endothelial Response to the Iontophoresis of Vasoactive Solutions with Simultaneous Loading of Pressure

Assessment of the endothelial function was carried out over two sessions using the LDF VP2T probe (Moor[®] Instruments Ltd.) to monitor the blood flow response to the iontophoresis of the vasoactive agents, acetylcholine (ACh) (Miochol-E) and sodium nitroprusside (SNP) (Nitropurssiat Fides). The first session involved the assessment of the dorsal surface of the foot and the second session assessed the plantar surface. All subjects were instructed to avoid food ingestion and caffeine-based drinks for at least two hours prior to attending both sessions. Participants were asked to rest for 10-20 minutes in a temperature-controlled room of $24^{\circ} \pm 1^{\circ}\text{C}$ prior to any iontophoresis procedure. Shoes and socks/tights were removed before subjects rested in a supine position with the right foot placed in the customised boot fixed to the bed in order to support the foot comfortably during the procedure. The boot also holds the foot in a stable position for pressure delivery.

The skin over the area of interest was cleansed with an adhesive tape, gently rubbed with an alcohol swab then washed with deionized water. The iontophoresis chamber was attached to the skin using double-sided adhesive pads and the 2% solution of the vasoactive drug with 2% methylcellulose in deionised water was added. The polarity of the chamber electrode always matched the charge of the vasoactive drug used and a reference electrode with the reverse charge was attached to a conductive hydrogel pad to be stuck onto the anterior surface of participant's right leg. The LDF probe housed in the pressure delivery equipment was then placed in position with the chamber's central aperture. Fine-tuning for the dorsal surface testing was achieved with the snake arm and for the plantar surface, with the adjustable bar on the boot backplate.

The spring used for the pressure delivery equipment changed according to the pressure values tested as detailed in Section 3.2.5.

Iontophoresis was carried out with both ACh and SNP on both foot surfaces using the protocol tested in Section 3.3.2.2. The LDF probe was utilised to monitor changes in the blood flow as well as the mean to transfer the premeasured pressure. Iontophoresis was performed with no pressure applied then during loading with the average PP on the tested foot surface (calculated on the test day) in participants' own comfortable shoes then under PP obtained in the size-matched orthopaedic shoes. In order to test the same area on the foot surface under different testing conditions, a waiting interval was required for the vasoactive agent to clear away. A different area was used for each tested condition because of time constraints.

3.5 STATISTICAL METHODS

The collected data were analysed using the Statistical Package for the Social Sciences (SPSS) Version 22, IBM Corp. Quantitative data were expressed as mean \pm standard deviation (SD) and qualitative data were expressed as frequency and percentage.

The following statistical tests were undertaken:

- Independent-samples t-test of significance was used when comparing two means
- Chi-square (χ^2) test of significance was used to compare proportions between qualitative parameters
- A one-way analysis of variance (ANOVA) when comparing more than two means
- Post Hoc test: Least Significant Difference (LSD) was used for multiple comparisons between different variables
- Mann Whitney U test: for two-group comparisons in non-parametric data
- Kruskal Wallis test: for multiple-group comparisons in non-parametric data
- Interclass Correlation Coefficient (ICC) for assessment of repeatability
- Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables
- Spearman rank correlation test to assess the degree of association between two sets of variables

The confidence interval was set to 95% and the margin of error accepted was set to 5%.

The Probability (P) was considered significant as the following:

- $P \leq 0.05$ was considered significant
- $P \leq 0.001$ was considered as highly significant
- $P > 0.05$ was considered no significant differences

4.1 STUDY GROUPS CHARACTERISTICS

Twenty subjects with Type 2 Diabetes agreed to take part in the study. However, two did not attend and three had their pressure data collected but were unable to complete the iontophoresis procedure. Therefore, 18 subjects with Type 2 Diabetes were investigated for in-shoe foot pressure and 15 had the entire protocol completed. Twenty-two non-diabetic volunteers were invited to participate in the study; although, two subjects were unable to lie still to complete the iontophoresis protocol, ending with a total of 20 subjects without diabetes were included for the blood flow data in the control group.

Control group and diabetes group characteristics matched and showed no significant differences ($p>0.05$) in demographic data, body features, shoe size, smoking history, alcohol intake and their exercise activities. Characteristics of both study groups are demonstrated in Table 4.1. There were participants in both study groups who suffered from high blood pressure. Due to the common combination of high blood pressure and Type 2 Diabetes, as well as its frequent presentation in this study age group, hypertension was the only cardiovascular risk factor that was not excluded on recruitment in both study groups. Medication history for both study groups and different modalities of diabetes control noted in subjects with diabetes are illustrated in Table 4.2 and Table 4.3, respectively. The descriptive statistics and significance values for the investigated parameters are supplied in the tables included in Appendix 16.

Table 4.1 Control and diabetes groups characteristics

Regarding; demographic data, body features, shoe size, smoking history, alcohol intake and regular exercise activities

Characteristics		Control group (n:22)	Diabetes group (n:18)	p
Sex	Female	11 (50.0%)	8 (44.4%)	0.726 #
	Male	11 (50.0%)	10 (55.6%)	
Age (years)	Mean±SD	63.38±7.09	65.73±6.01	0.271
	Range	51.24-73.65	52.55-74.29	
Height (m)	Mean±SD	1.67±0.09	1.66±0.10	0.615
	Range	1.52-1.83	1.51-1.81	
Weight (kg)	Mean±SD	75.97±12.40	81.69±13.13	0.166
	Range	55-100	57.7-108.9	
BMI [weight/(height)^2]	Mean±SD	27.12±3.38	29.81±4.56	0.039
	Range	20.65-32.65	21.45-39.26	
Shoe size	Mean±SD	7.09±2.14	7.22±2.13	0.847
	Range	4-10	4-10	
Smoking	No	21 (95.5%)	18 (100.0%)	0.919 #
	Yes	1 (4.5%)	0 (0.0%)	
Alcohol intake	Daily/Regularly	5 (22.7%)	1 (5.6%)	0.105 #
	Weekly/ Moderate	2 (9.1%)	1 (5.6%)	
	Mild	2 (9.1%)	3 (16.7%)	
	Limited/ Occasional	7 (31.8%)	6 (33.4%)	
	No	6 (27.3%)	7 (38.9%)	
Exercise activities	Golf	1 (4.5%)	0 (0.0%)	0.352 #
	Gym and Golf	0 (0.0%)	1 (5.6%)	
	Gym and Swimming	1 (4.5%)	0 (0.0%)	
	Kayaking and Walking	0 (0.0%)	1 (5.6%)	
	Pilates	1 (4.5%)	0 (0.0%)	
	Running and Cycling	1 (4.5%)	0 (0.0%)	
	Skating, dancing and Swimming	1 (4.5%)	0 (0.0%)	
	Walking	10 (45.5%)	10 (55.6%)	
	Walking and Cycling	3 (13.6%)	0 (0.0%)	
	Walking and Gym machines	1 (4.5%)	0 (0.0%)	
	Walking and Swimming	0 (0.0%)	1 (5.6%)	
	Walking and Exercise classes	0 (0.0%)	1 (5.6%)	
	Walking and Zumba	0 (0.0%)	1 (5.6%)	
	No exercise	3 (13.6%)	3 (16.7%)	

BMI: Body mass index

#: is for comparison done by Chi-square test. Other characteristics are compared using Independent Sample t-test. p>0.05 No Significance (NS)); *: p<0.05 Significant (S); **: p<0.001 Highly Significant (HS)

Table 4.2 Medication history

Medications	Control group (n:22)	Diabetes group (n:18)
Calcium	1 (4.5%)	0 (0.0%)
Anti-hypertensives	6 (27.3%)	17 (94.4%)
Statins	4 (18.2%)	16 (88.9%)
Thyroxin	1 (4.5%)	1 (5.6%)
No medications	14 (63.6%)	0 (0.0%)

Table 4.3 Mode of management of Diabetes Mellitus

Mode of management (n:18)	Diet control	4 (22.2%)
	Insulin	5 (27.8%)
	Metformin	5 (27.8%)
	Other Oral hypoglycaemics	10 (55.6%)

4.2 IN-SHOE FOOT PRESSURE ASSESSMENT

In-shoe walking pressures exerted on dorsal and plantar surfaces of participants' right foot were recorded within participants' own comfortable shoes which they selected to use for the assessment session as well as within the size-matched orthopaedic shoes provided.

Peak Pressure (PP), Pressure-Time Integral (PTI), Contact Area (CA) and Maximum Force (MF) were examined for differences between the two tested shoe conditions within each study group and differences between groups across the 21-areas/foot masks defined in Table 3.11.

4.2.1 Peak Pressure

The first pressure parameter studied, was Peak Pressure (PP), defined as the highest pressure in any sensor across a given mask (area). Both study groups showed a significantly higher total dorsal PP in participants' own shoes than within the orthopaedic shoes ($p < 0.001$ in both groups). Figure 4.1 illustrates the differences between the two tested shoe conditions across foot areas in each study group. Own shoe PP was significantly different from that recorded in the orthopaedic shoes, across all foot areas

under study, except area DT Med in diabetes group which was not significantly different ($p=0.222$) between the two tested shoe conditions.

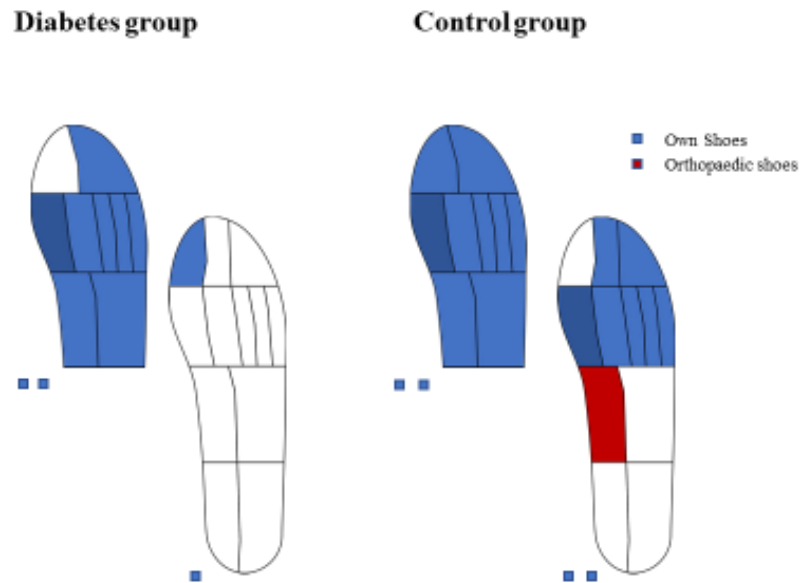


Figure 4.1 Differences between Orthopaedic Shoes and Own Shoes PP means in the study groups
The small squares represent differences in Total PP; one for $p < 0.05$ Significant (S),
two for $p < 0.001$ Highly Significant (HS)

The highest dorsal PPs were recorded on area DMH1 in own shoes in both study groups (Mean \pm SD 55.51 \pm 27.94 KPa in control group and Mean \pm SD 65.43 \pm 32.86 KPa in diabetes group). Other significantly high dorsal PPs in participants' own shoes were seen on area DT Lat (Mean \pm SD 46.76 \pm 22.60 KPa) in control group (Figure 4.2) and area DM midfoot (Mean \pm SD 52.10 \pm 24.84 KPa) in diabetes group (Figure 4.3).

The highest dorsal PP areas in the orthopaedic shoes were area DM midfoot in both groups (Mean \pm SD 36.76 \pm 16.04 KPa in control group and Mean \pm SD 41.01 \pm 13.46 KPa in diabetes group) followed by area DT Lat (Mean \pm SD 32.33 \pm 14.49 KPa) in control group and area DMH1 in diabetes group (Mean \pm SD 37.57 \pm 14.40 KPa).

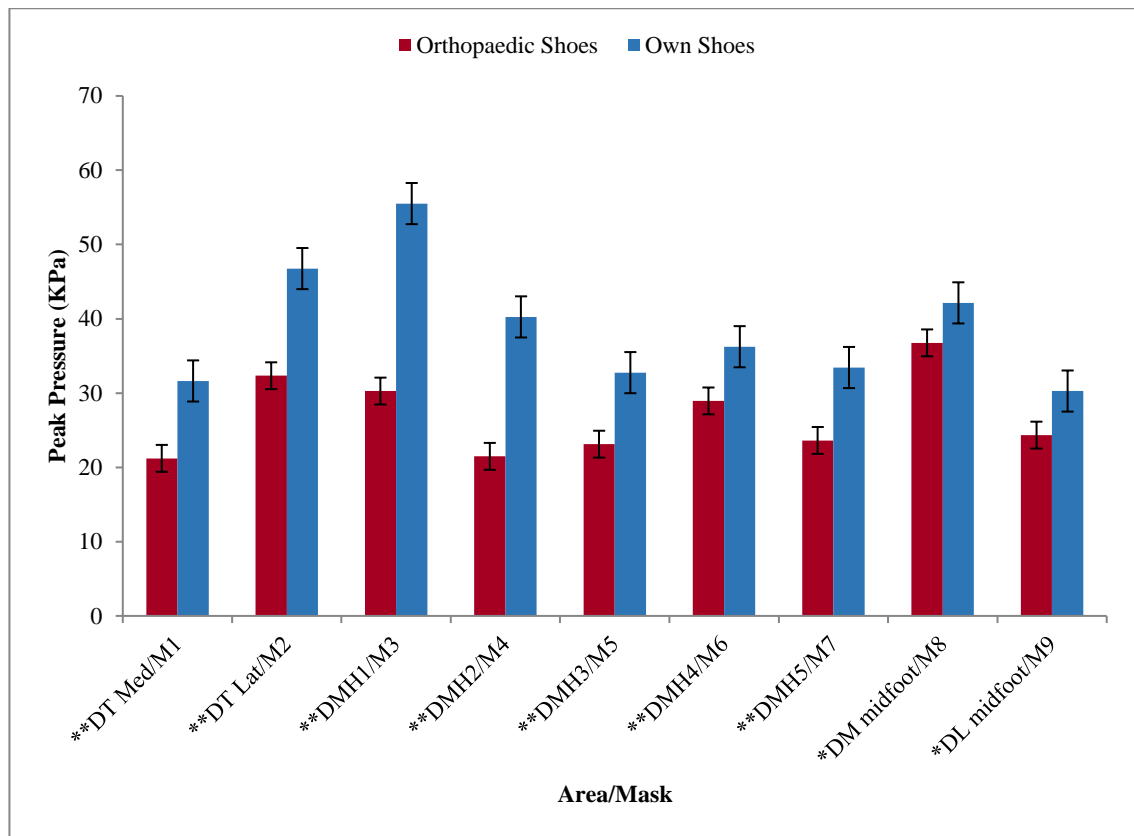


Figure 4.2 Orthopaedic Shoes and Own Shoes Dorsal PP means in control group
 *: $p < 0.05$ Significant (S); **: $p < 0.001$ Highly Significant (HS)

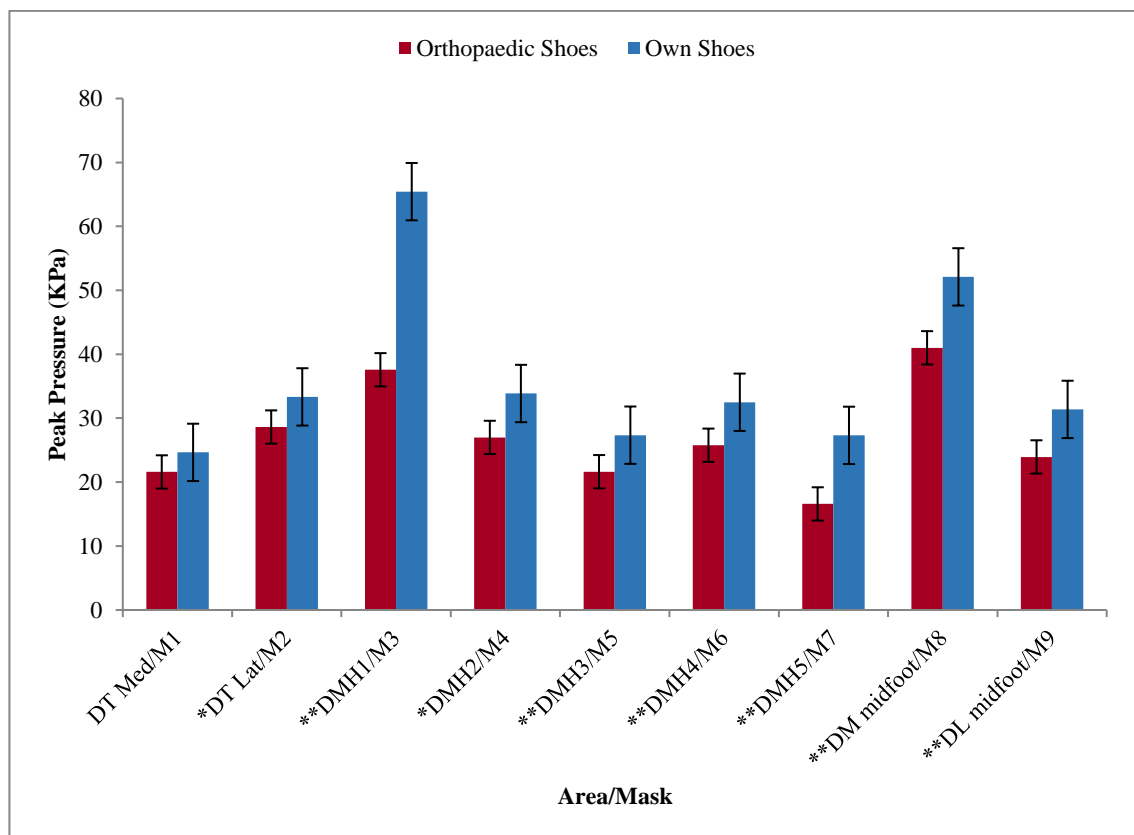


Figure 4.3 Orthopaedic Shoes and Own Shoes Dorsal PP means in diabetes group
 *: $p < 0.05$ S; **: $p < 0.001$ HS

Overall Plantar PP was significantly higher in participants' own shoes than the orthopaedic shoes in both study groups ($p < 0.001$ in control group and $p = 0.006$ in diabetes group). The control group (Figure 4.4) showed significantly higher Plantar PP in participants' own shoes on metatarsal heads areas and toes areas except PT1, plus a significantly higher PP in the orthopaedic shoes on area PM midfoot. However, diabetes group showed no significant differences between the two shoes across all plantar foot areas except PT1 (Figure 4.5).

Control group had the highest own shoe plantar PP recorded on area PMH1 (Mean \pm SD 241.55 \pm 82.79 KPa) which was also significantly different from the orthopaedic shoes. The highest orthopaedic shoe PPs in the control group were seen on heel areas which were not significantly different from own shoes plantar PP. Diabetes group had the same highest plantar PP areas' distribution, but all were not significantly different between the two shoe conditions.

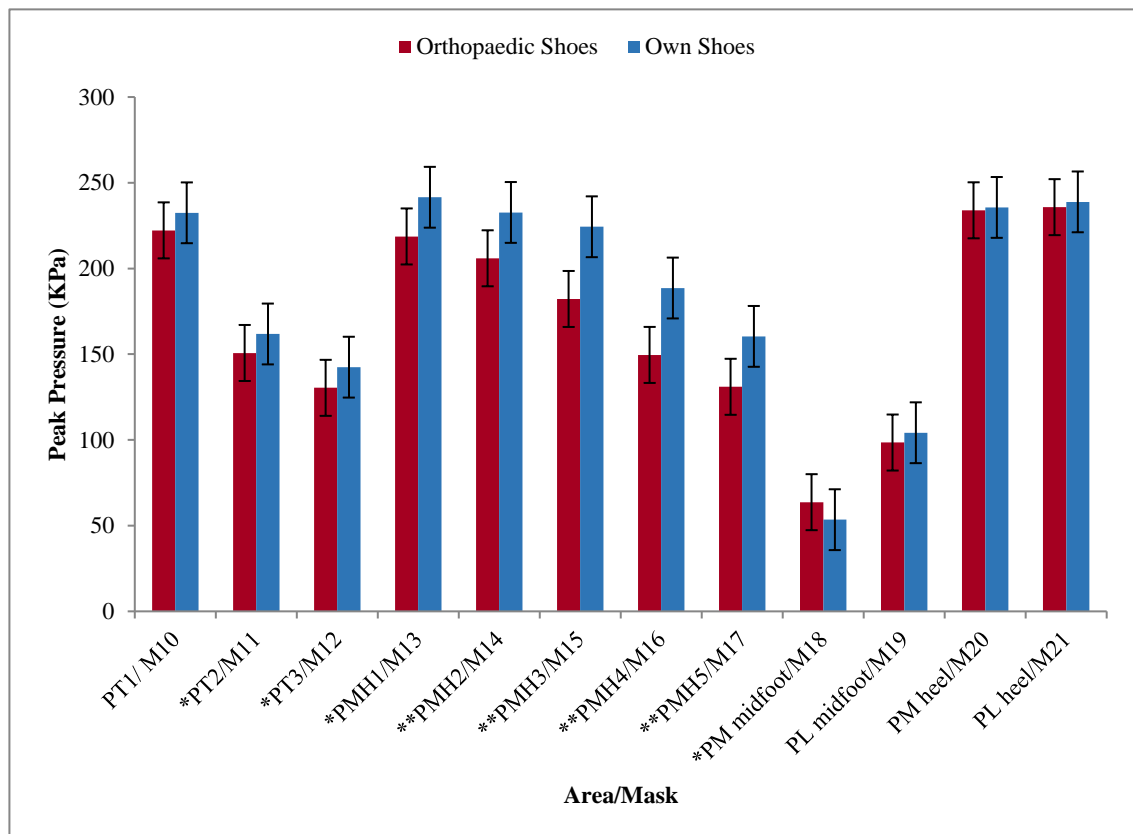


Figure 4.4 Orthopaedic Shoes and Own Shoes Plantar PP means in control group

*: $p < 0.05$ S; **: $p < 0.001$ HS

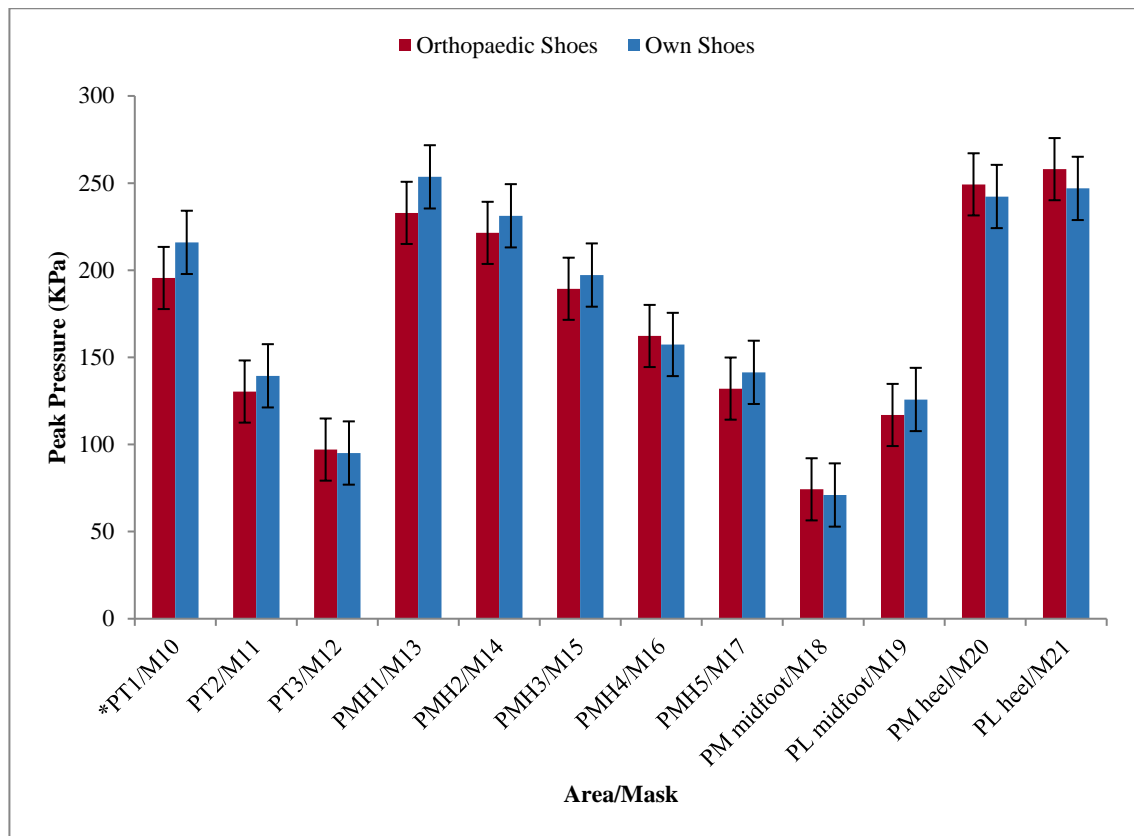


Figure 4.5 Orthopaedic Shoes and Own Shoes Plantar PP means in diabetes group
 *: $p < 0.05$ S; **: $p < 0.001$ HS

Comparing PP between groups in each tested shoe is summarised in Figure 4.6. No significant differences between groups were observed in the whole dorsal surface PP within participants' own shoes as well as the orthopaedic shoes.

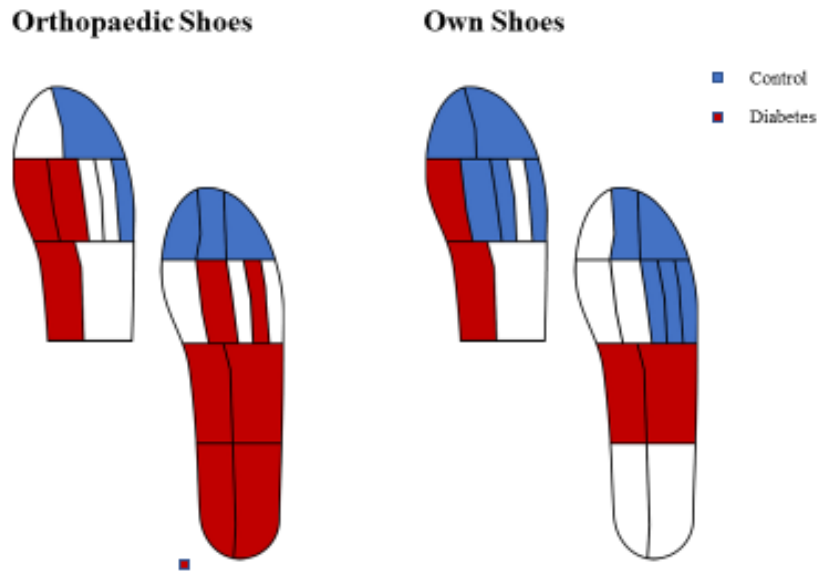


Figure 4.6 Differences between study groups in PP means within own and orthopaedic shoe conditions
 The small squares represent differences in Total PP; one for $p < 0.05$ (S), two for $p < 0.001$ (HS)

However, significant differences were noted in most of the dorsal foot areas within own shoes except DMH4 and DL midfoot. Significantly higher means recorded with diabetes group within own shoes on area DMH1 (Mean \pm SD 65.43 \pm 32.86 KPa) then area DM midfoot (Mean \pm SD 52.10 \pm 24.84 KPa). While all toes areas, 2nd, 3rd and 5th metatarsal areas were significantly higher with control in own shoes (Figure 4.7). The comparison of dorsal PP between groups in the orthopaedic shoes (Figure 4.8) followed a similar pattern except in areas DT Med/and DMH3 which changed to no significant differences between groups in the orthopaedic shoes. Same as in own shoes but with a reversed order, where the orthopaedic shoes showed a significantly higher PP in diabetes group on area DM midfoot with Mean \pm SD of 41.01 \pm 13.46 KPa followed by area DMH1 with Mean \pm SD of 37.57 \pm 14.40 KPa. Area DMH2 was also, significantly higher in diabetes group within the orthopaedic shoes (Mean \pm SD 26.99 \pm 13.60 KPa).

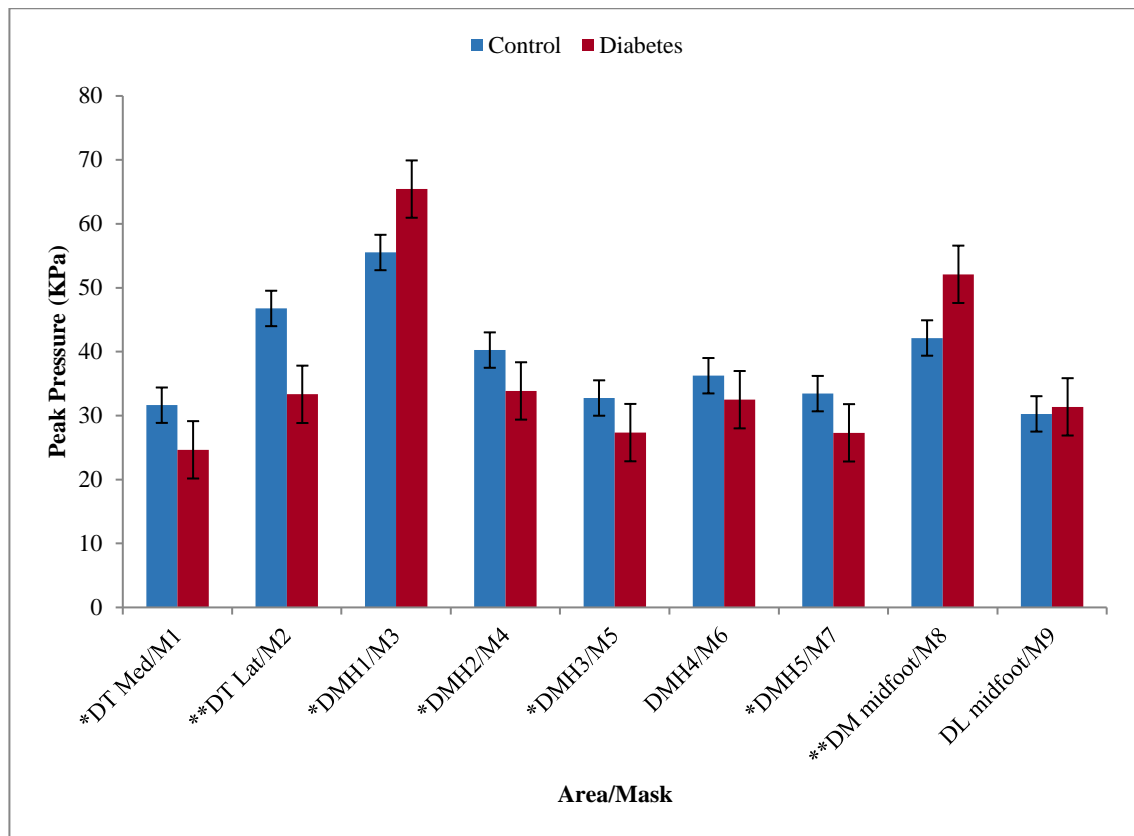


Figure 4.7 Control group and diabetes group Dorsal PP means in Own Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

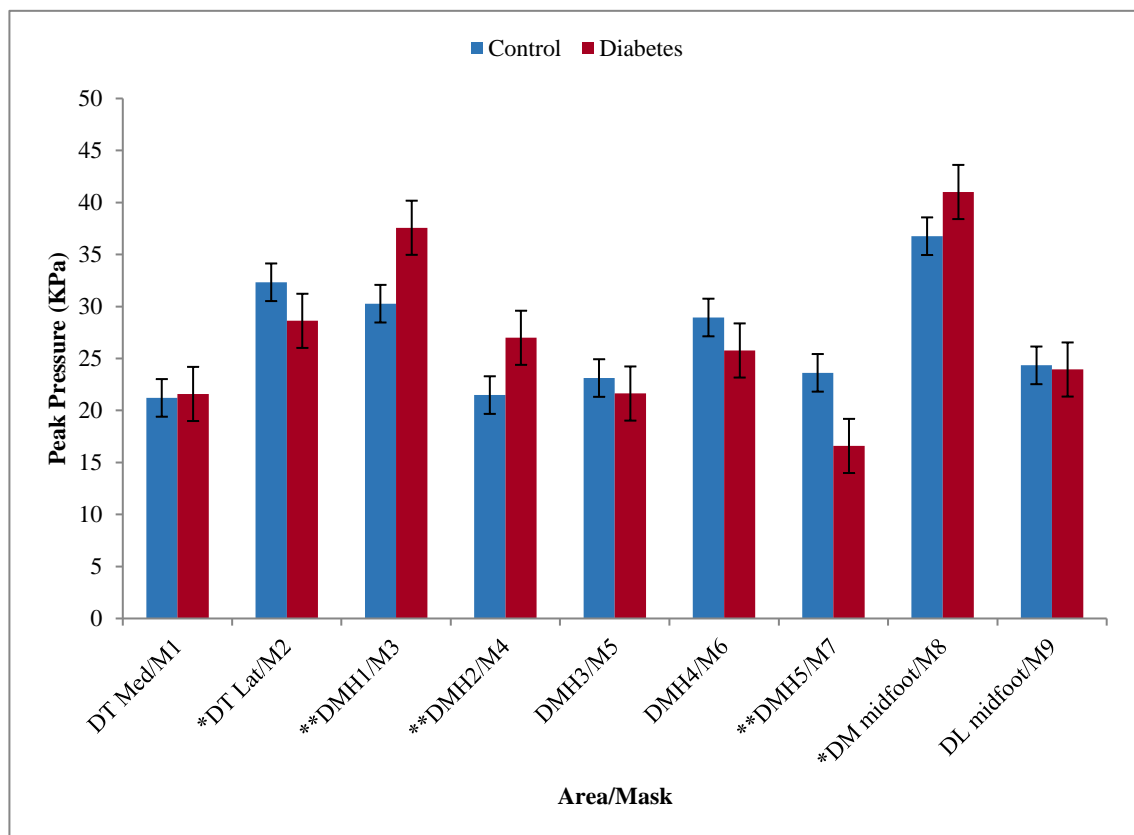


Figure 4.8 Control group and Diabetes group Dorsal PP means in the Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

Plantar surface total PP was not significantly different between groups in participants' own shoes but significantly higher in diabetes group within the orthopaedic shoes ($p=0.013$). By looking at different plantar areas within own shoes (Figure 4.9), it was noted that heel areas showed no significant differences between groups and the highest plantar PP was recorded on area PMH1 (Mean \pm SD 253.56 \pm 95.54 KPa in diabetes group and Mean \pm SD 241.55.56 \pm 82.79 KPa in control group) which also had no significant differences between groups. Yet, heel areas had the highest plantar PPs in the orthopaedic shoes (Figure 4.10) that were significantly higher among diabetes group (PL heel Mean \pm SD 257.99 \pm 45.13 KPa and PM heel Mean \pm SD 249.26 \pm 40.56 KPa) when compared to control (PL heel Mean \pm SD 235.78 \pm 46.22 KPa and PM heel Mean \pm SD 233.94 \pm 48.77 KPa). The midfoot areas had significantly higher plantar PP in diabetes group than control in both shoe conditions. Toes and metatarsal heads areas within participants' own shoes had significantly higher Plantar PP with control group except for areas PT1, PMH1 and PMH2 which were not significantly different between groups. All toes areas were significantly different between groups within the orthopaedic shoes with higher plantar PP recorded with the control group. Metatarsal heads areas mostly showed no significant differences between groups in Plantar PP within the orthopaedic shoes except for areas PMH2 and PMH4 which were significantly higher in diabetes group.

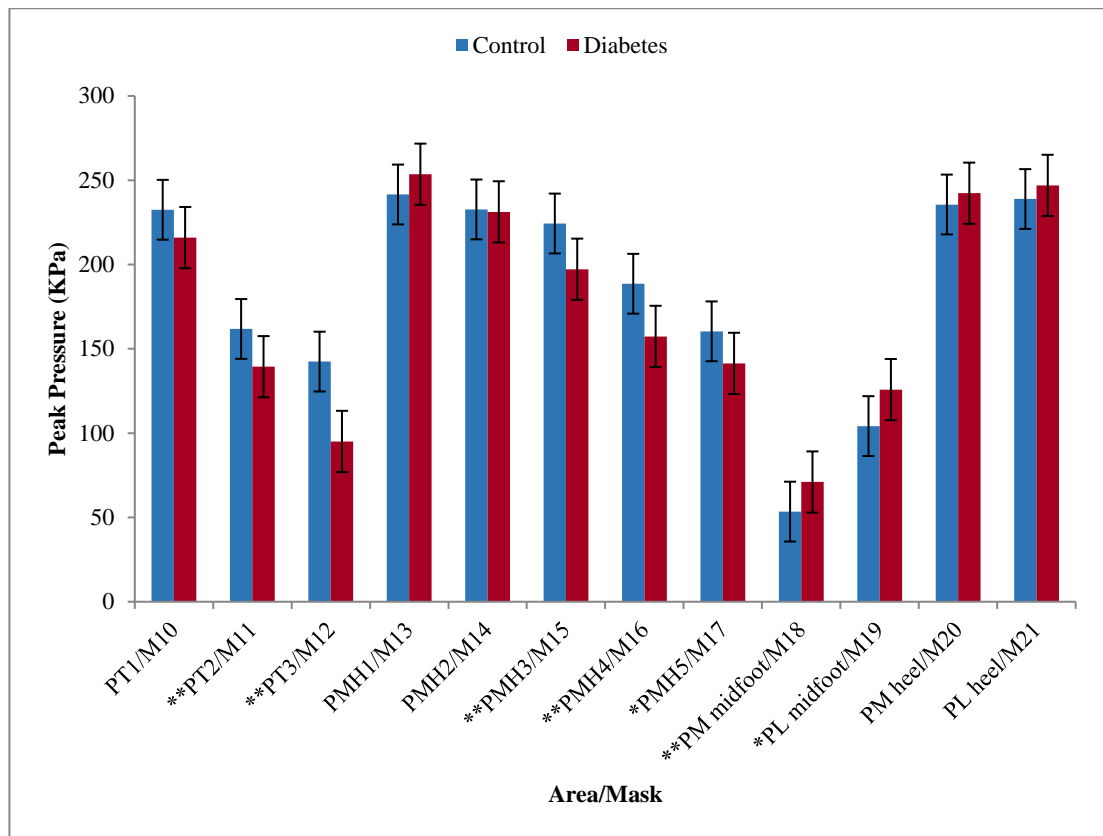


Figure 4.9 Control group and diabetes group Plantar PP means in Own Shoes
 *: $p < 0.05$ S; **: $p < 0.001$ HS

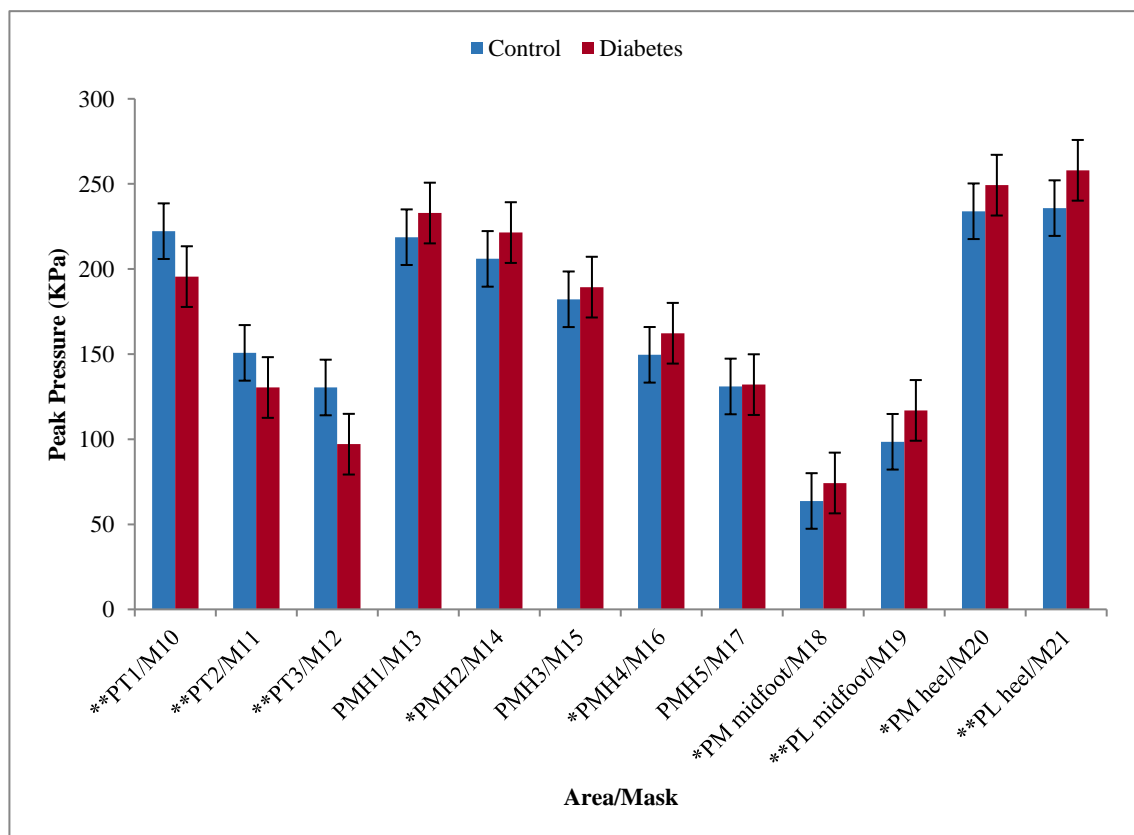


Figure 4.10 Control group and diabetes group Plantar PP means in the Orthopaedic Shoes
 *: $p < 0.05$ S; **: $p < 0.001$ HS

4.2.2 Pressure Time Integral

Another parameter examined, was Pressure Time Integral (PTI) in KPa.s (KPa*seconds). It is defined as the time integral of the peak pressure measured in any sensor within the specified region during one-foot step, calculated as the area under the peak pressure versus time curve of a particular region (Waaijman and Bus, 2012). The differences between PTI in the two tested shoes across different foot areas in each study group are illustrated in Figure 4.11.

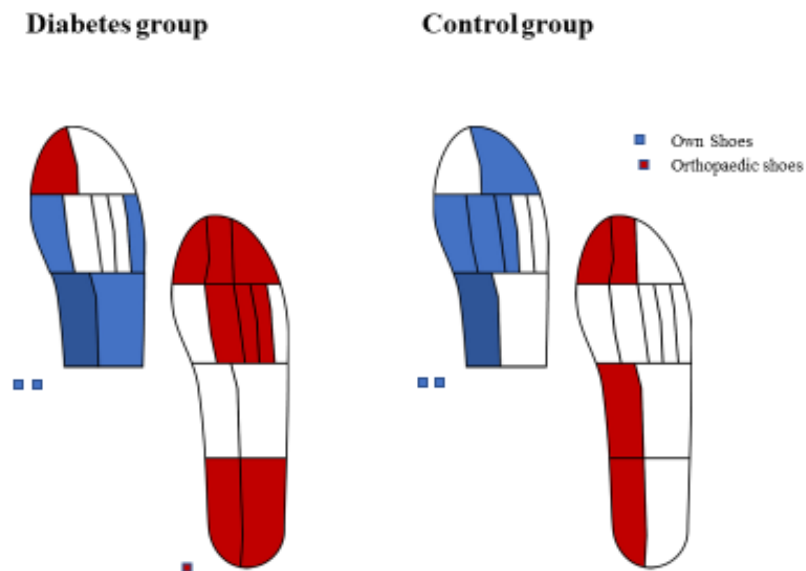


Figure 4.11 Differences between Orthopaedic Shoes and Own Shoes PTI means in the study groups
The small squares represent differences in Total PP; one for $p < 0.05$ (S), 2 for $p < 0.001$ (HS)

Total dorsal surface PTI was significantly higher in subjects' own shoes ($p < 0.001$) in both study groups. Control group had significantly higher dorsal PTI, in participants' own shoes on areas DT Lat, DMH1, DMH2, DMH3 and DM midfoot. In diabetes group, significant differences with also higher PTI in own shoes were found on dorsal areas DT Med, DMH1, DMH5, DM midfoot, DL midfoot.

Although DT Med showed no significant differences in PTI between the two shoes in the control group plus no significant differences in PP in diabetes group, it had a significantly higher PTI in the orthopaedic shoes in diabetes group. Both study groups showed the highest PTI recorded on area DM midfoot within own shoes (Mean \pm SD 95.59 \pm 74.50 KPa.s in control group and Mean \pm SD 145.62 \pm 101.63 KPa.s in diabetes group) as well as orthopaedic shoes (Mean \pm SD 79.39 \pm 43.87 KPa.s in control group and Mean \pm SD 106.91 \pm 61.94 KPa.s in diabetes group) with significantly higher PTI in own shoes than orthopaedic shoes in both groups.

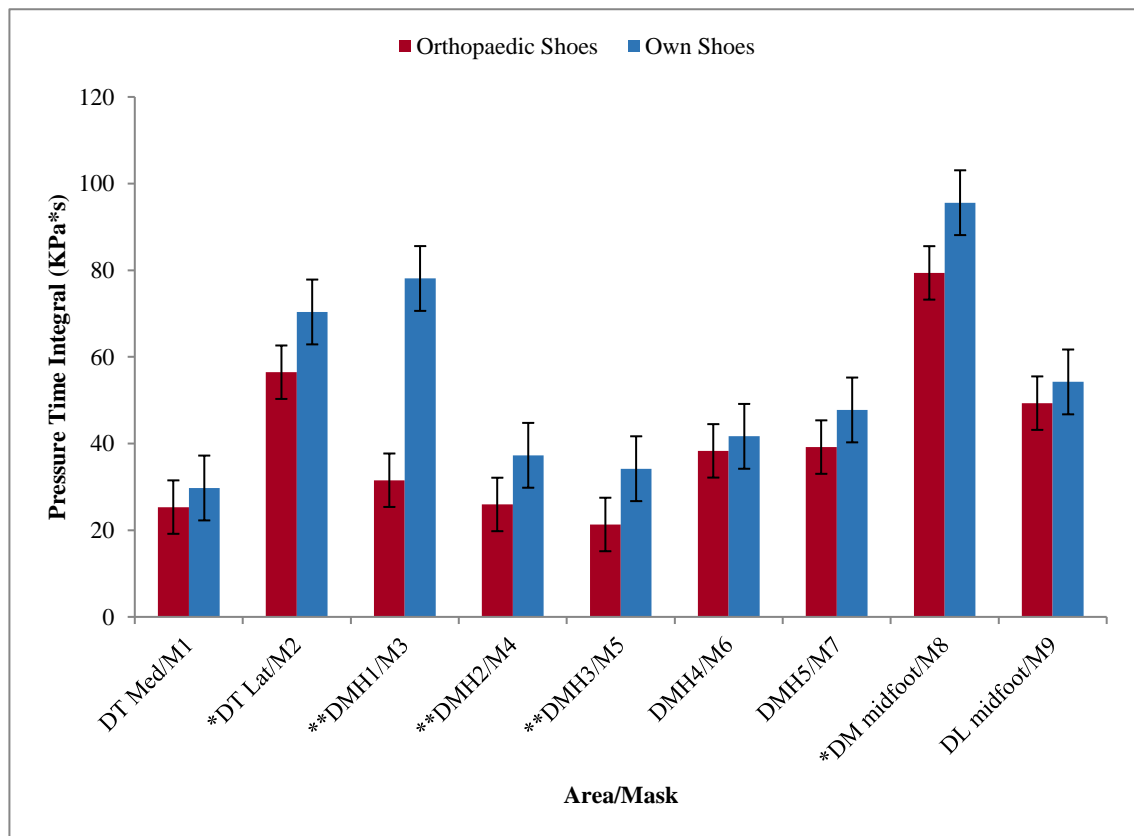


Figure 4.12 Orthopaedic Shoes and Own Shoes PTI means on the dorsal surface in control group
*: p <0.05 S; **: p <0.001 HS

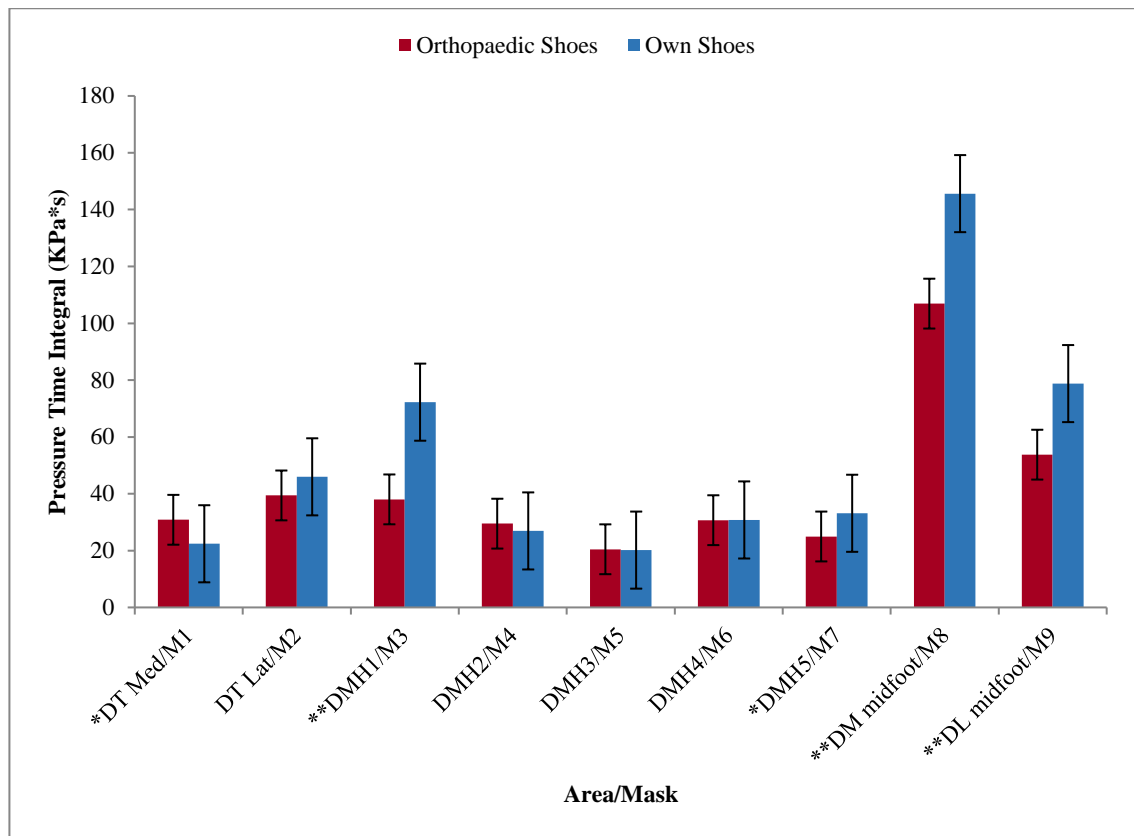


Figure 4.13 Orthopaedic Shoes and Own Shoes PTI means on the dorsal surface in diabetes group
 *: $p < 0.05$ S; **: $p < 0.001$ HS

Total plantar surface PTI was not significantly different between the two shoes in the control group ($p=0.507$). However, the orthopaedic shoes showed a significantly higher plantar PTI than own shoes in the diabetes group ($p = 0.044$). The plantar surface showed significantly higher PTI within the orthopaedic shoes in plantar areas PT1, PT2, PM midfoot and PM heel in control group. Plantar areas with also significantly higher PTI within the orthopaedic shoes were found on areas PT1, PT2, PT3, PMH2, PMH3, PMH4, PM heel and PL heel in the diabetes group.

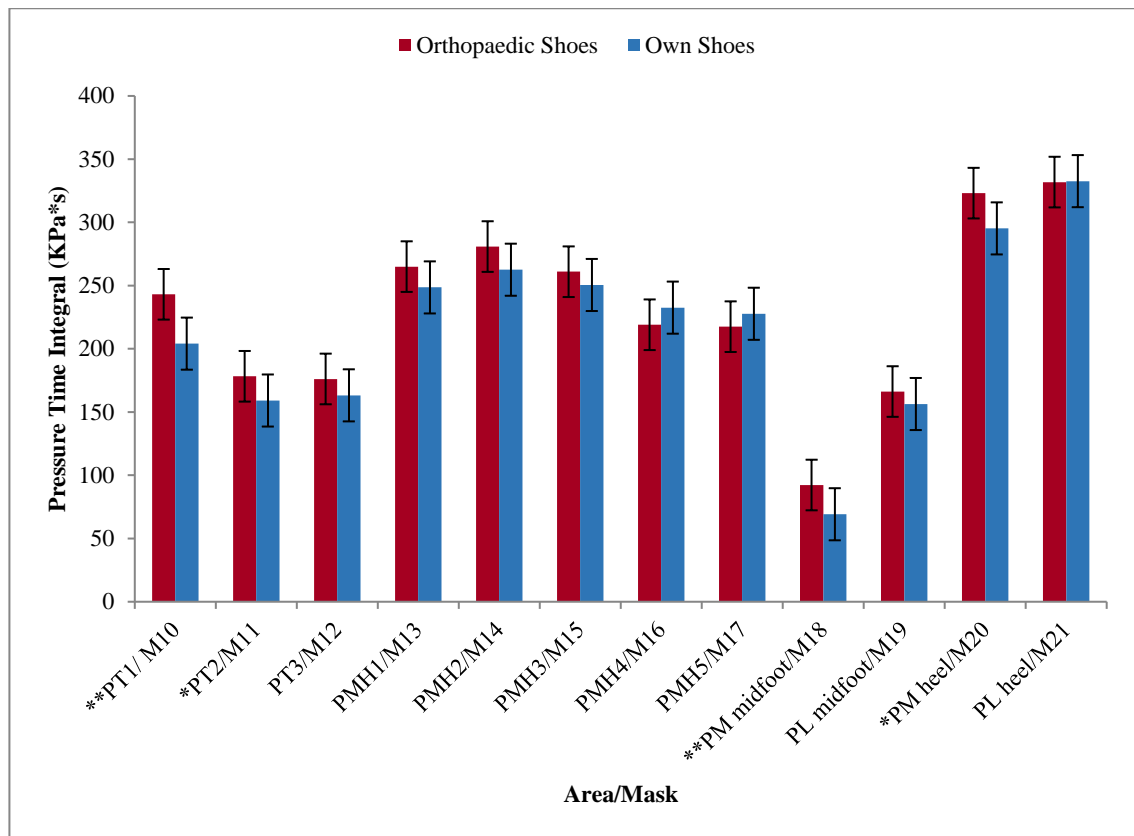


Figure 4.14 Orthopaedic Shoes and Own Shoes PTI means on the plantar surface in control group
*: $p < 0.05$ S; **: $p < 0.001$ HS

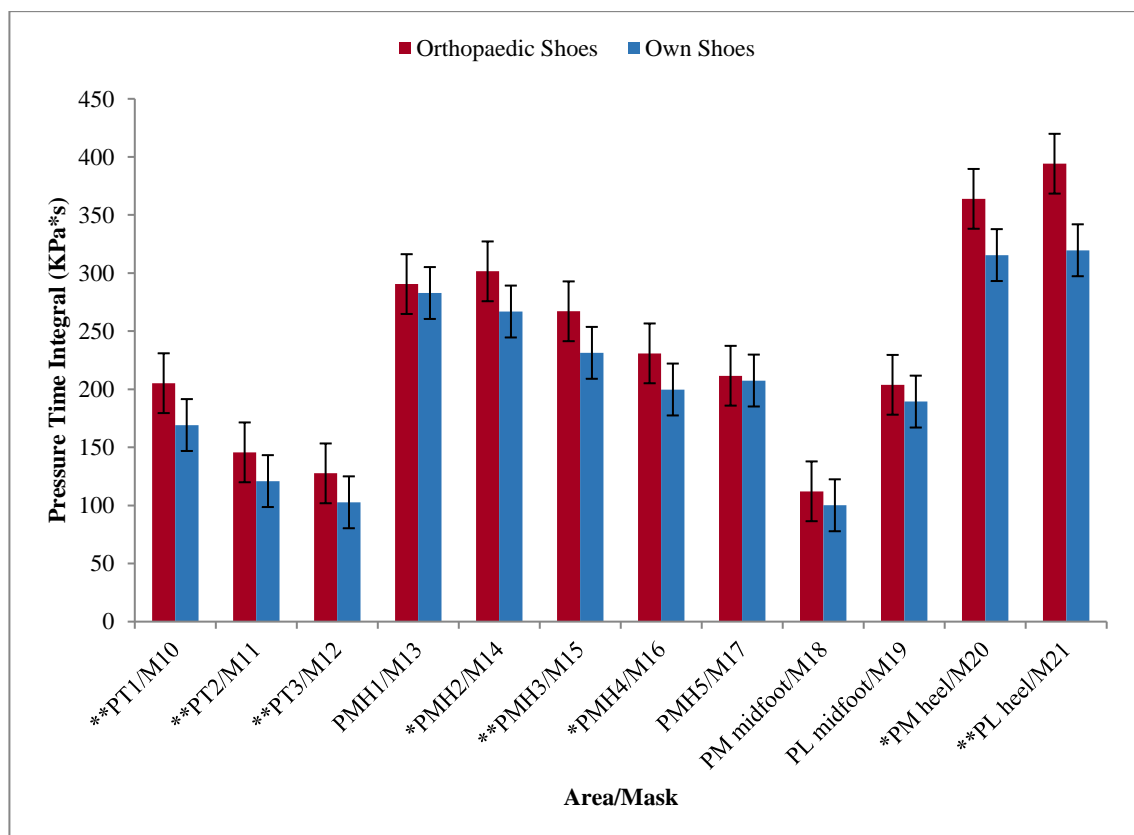


Figure 4.15 Orthopaedic Shoes and Own Shoes PTI means on the plantar surface in diabetes group
*: $p < 0.05$ S; **: $p < 0.001$ HS

The whole dorsal surface PTI showed no significant differences between groups within own shoes as well as the orthopaedic shoes (Figure 4.16).

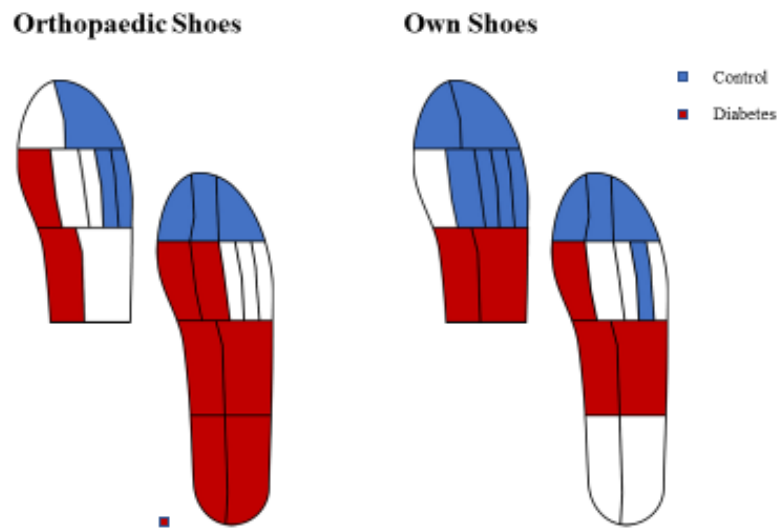


Figure 4.16 Differences between study groups PTI means within own and orthopaedic shoe conditions
The small squares represent differences in Total PP; one for $p < 0.05$ (S), two for $p < 0.001$ (HS)

Yet, own shoes dorsal PTI showed significant differences between groups on all areas on the dorsal surface except area DMH1. Mean values were mostly higher in control group except for midfoot areas that were significantly higher in diabetes group. Also, the orthopaedic shoes dorsal PTI showed significant differences between groups in areas DMH1 and DM midfoot with higher means recorded in the diabetes group and areas DT Lat, DMH4 and DMH5 with higher means in the control group. Again, DM midfoot had the highest dorsal PTI in both study groups within the two shoe conditions.

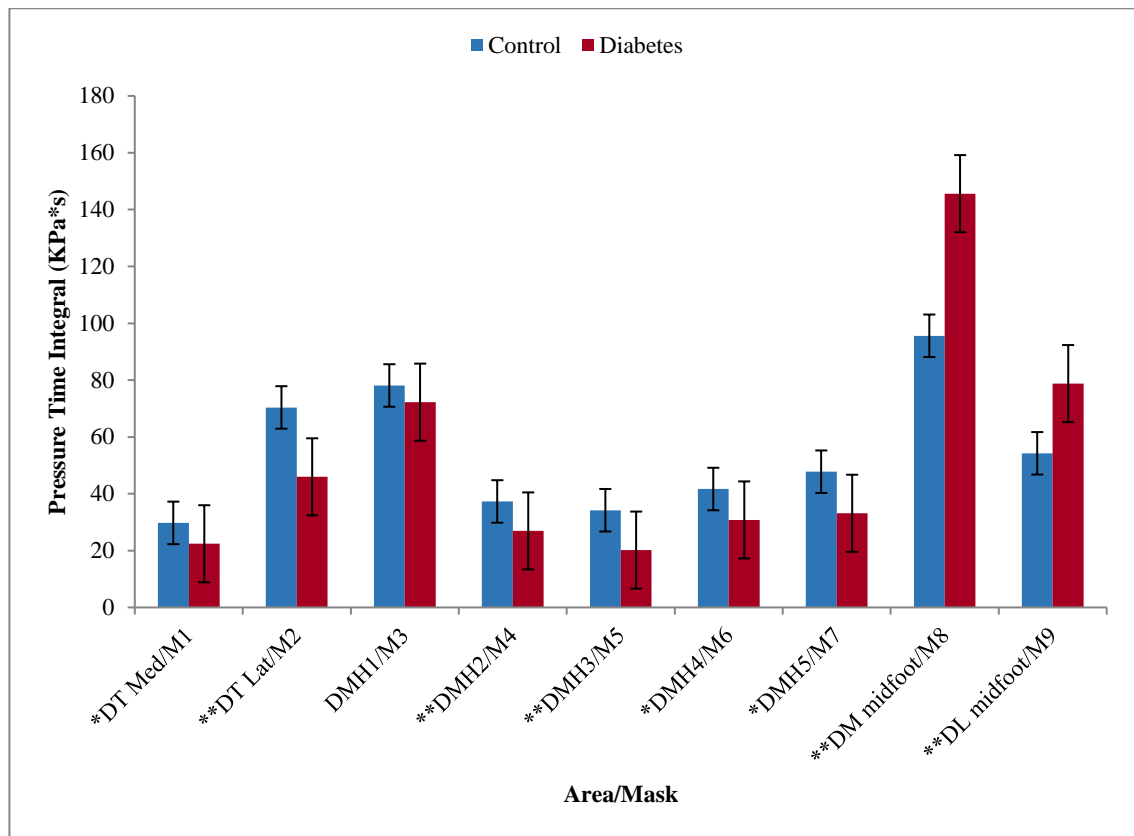


Figure 4.17 Control group and diabetes group PTI means on the dorsal surface in Own Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

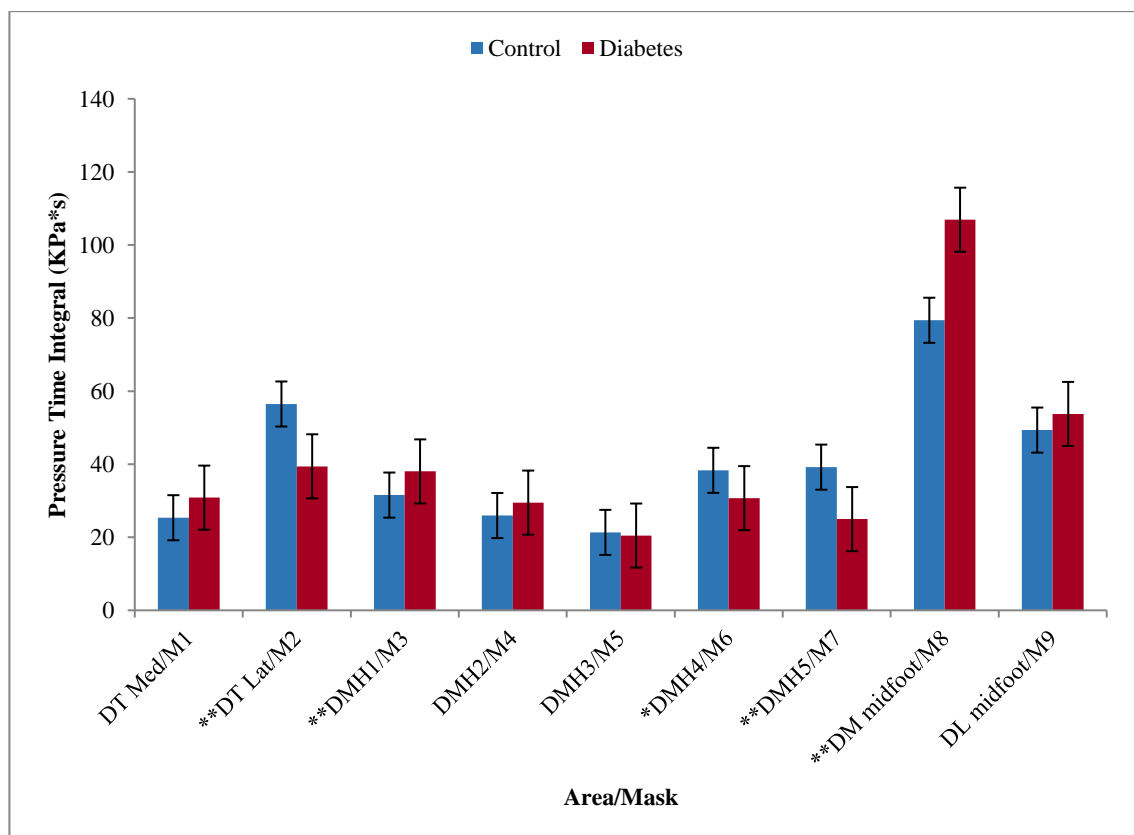


Figure 4.18 Control group and diabetes group PTI means on the dorsal surface in Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

On the plantar surface, the significant difference between the study groups in the whole surface PTI was only present within the orthopaedic shoes ($p=0.002$). Yet, the own shoes PTI showed significant differences between groups in areas PT1, PT2, PT3, PMH1, PMH4, PM midfoot and PL midfoot. Diabetes group had significantly higher PTI on PMH1 and midfoot areas when compared with control in own shoe condition. Plantar toes areas, (PT1, PT2, PT3), were significantly higher in the control group within orthopaedic shoes, the same as they were in own shoes. Significant differences with higher means in the diabetes group were found in plantar areas PMH1, PMH2, PM midfoot, PL midfoot PM heel and PL heel within the orthopaedic shoes.

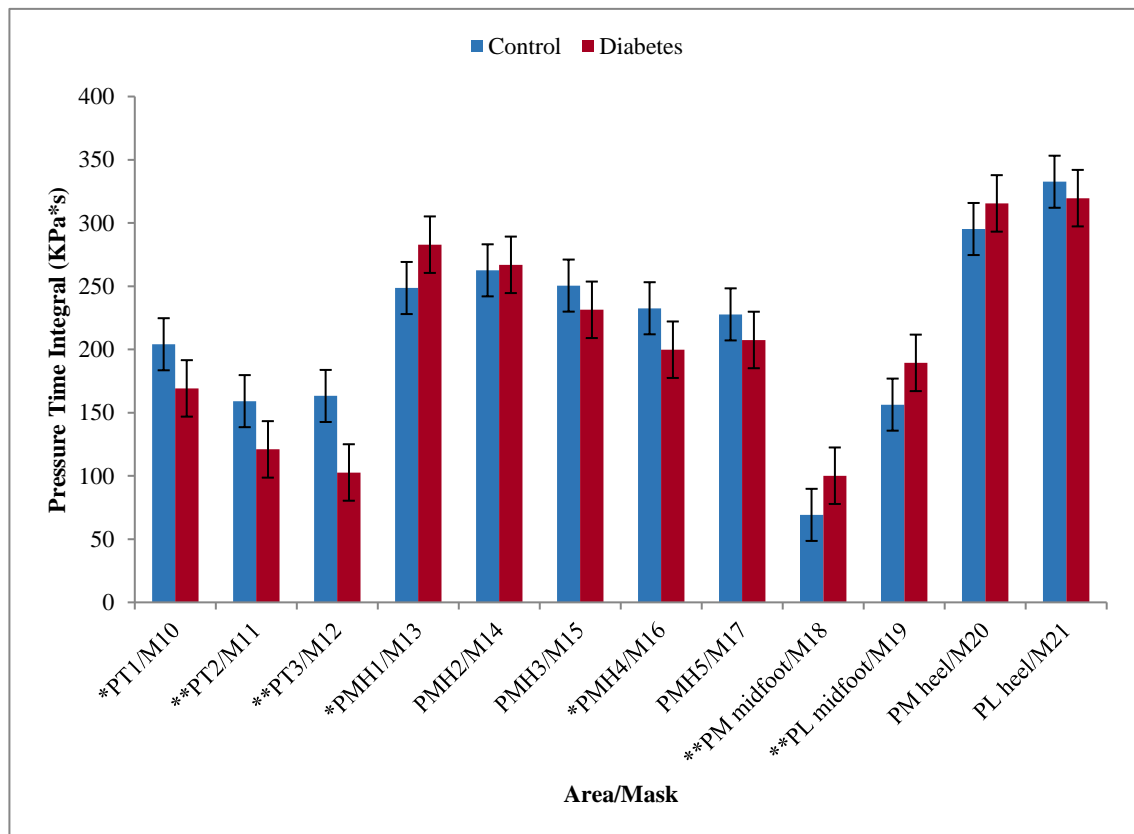


Figure 4.19 Control group and diabetes group PTI means on the plantar surface in Own Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

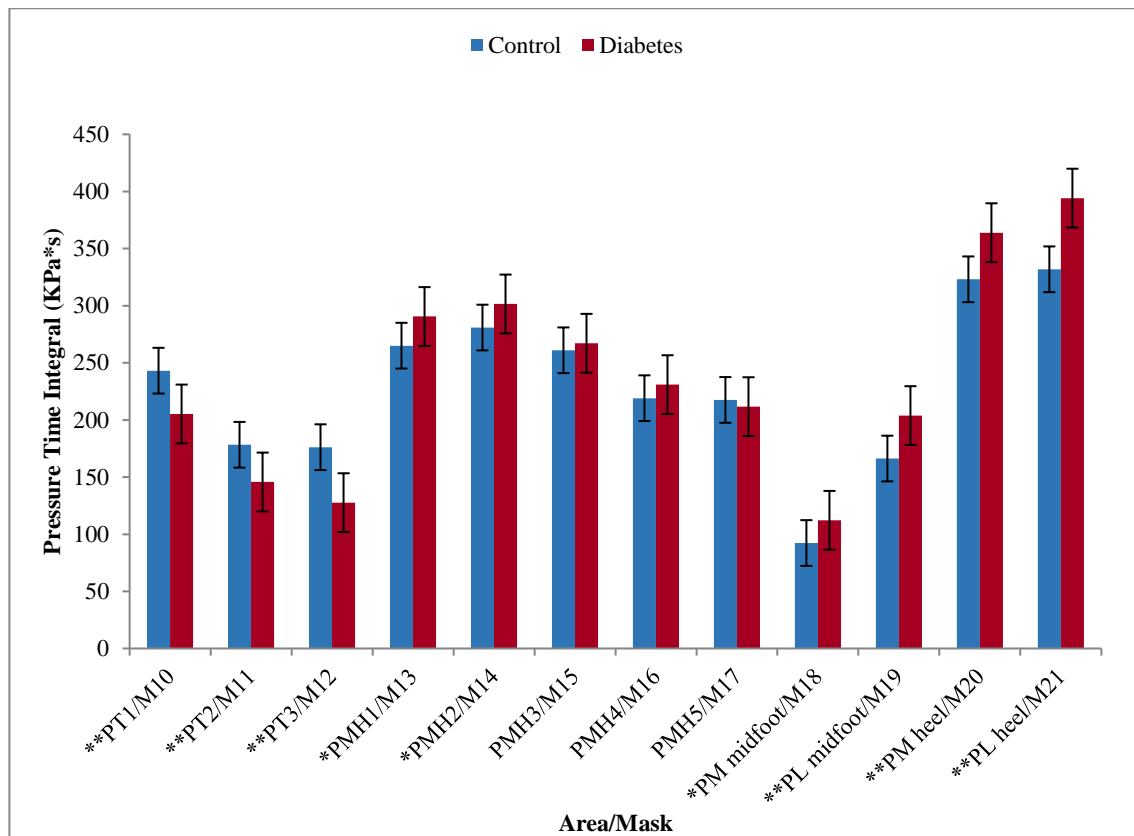


Figure 4.20 Control group and diabetes group PTI means on the plantar surface in Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

4.2.3 Contact Area

Total Contact area (CA) on the dorsal surface showed significant differences between the two tested shoe conditions with higher CA within the orthopaedic shoes than own shoes in both study groups (Figure 4.21).

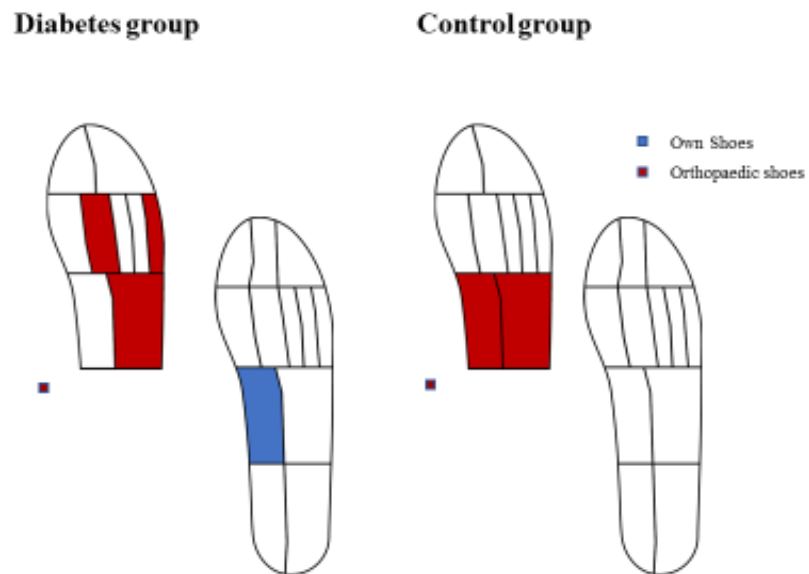


Figure 4.21 Differences between Orthopaedic Shoes and Own Shoes CA means in the study groups
 The small squares represent differences in Total PP; one for $p < 0.05$ (S), two for $p < 0.001$ (HS)

Significant differences between the two tested shoes were only noted in CA on the midfoot areas (DM midfoot and DL midfoot) in the control group, with higher CA means recorded in the orthopaedic shoes. In diabetes group, significant differences between the two tested shoes were found in dorsal areas DMH2, DMH5 and DL midfoot with higher means also noted in the orthopaedic shoes.

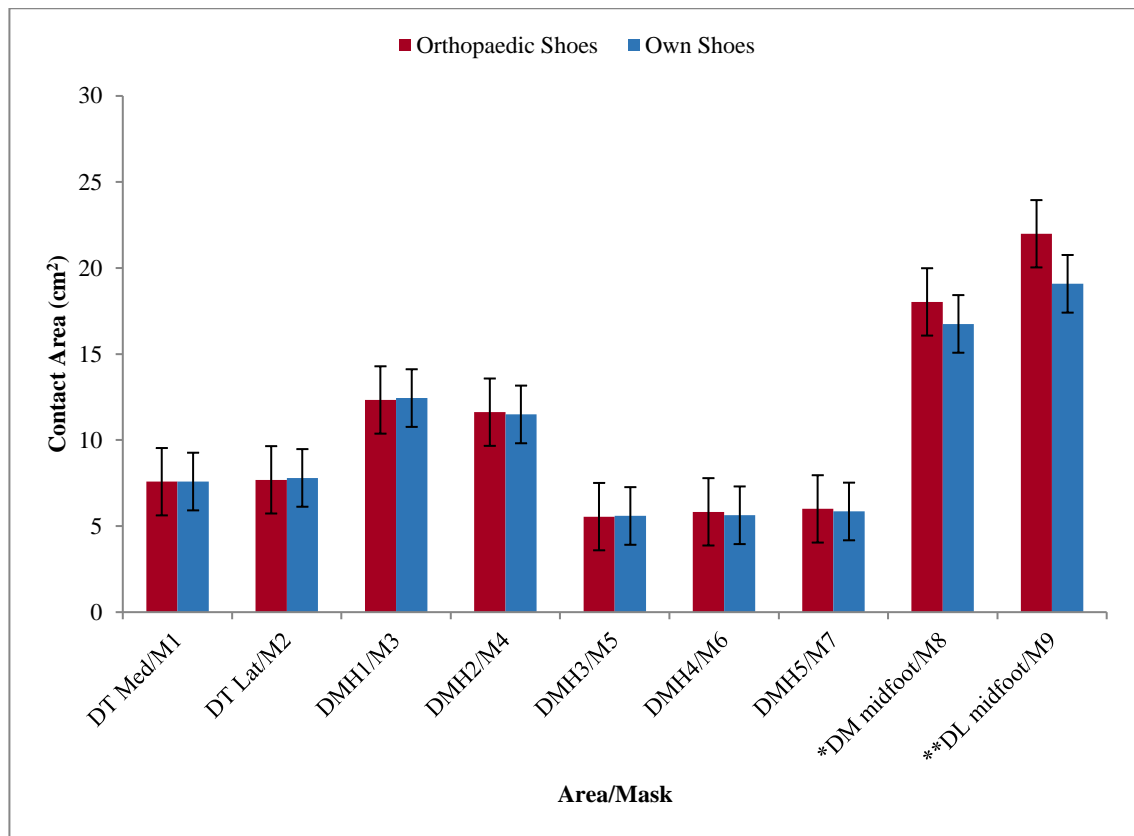


Figure 4.22 Orthopaedic Shoes and Own Shoes CA means on the dorsal surface in control group
*: $p < 0.05$ S; **: $p < 0.001$ HS

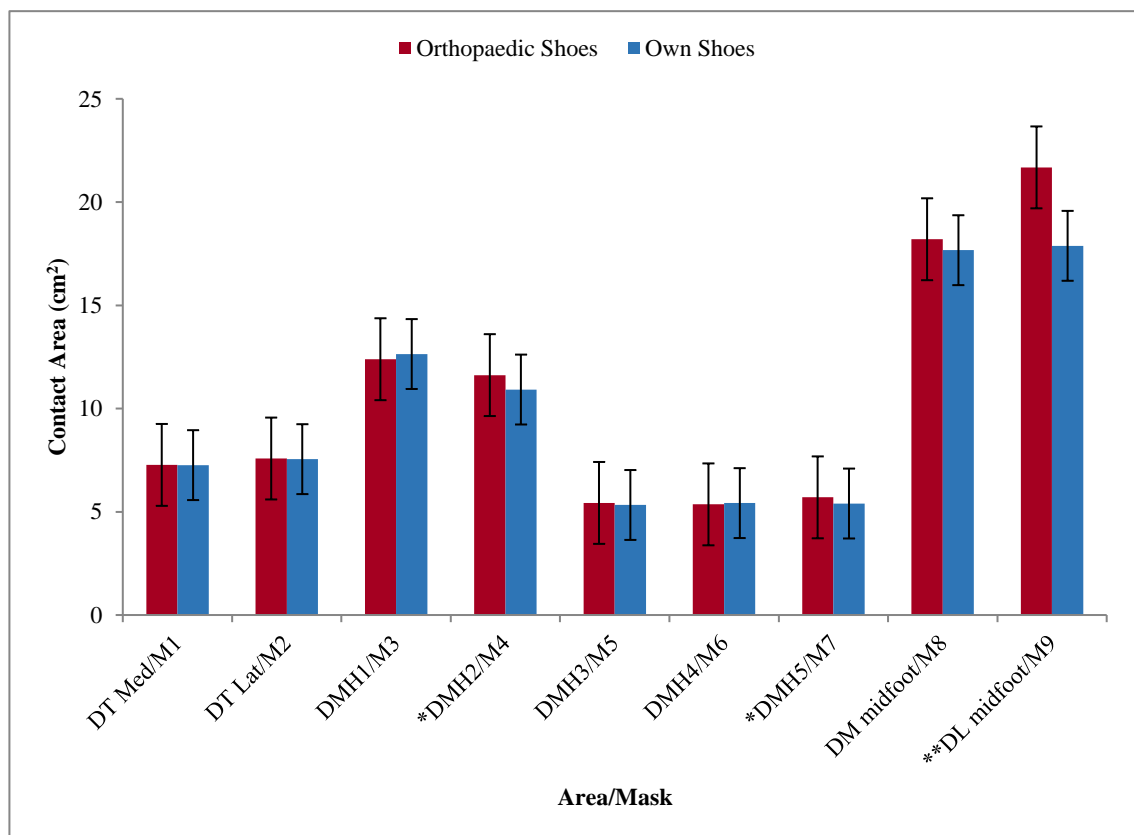


Figure 4.23 Orthopaedic Shoes and Own Shoes CA means on the dorsal surface in diabetes group
*: $p < 0.05$ S; **: $p < 0.001$ HS

No significant differences were noted between the two shoes on the plantar surface in total CA with both study groups. Also, same no significant differences were noted across all plantar areas in the control group. While only area PM midfoot in the diabetes group showed significant difference with higher means found in own shoes.

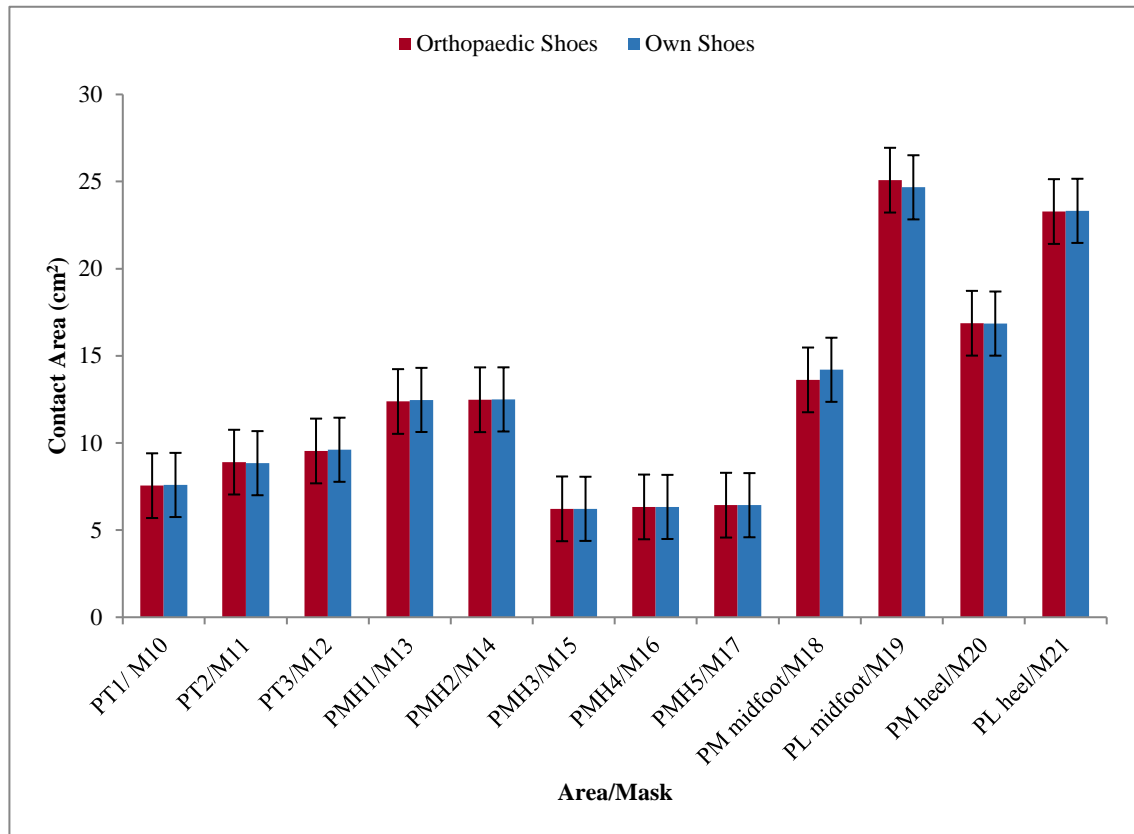


Figure 4.24 Orthopaedic Shoes and Own Shoes CA means on the plantar surface in control group
 *: $p < 0.05$ S; **: $p < 0.001$ HS

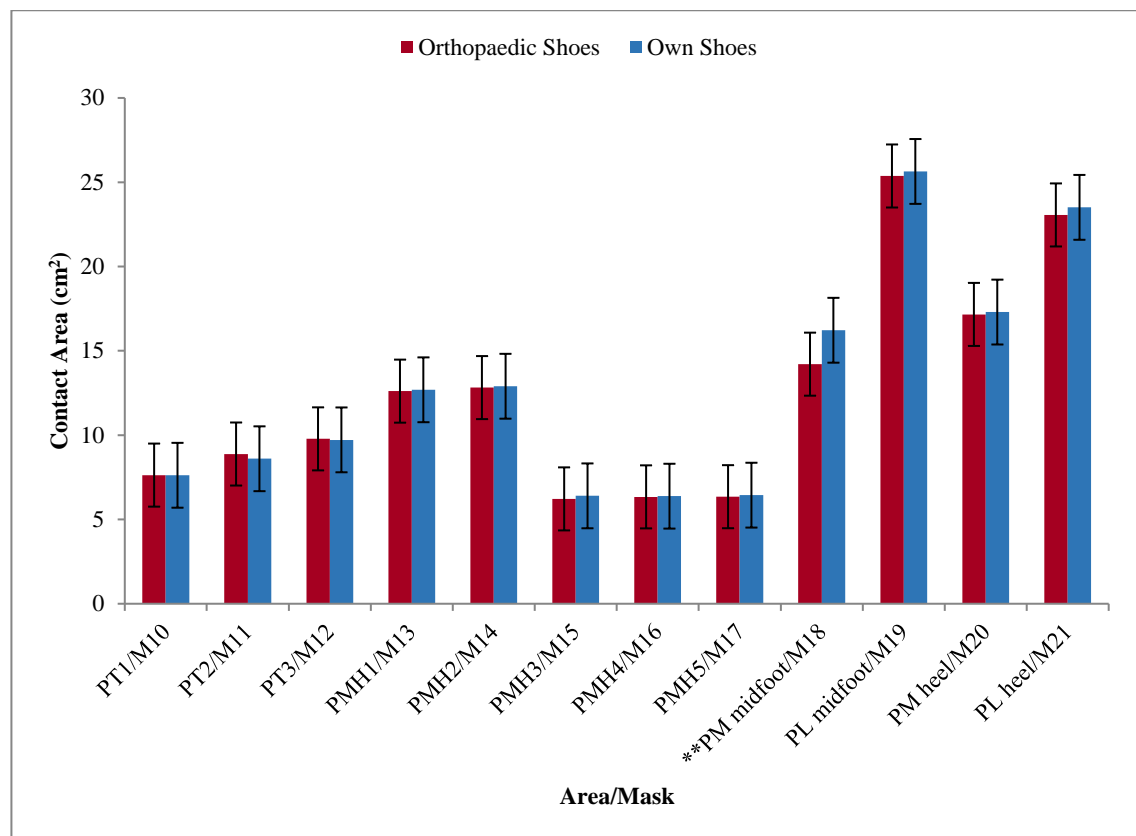


Figure 4.25 Orthopaedic Shoes and Own Shoes CA means on the plantar surface in diabetes group
*: $p < 0.05$ S; **: $p < 0.001$ HS

Both shoe conditions showed no significant differences between groups in total CA on dorsal as well as plantar surfaces (Figure 4.26).

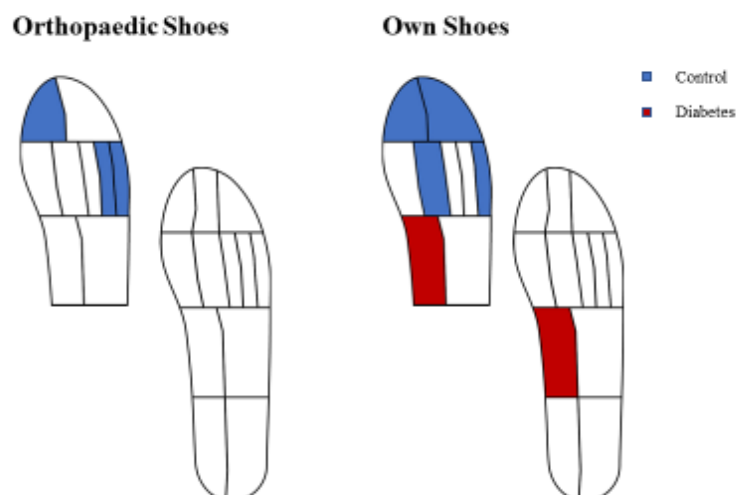


Figure 4.26 Differences between study groups CA means within own and orthopaedic shoe conditions
The small squares represent differences in Total PP; one for $p < 0.05$ (S), two for $p < 0.001$ (HS)

However, on examining individual foot areas, own shoes had significant differences in CA between groups in dorsal areas DT Med, DT Lat, DMH2, DMH5 and DM midfoot. One significant difference between groups was found on the plantar surface in own shoes which was recorded in area PM midfoot with higher mean noted in the diabetes group.

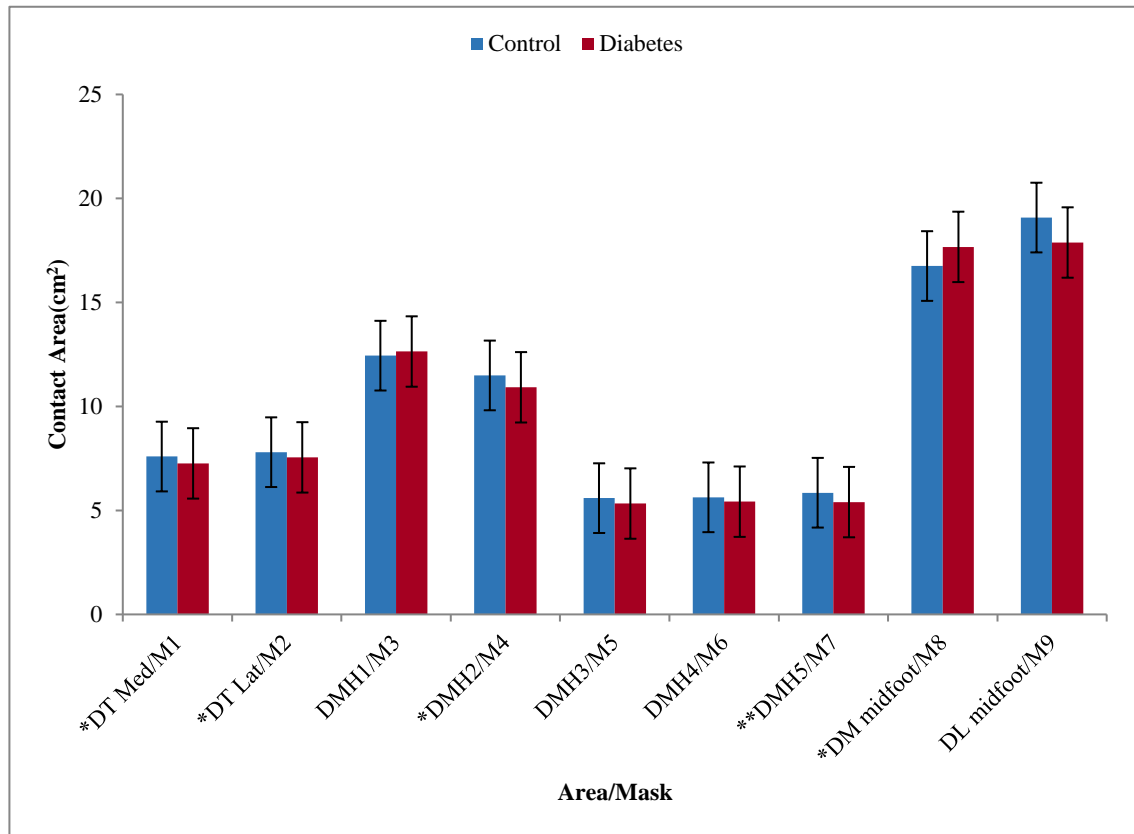


Figure 4.27 Control group and diabetes group CA means on the dorsal surface in Own Shoes
 *: $p < 0.05$ S; **: $p < 0.001$ HS

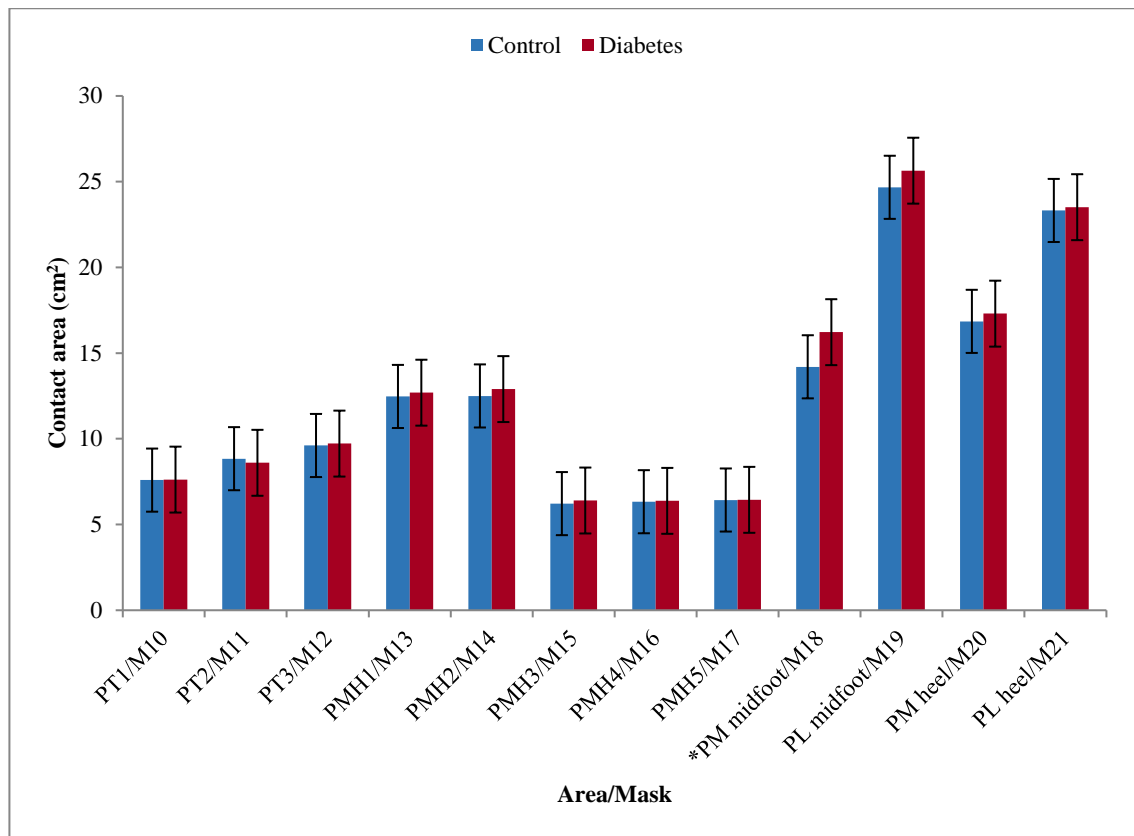


Figure 4.28 Control group and diabetes group CA means on the plantar surface in Own Shoes
 *: $p < 0.05$ S; **: $p < 0.001$ HS

Comparing CA between groups in the orthopaedic shoes showed significant differences with higher means in control group in areas DT Med, DMH4 and DMH5 on the dorsum and no significant differences between groups in all plantar areas.

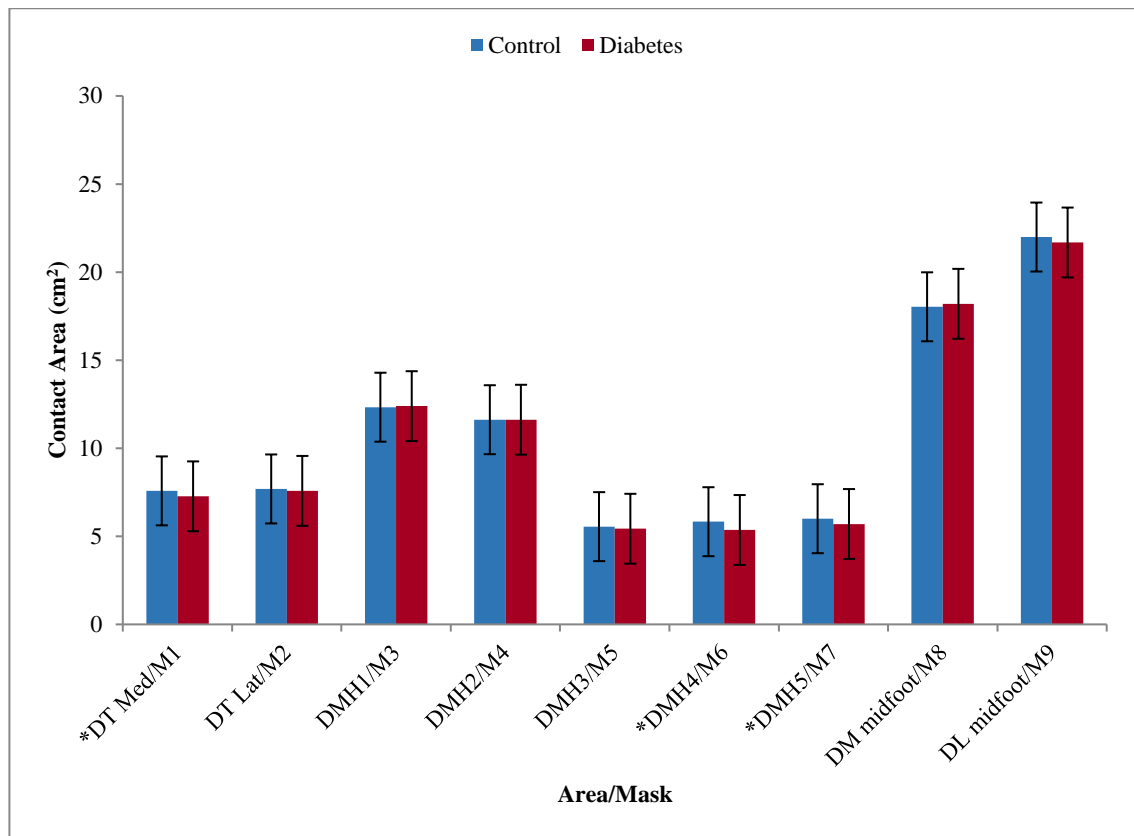


Figure 4.29 Control group and diabetes group CA means on the dorsal surface in the Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

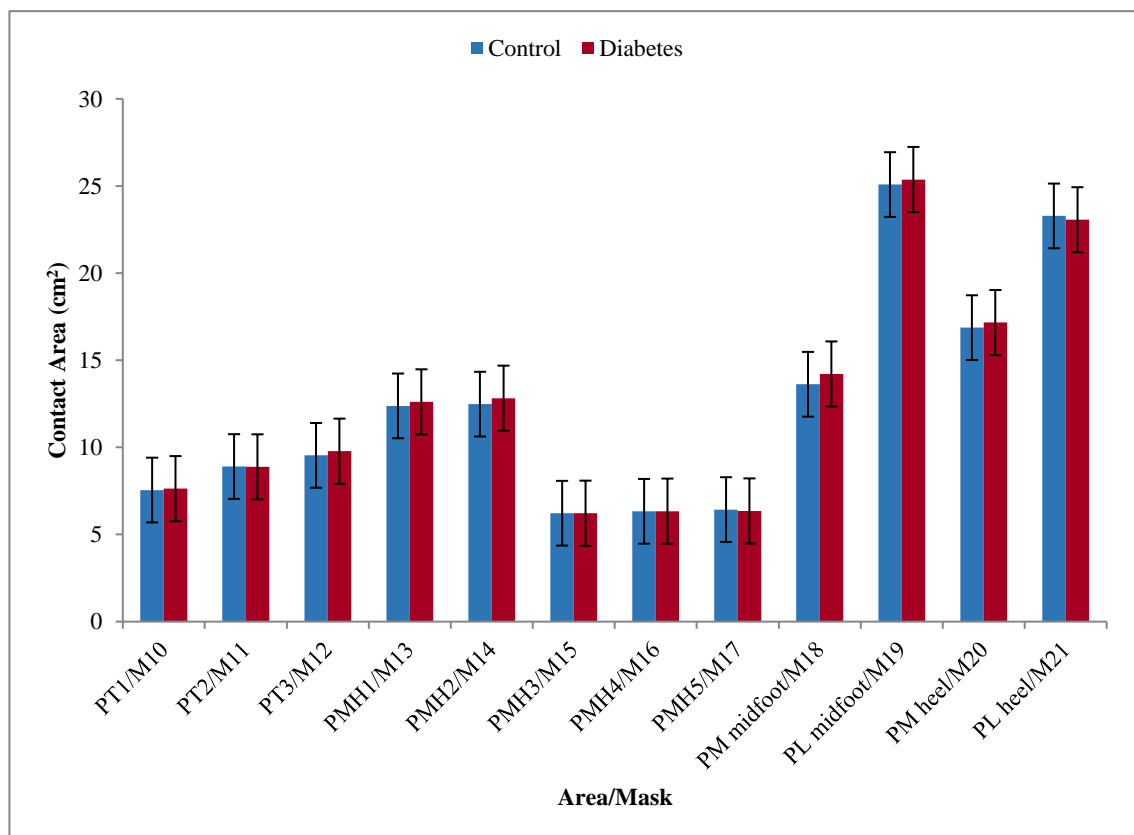


Figure 4.30 Control group and diabetes group CA means on the plantar surface in the Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

4.2.4 Maximum Force

Maximum Force (MF) measured in Newtons (N), showed significant differences between orthopaedic and own shoes in the control group for the whole surface MF on both foot surfaces (Figure 4.31).

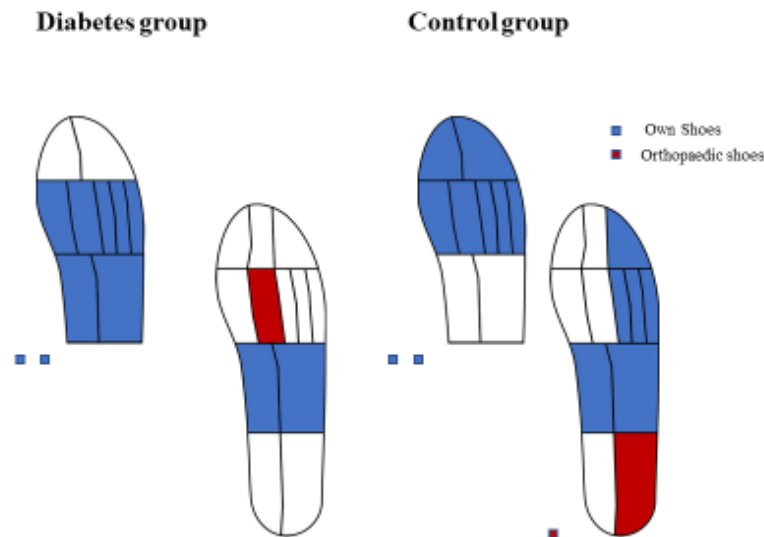


Figure 4.31 Differences between Orthopaedic Shoes and Own Shoes MF means in the study groups
The small squares represent differences in Total PP; one for $p < 0.05$ (S), two for $p < 0.001$ (HS)

Most areas on the dorsal surface in the control group were similar to total MF, showing significantly higher means in participants' own shoes, except midfoot areas which were not significantly different between the two shoes.

The plantar surface in the control group had significantly different MF in areas PT3, PMH3, PMH4, PMH5, PM midfoot, PL midfoot and PL heel that were all higher in own shoes except PL heel which was higher in the orthopaedic shoes along with the whole plantar surface MF.

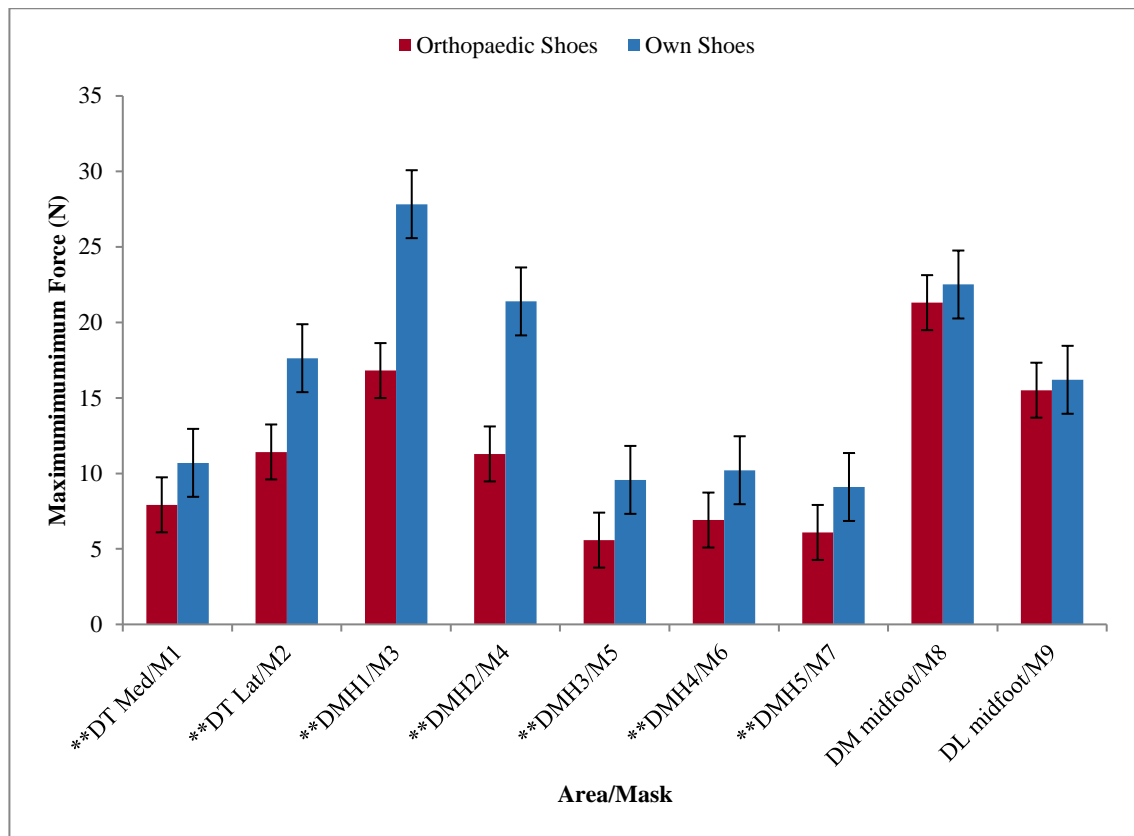


Figure 4.32 Orthopaedic Shoes and Own Shoes MF means on the dorsal surface in control group
*: $p < 0.05$ S; **: $p < 0.001$ HS

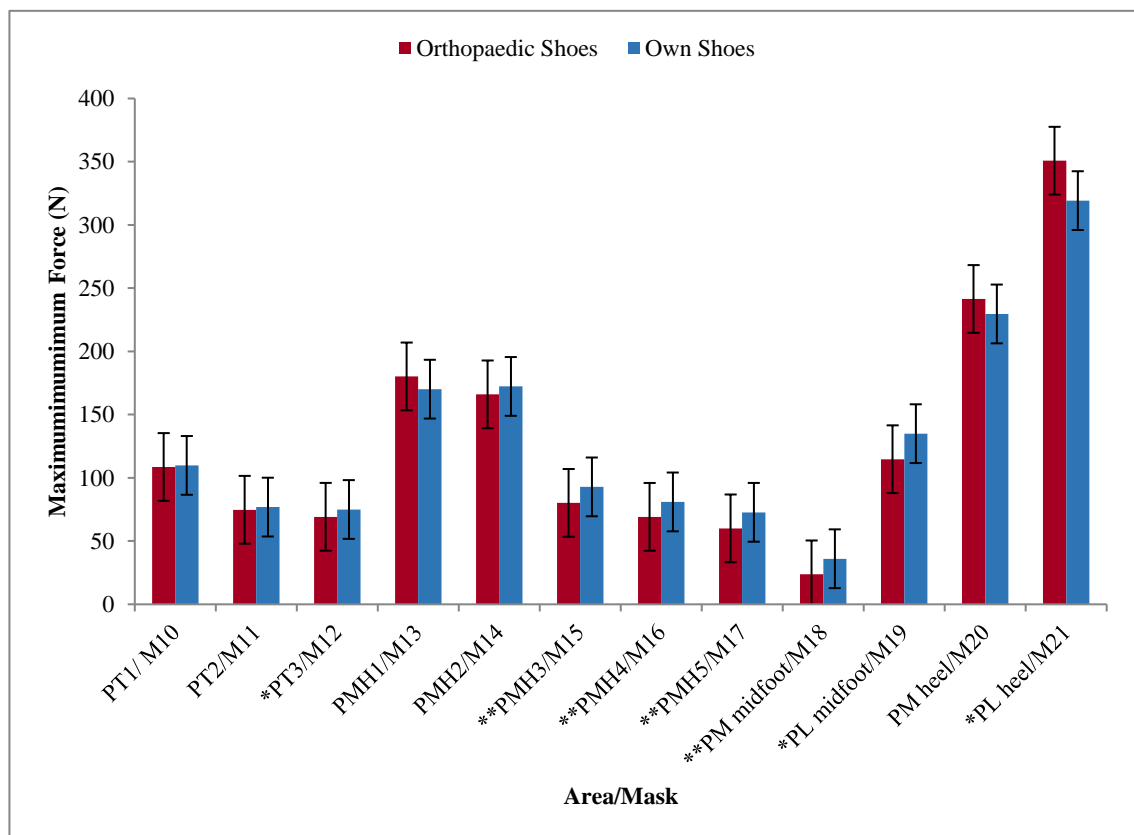


Figure 4.33 Orthopaedic Shoes and Own Shoes MF means on the plantar surface in control group
*: $p < 0.05$ S; **: $p < 0.001$ HS

Total dorsal MF was significantly different between the two shoes in the diabetes group, but no significant differences were noted for the whole plantar surface MF. Diabetes group showed significant differences between the two tested shoes' MF on the dorsal surface in metatarsal and midfoot areas, with higher means recorded in participants' own shoes, and no significant differences between the two shoes on toes areas.

Plantar surface in diabetes group showed significantly higher MF in orthopaedic shoes in PMH2 and significantly higher MF in own shoes in PM midfoot and PL midfoot.

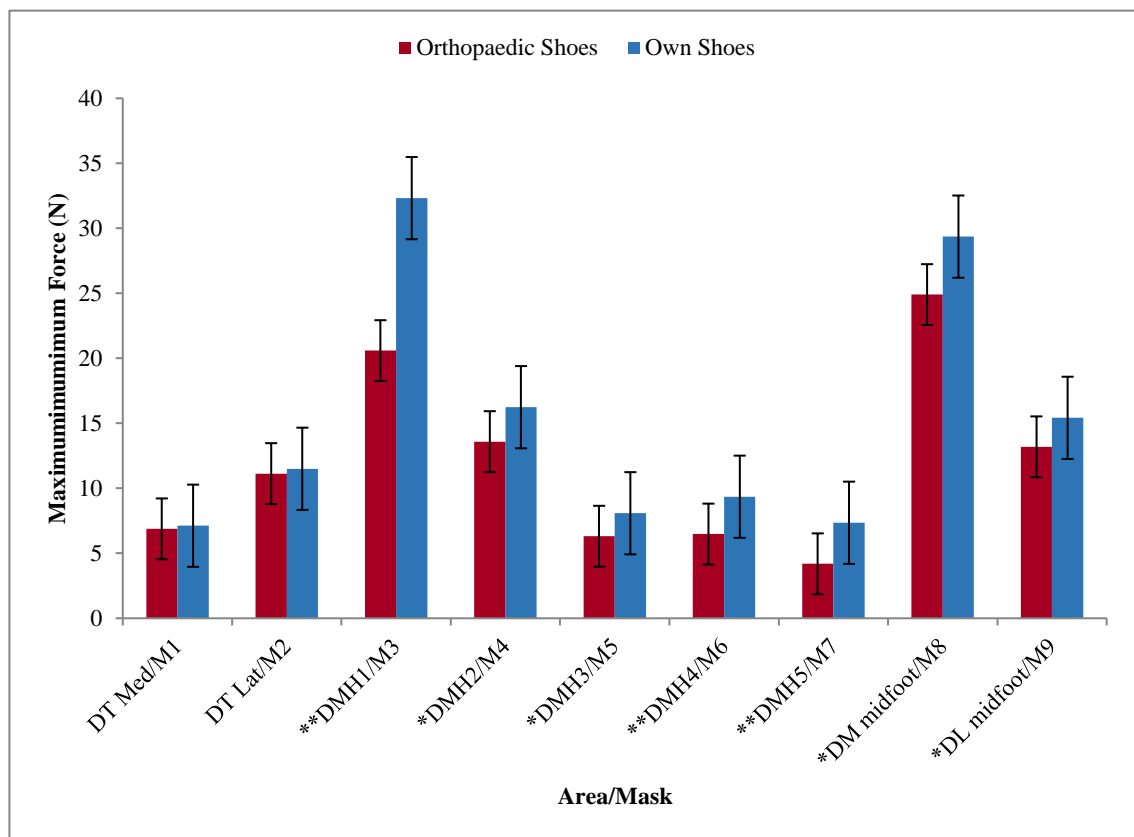


Figure 4.34 Orthopaedic Shoes and Own Shoes MF means on the dorsal surface in diabetes group
 *: $p < 0.05$ S; **: $p < 0.001$ HS

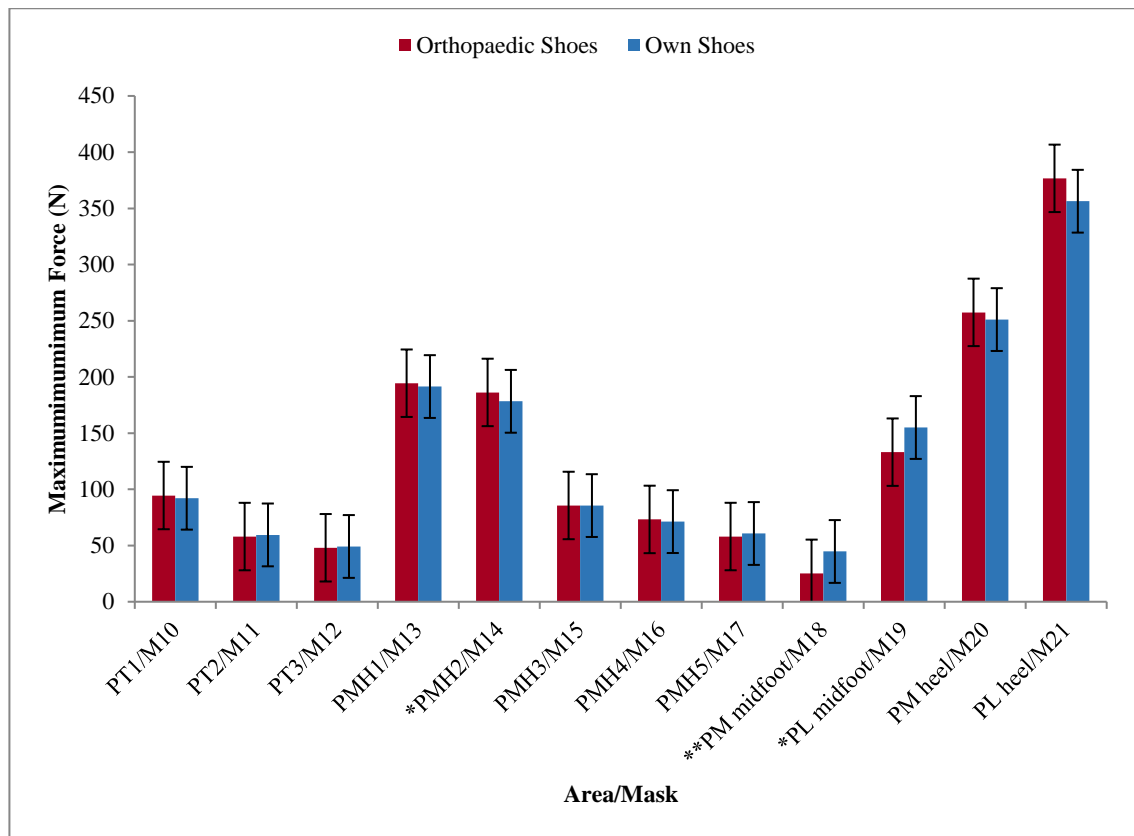


Figure 4.35 Orthopaedic Shoes and Own Shoes MF means on the plantar surface in diabetes group
*: $p < 0.05$ S; **: $p < 0.001$ HS

MF had no significant differences between groups for whole dorsal surface MF in both tested shoes (Figure 4.36).

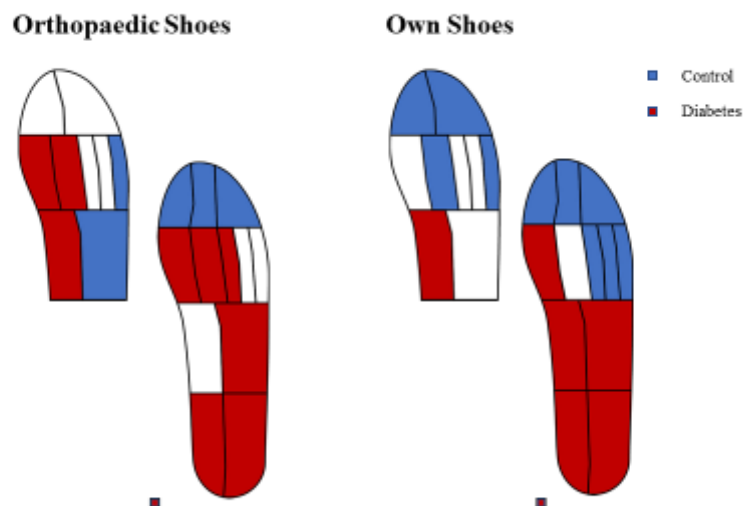


Figure 4.36 Differences between study groups MF means within own and orthopaedic shoe conditions
The small squares represent differences in Total PP; one for $p < 0.05$ (S), two for $p < 0.001$ (HS)

However, own shoes showed significant differences on dorsal areas DT Med, DT Lat, DMH2, DMH5 and DM midfoot with significantly higher means found in control group except for DM midfoot that was significantly higher in diabetes group.

Whole plantar surface MF and all individual plantar areas except PMH2 were significantly different between groups within own shoes with higher means recorded in diabetes group in PMH1, midfoot and heel areas. Toes and metatarsal areas PMH3, PMH4 and PMH5 in own shoes had significantly higher MFs with control than diabetes.

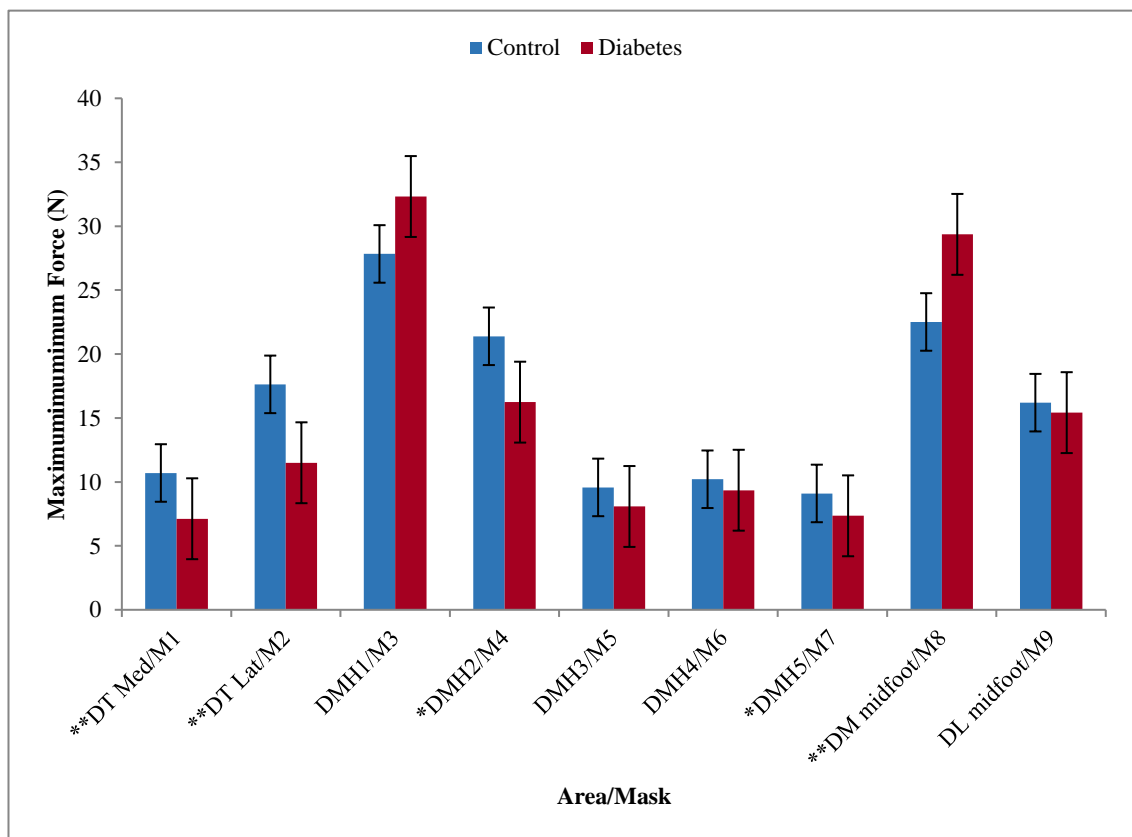


Figure 4.37 Control group and diabetes group MF means on the dorsal surface in Own Shoes
 *: $p < 0.05$ S; **: $p < 0.001$ HS

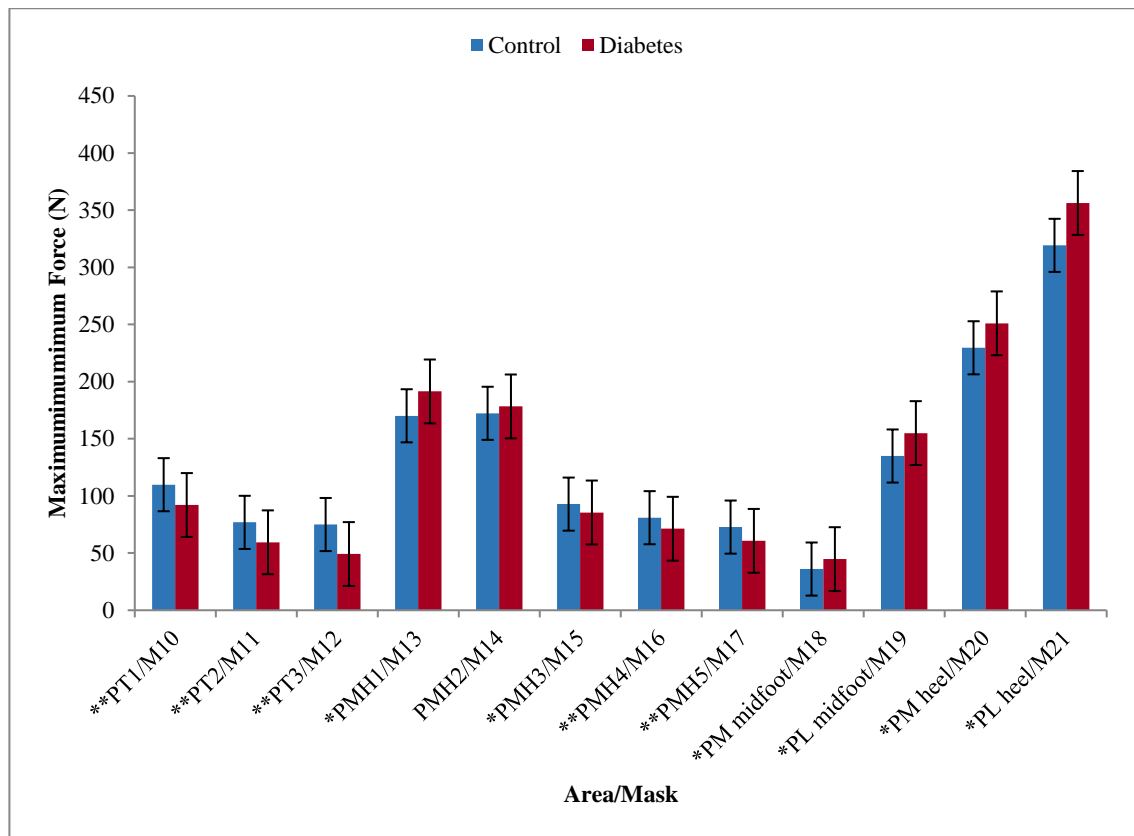


Figure 4.38 Control group and diabetes group MF on the plantar surface in Own Shoes
 *: $p < 0.05$ S; **: $p < 0.001$ HS

Though, between groups comparison for the whole surface MF within the orthopaedic shoes had no significant differences on the dorsal surface, a significantly higher total MF with diabetes than control was found on the plantar surface.

MF revealed significant differences in dorsal areas DMH1, DMH2, DMH5, DM midfoot and DL midfoot. Significant differences on the medial side of the dorsal surface (DMH1, DMH2 and DM midfoot) in the orthopaedic shoes had significantly higher means in the diabetes group. Dorsal toes areas, DMH3 and DMH4 had no significant differences between groups in the orthopaedic shoes.

Significant differences in MF between groups in the orthopaedic shoes were noted in most of the plantar areas except PMH4, PMH5 and PM midfoot which showed no significant differences between groups in the orthopaedic shoes. Plantar toes areas in the orthopaedic shoes were significantly higher in the control group while other significantly different plantar areas were higher in the diabetes group.

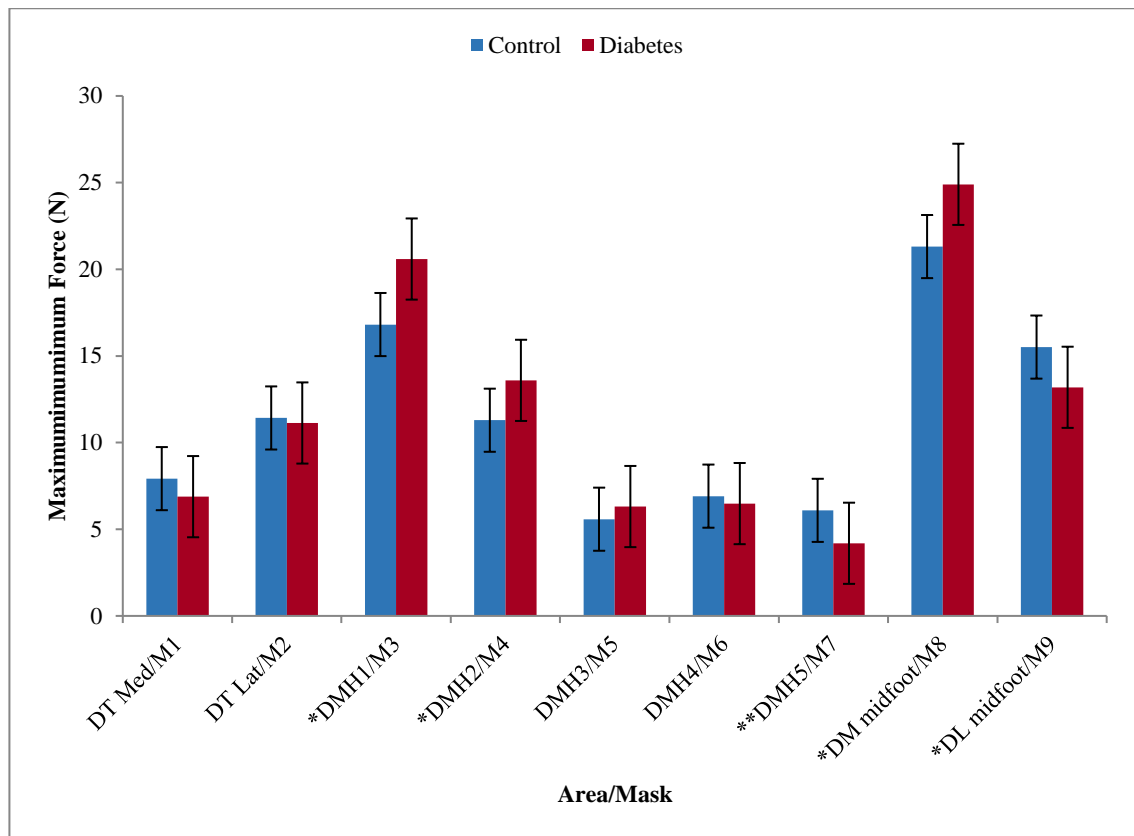


Figure 4.39 Control group and diabetes group MF means on the dorsal surface in the Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

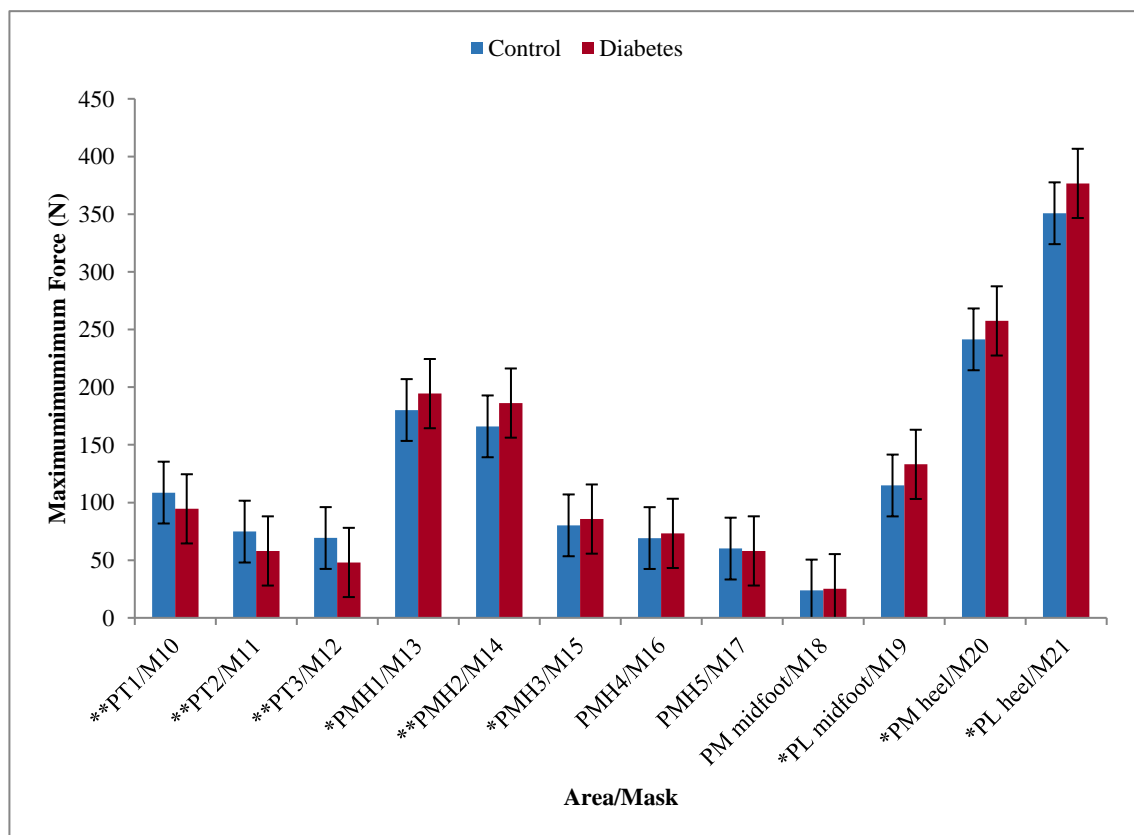


Figure 4.40 Control group and diabetes group MF means on the plantar surface in the Orthopaedic Shoes
*: $p < 0.05$ S; **: $p < 0.001$ HS

4.2.5 Time for Peak Pressure

Software developed in our laboratory was used to extract the time of occurrence of Dorsal Peak Pressure during the gait cycle. There were no significant differences between the tested shoes in both study groups as well as between groups in both shoe conditions.

All data extracted from the in-house software was examined for the frequency of occurrence of dorsal PP as a percentage of the gait cycle. The gait cycle phases with approximated timing of each event were demonstrated in Figure 4.41.

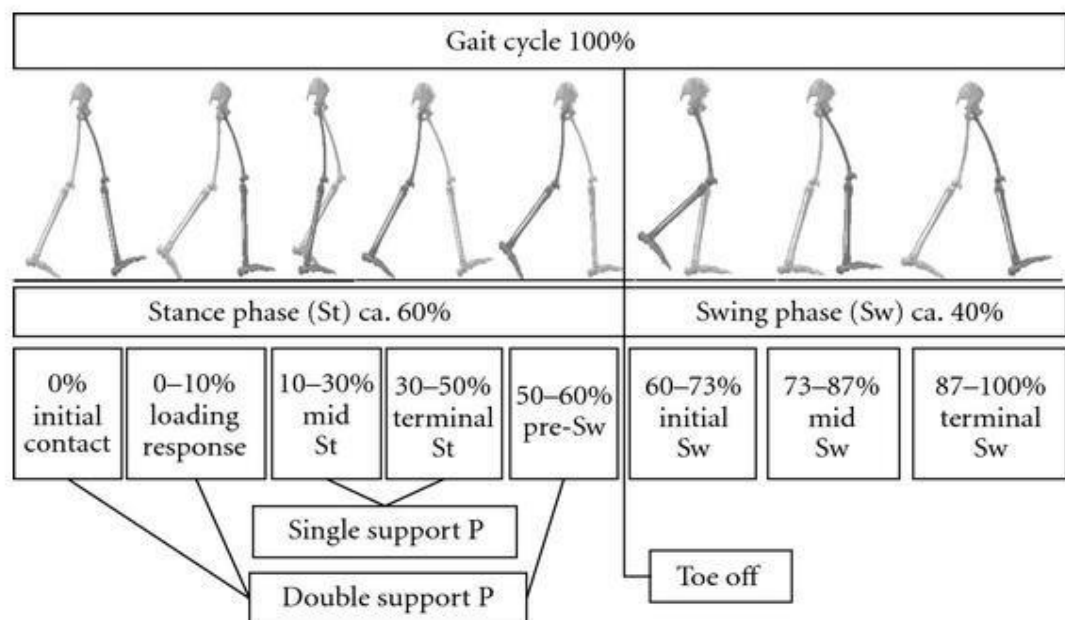


Figure 4.41 Phases of gait cycle (Hartmann et al., 2010)

Dorsal PP existed through most of the stance phase as well as initial swing with sporadic presence in the mid and late swing. Dorsal PP also followed a 2-peak pattern similar to Plantar PP. However, the first Dorsal PP seemed to occur earlier in the stance phase than Plantar PP. Also, the time of the second Dorsal PP shifted from being terminal stance/pre-swing in Plantar PP to a later timing that was more pre-swing/initial swing peak. Moreover, there was a noted existence of dorsal PP but with less frequency in the terminal stance period. Dorsal force followed a similar timing for peaks as the dorsal PP.

Using all participants' data as one group and comparing own shoes with orthopaedic shoes, showed a significant difference in the time of dorsal PP ($p=0.047$) with more frequent peaks occurring late in the swing phase within the orthopaedic shoes.

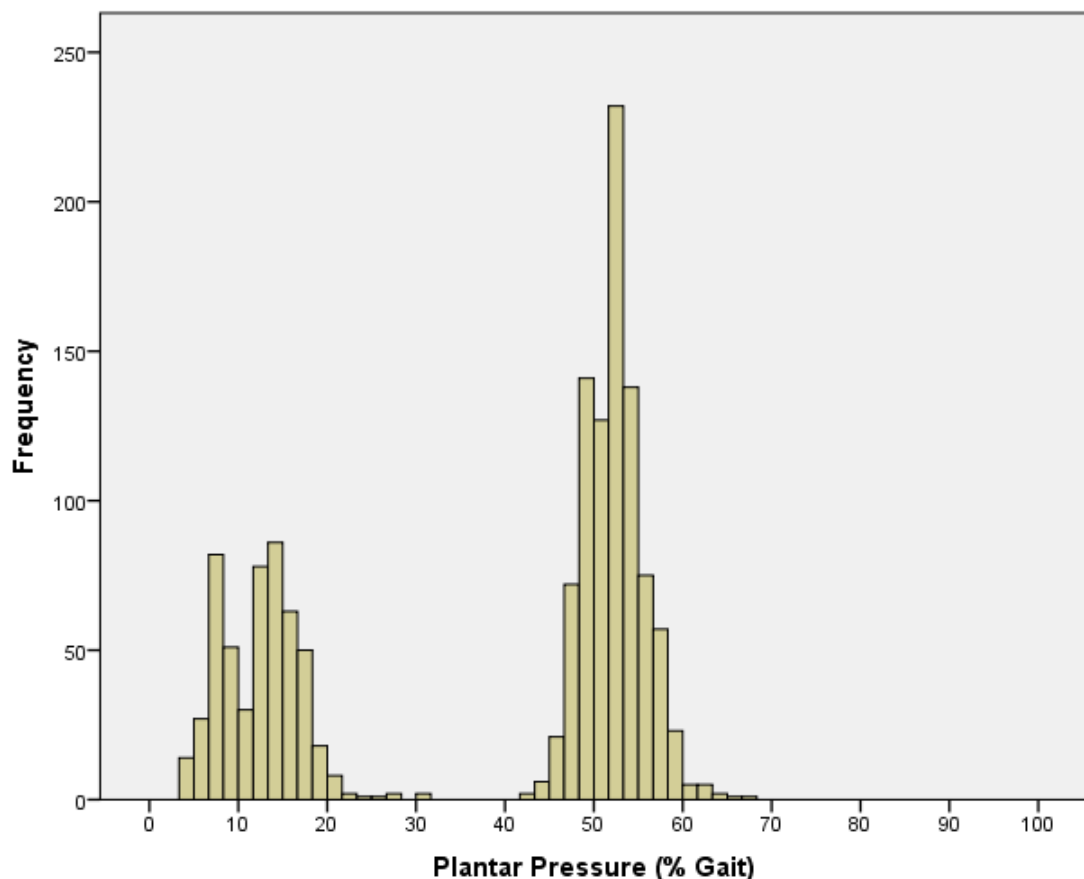


Figure 4.42 Frequency of Plantar PP during the gait cycle in all tested conditions

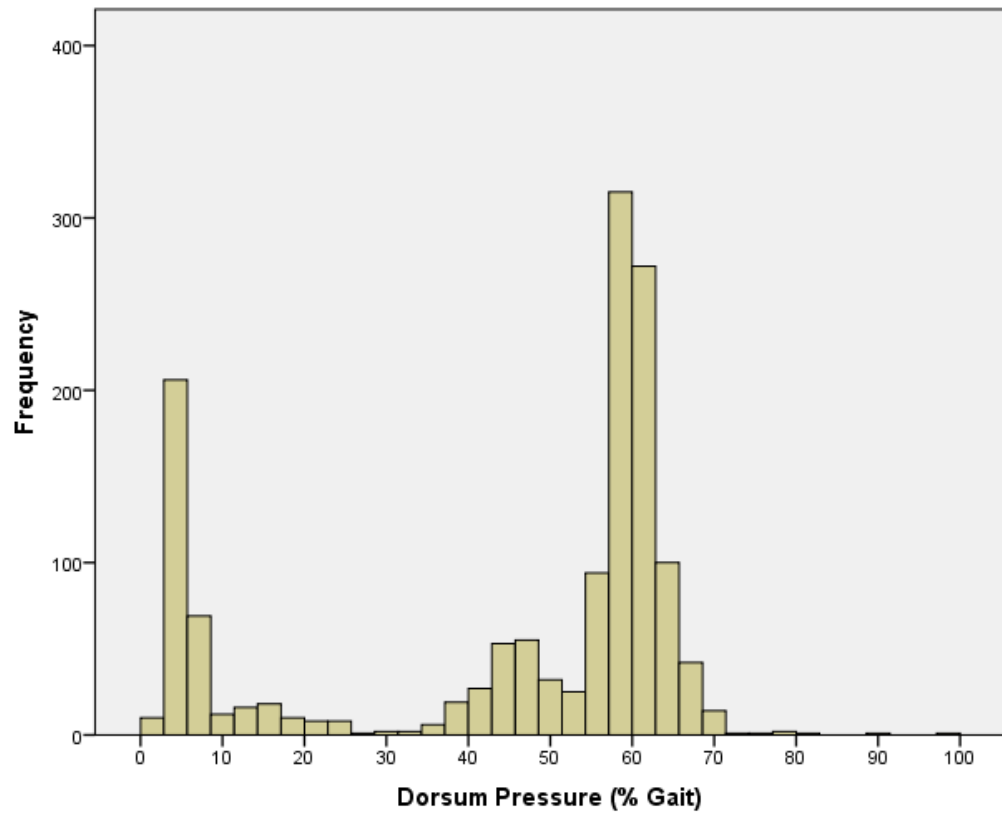


Figure 4.43 Frequency of Dorsal PP during the gait cycle in all tested conditions

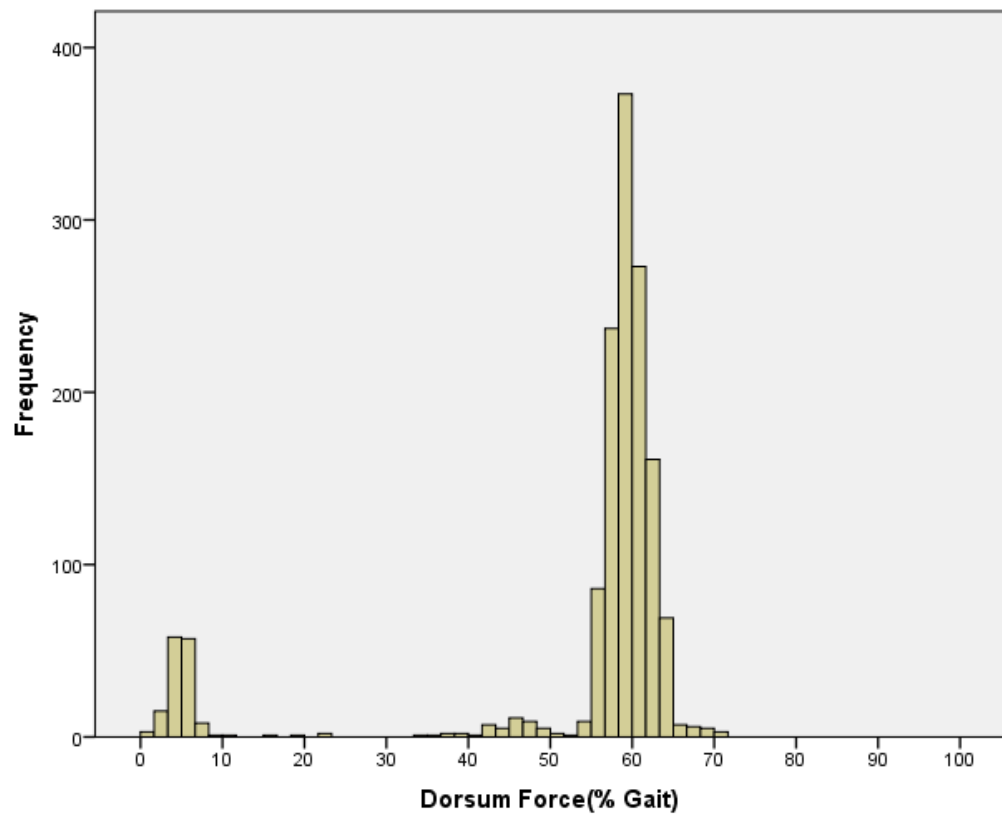


Figure 4.44 Frequency of Dorsal Maximum Force during the gait cycle in all tested conditions

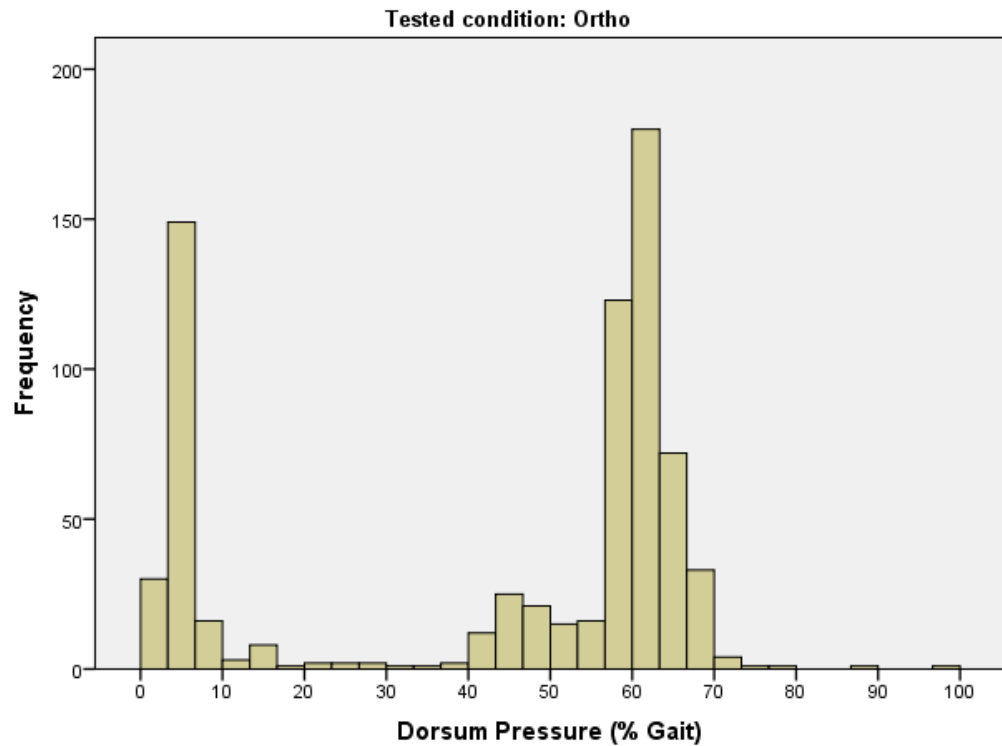


Figure 4.45 Frequency of Dorsal PP during the gait cycle in Orthopaedic Shoes across all participants

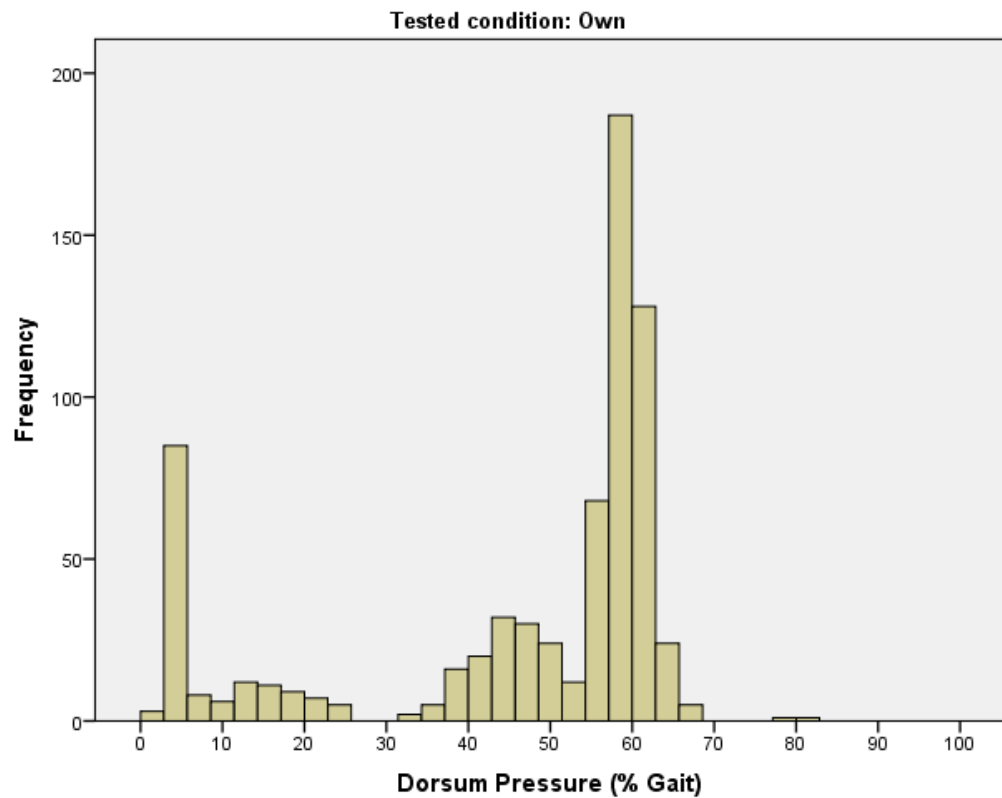


Figure 4.46 Frequency of Dorsal PP during the gait cycle in Own Shoes across all participants

4.3 ASSESSMENT OF THE IMPACT OF PRESSURE ON ENDOTHELIAL FUNCTION

Endothelial function under loading pressure was assessed via monitoring the response of foot skin superficial blood vessels to the iontophoresis of acetylcholine (ACh) an endothelium-dependent vasoactive agent, and sodium nitroprusside (SNP) an endothelium-independent vasodilator, measured with the single-point laser Doppler flowmetry (LDF). Iontophoresis was carried out on dorsal and plantar surfaces of the right foot of subjects from both study groups using the protocol tested and detailed in section 3.3.2.2. Iontophoresis of the vasoactive agents was conducted on each foot surface with no pressure applied, under the average PP in participants' own comfortable shoes then the average PP in the size-matched orthopaedic shoes which were calculated from the foot surface PP values in that shoes on the testing session.

We had the same issue of movement artefacts with some participants' recordings as in the testing for the protocol repeatability. Thus, noise filtration was performed prior to any data extraction for analysis and values for the median of perfusion in each iontophoresis period were examined plus the mean values. Peak of Maximum Response was calculated from the average of all maximum perfusion values of the 16 periods of the iontophoresis protocol. We also examined the changes from baseline in endothelial response to the iontophoresis of vasoactive agent reflected in the percentage of change in blood flow from baseline (0 μ A current) which was calculated as:

$$\text{Change in response} = \frac{(\text{Peak of Maximum Response} - \text{Baseline blood flow})}{\text{Baseline blood flow}} \times 100 = \%$$

According to the Central Limit Theorem, “as the number of the sample increases, the closer its variation is to the variance of the society”. Thus, distribution can be considered approximate naturally when the sample size becomes 30 or more (Walpole *et al.*, 2017). As our sample/ study population was more than 30 (40 for pressure data and 35 completed the whole study with iontophoresis), we followed the Central Limit Theory and assumed normal distribution of our data. However, to confirm the results of data analysis, perfusion data for Peak of Maximum Response and Change in response that mostly did not follow a normal distribution pattern were also examined using non-parametric statistical tests. Median flux values showed similar analysis results as Mean flux values, and this was also confirmed when using non-parametric statistical test analysis of Peak of Maximum response and change in response. Data for both study groups were extracted, analysed and the findings are illustrated in the following section. Tables for the descriptive statistics and significance values supplied in the tables included in Appendix 16.

4.3.1 Comparison of Flux Values Under the Three Tested Pressure Conditions Within the Study Groups

4.3.1.1 Blood Flow Changes in Control Group

Comparing the flux values recorded on the dorsal surface of the foot in the control group during the iontophoresis of ACh showed significant differences between the resting/no pressure condition and own shoes as well as orthopaedic shoes’ pressure applications ($p < 0.001$) at all the iontophoresis protocol periods, Peak of Maximum response and change in response.

Own shoes and orthopaedic shoes pressures application were only significantly different in the change in response ($p < 0.001$) with higher flux changes recorded under the orthopaedic shoes' PP (flux median change in response % $\text{Mean} \pm \text{SD}$ $192 \pm 80.08\%$) when compared to own shoe effect (flux median change in response % $\text{Mean} \pm \text{SD}$ $149.65 \pm 66.86\%$). Similar differences between the three tested pressure conditions were noted in response to SNP iontophoresis with the lone significant difference between orthopaedic shoes and own shoes' pressure applications found in the change in response from baseline flux ($p < 0.001$).

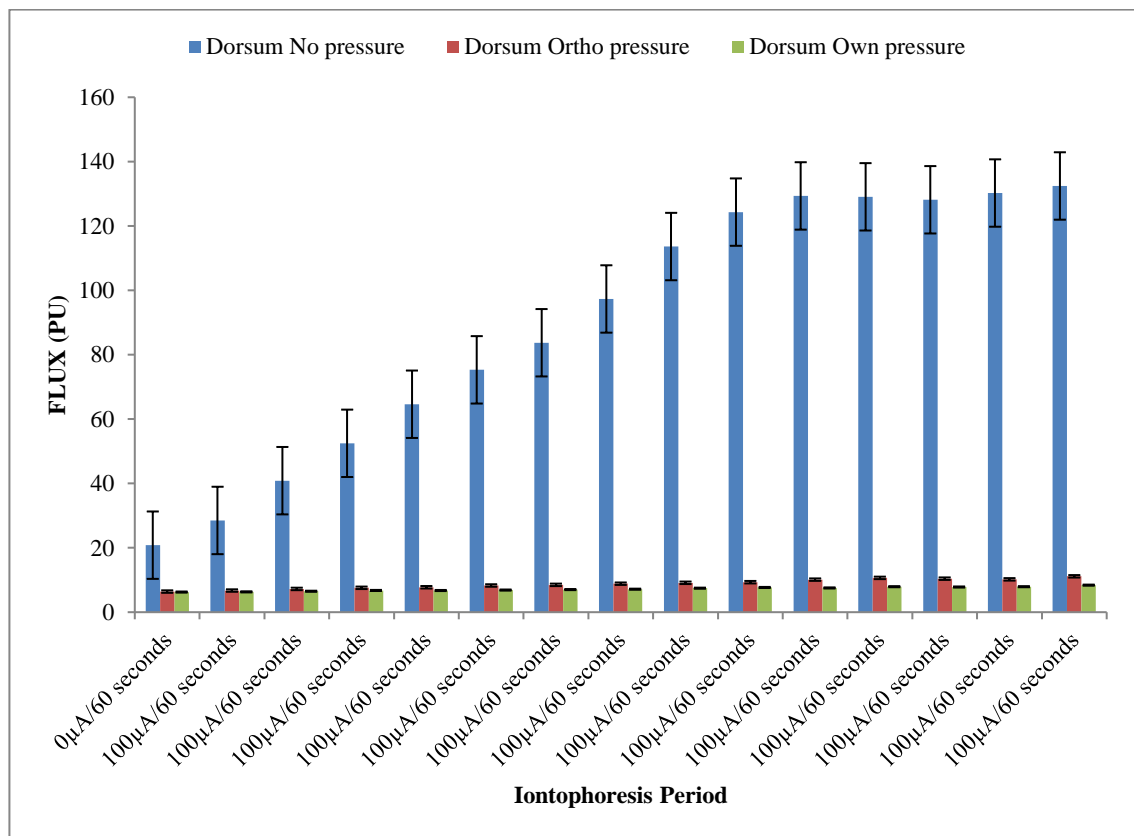


Figure 4.47 Means of flux median under the three tested pressure conditions in response to the iontophoresis of ACh on the dorsal surface in control group

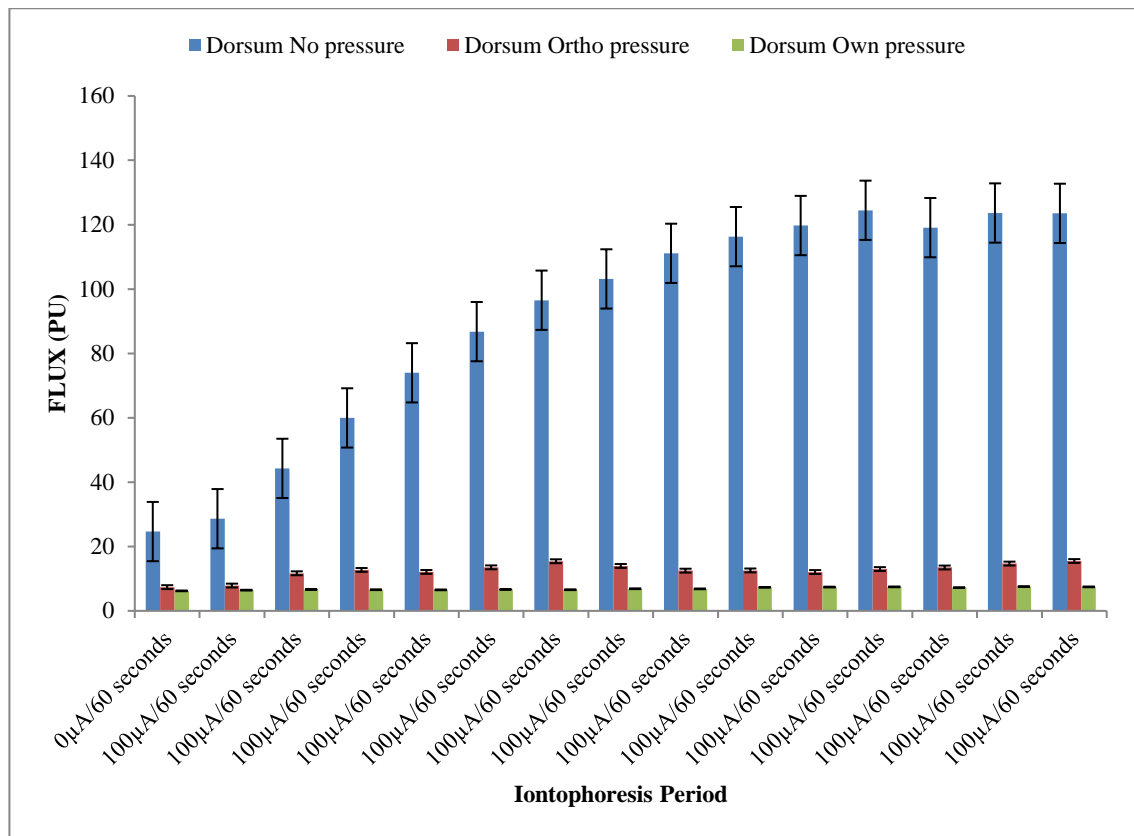


Figure 4.48 Means of flux median under the three tested pressure conditions in response to the iontophoresis of SNP on the dorsal surface in control group

Table 4.4 Comparison between the three tested pressure conditions changes in flux in response to the iontophoresis of ACh on the dorsal surface in control group using: Kruskal Wallis test

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
Peak of Maximum response (PU)	Median	137.3	17.5a	14.3a	<0.001**
	IQR	38.0	7.9	3.5	
Change in response % (Using Flux Median)	Median	725.81	184.32a	143.66ab	<0.001**
	IQR	448.36	76.88	64.19	
Change in response % (Using Flux Mean)	Median	682.84	165.96a	130.83ab	<0.001**
	IQR	414.58	72.72	58.58	

p >0.05 NS; *p <0.05 S; **p <0.001 HS. IQR: interquartile range

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

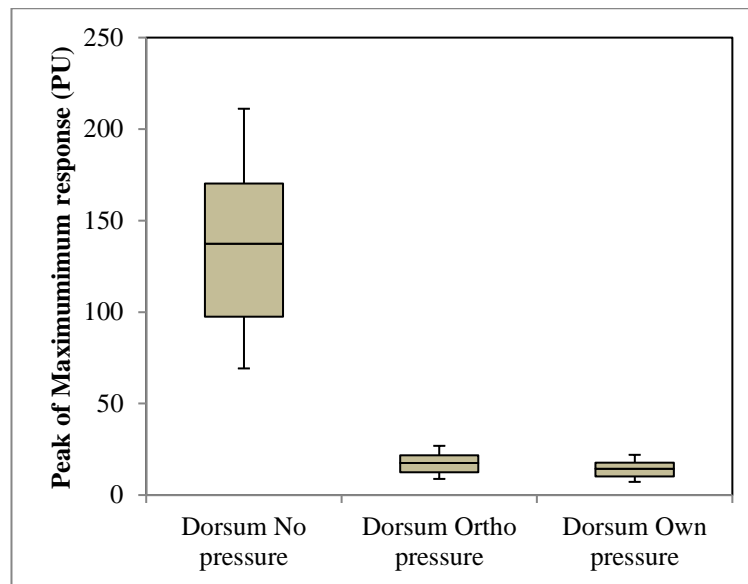


Figure 4.49 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of ACh on the dorsal surface in control group

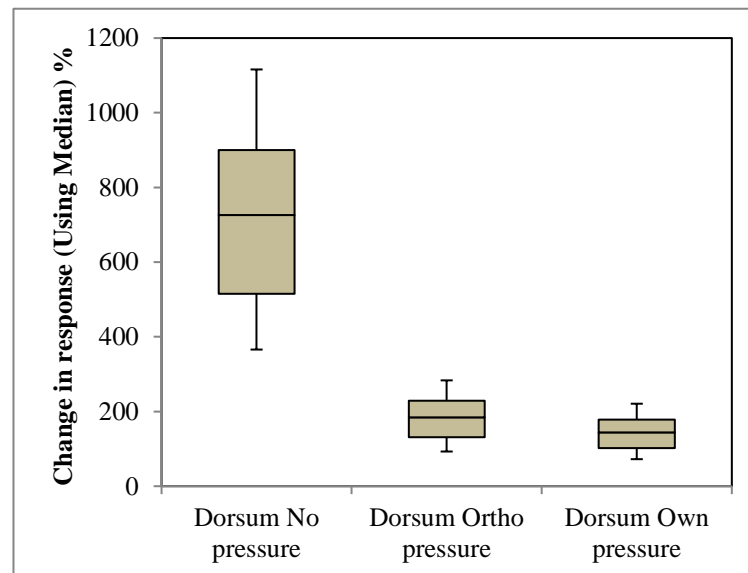


Figure 4.50 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of ACh on the dorsal surface in control group

Table 4.5 Comparison between the three tested pressure conditions changes in flux in response to the iontophoresis of SNP on the dorsal surface in control group using: Kruskal Wallis test

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
Peak of Maximum response (PU)	Median	135.7	23.0a	14.1a	<0.001**
	IQR	54.2	26.7	5.6	
Change in response % (Using Flux Median)	Median	497.15	191.68a	133.65ab	<0.001**
	IQR	237.84	119.32	49.79	
Change in response % (Using Flux Mean)	Median	482.65	167.99a	119.89ab	<0.001**
	IQR	234.56	114.29	42.17	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

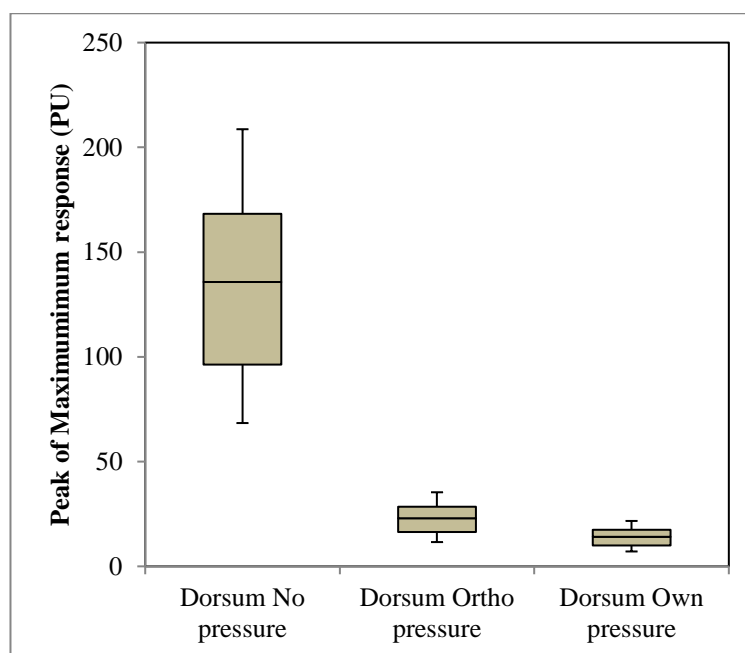


Figure 4.51 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of SNP on the dorsal surface in control group

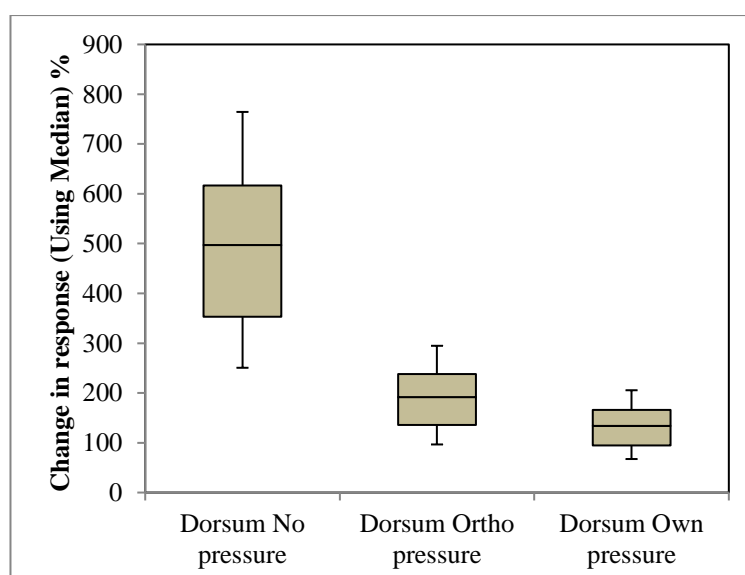


Figure 4.52 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of SNP on the dorsal surface in control group

Plantar surface showed the same relationship between the three tested conditions as on the dorsal surface. Significant reduction in blood flow ($p < 0.001$) was noted from no pressure/resting condition in all periods of the iontophoresis protocol as well as Peak of Maximum response in response to the iontophoresis of both the vasoactive drugs under own shoes' PP and orthopaedic shoes' PP. However, the higher changes in response under the orthopaedic shoes' pressures were not significantly different from those under own shoe pressure in response to the iontophoresis of ACh nor SNP.

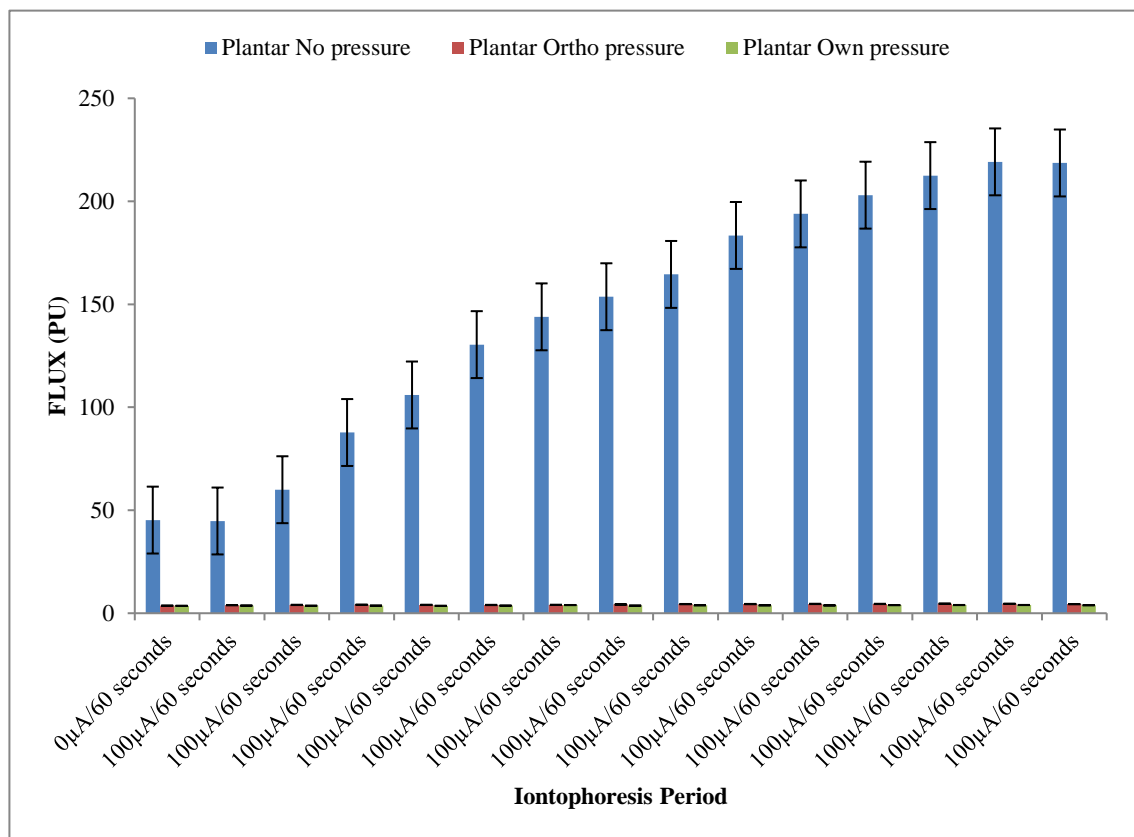


Figure 4.53 Means of flux median under the three tested pressure conditions in response to the iontophoresis of ACh on the plantar surface in control group

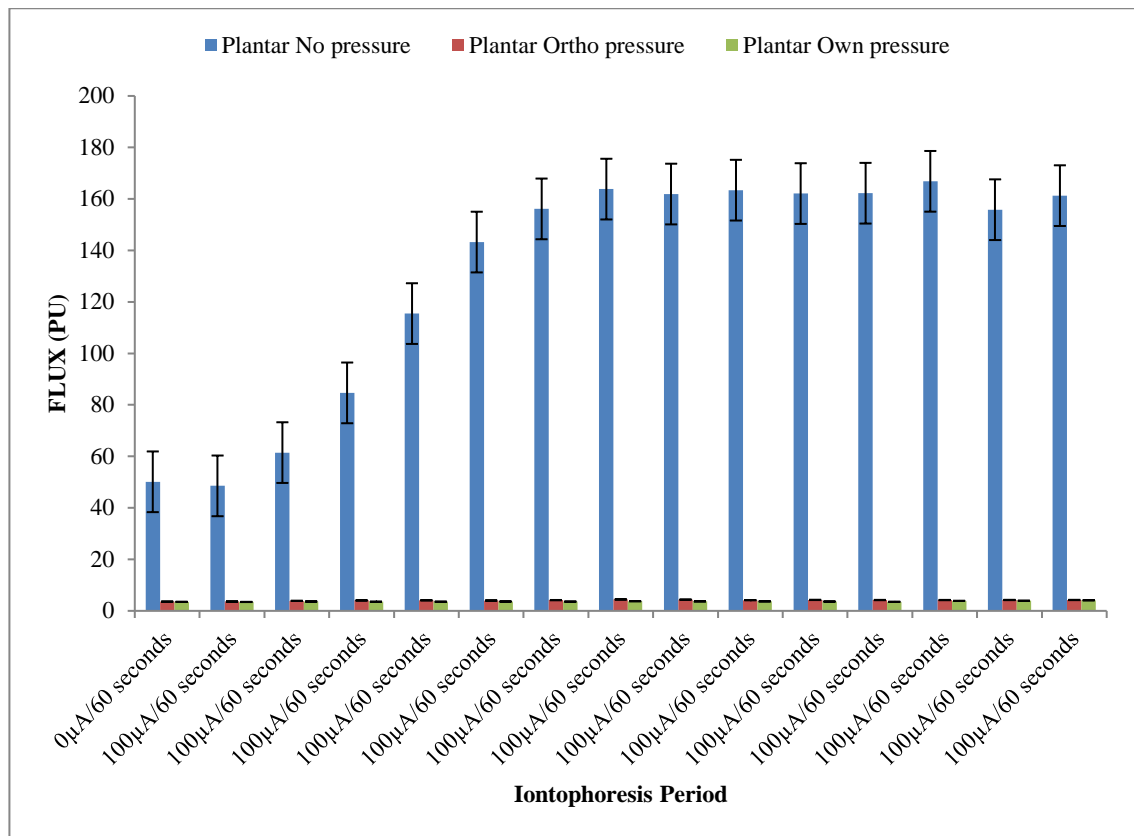


Figure 4.54 Means of flux median under the three tested pressure conditions in response to the iontophoresis of SNP on the plantar surface in control group

Table 4.6 Comparison between the three tested pressure conditions changes in flux during the iontophoresis of ACh on the plantar surface in control group using: Kruskal Wallis test

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
Peak of Maximum response (PU)	Median	214.5	8.6a	7.8a	<0.001**
	IQR	135.3	4.0	3.3	
Change in response % (Using Flux Median)	Median	471.80	140.35a	120.80a	<0.001**
	IQR	341.59	61.32	67.34	
Change in response % (Using Flux Mean)	Median	442.23	125.68a	111.71a	<0.001**
	IQR	330.58	51.66	61.98	

p > 0.05 NS; *p < 0.05 S; **p < 0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

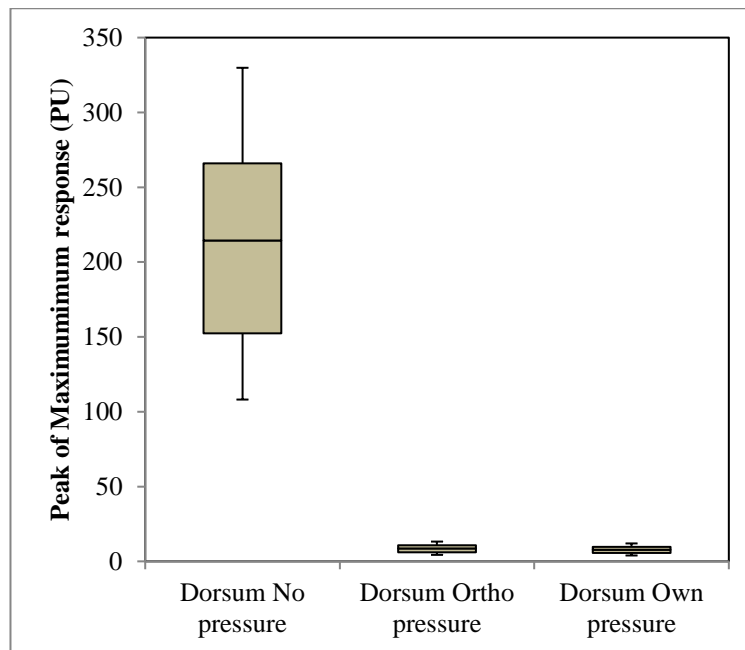


Figure 4.55 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of ACh on the plantar surface in control group

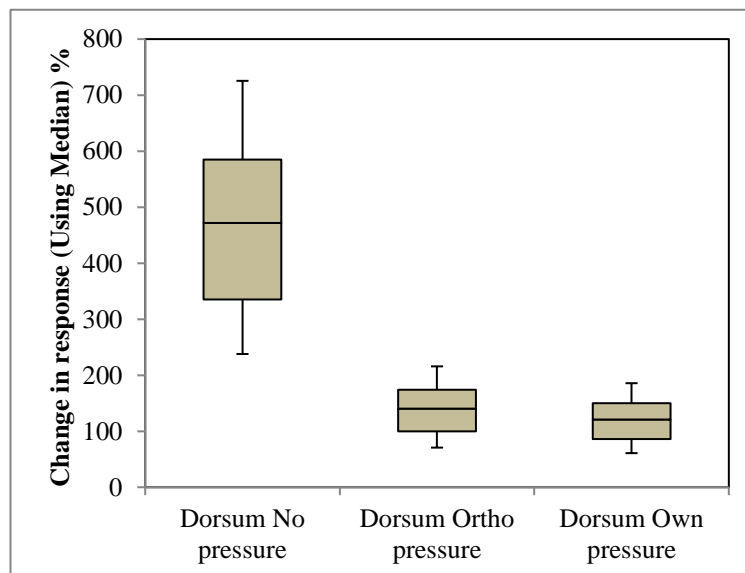


Figure 4.56 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of ACh on the plantar surface in control group

Table 4.7 Comparison between the three tested pressure conditions changes in flux during the iontophoresis of SNP on the plantar surface in control group using: Kruskal Wallis test

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
Peak of Maximum response (PU)	Median	195.0	7.7a	7.5a	<0.001**
	IQR	132.7	3.4	3.8	
Change in response % (Using Flux Median)	Median	619.20	123.24a	113.57a	<0.001**
	IQR	693.98	83.48	56.70	
Change in response % (Using Flux Mean)	Median	580.73	113.08a	99.44a	<0.001**
	IQR	657.85	78.95	40.63	

p > 0.05 NS; *p < 0.05 S; **p < 0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

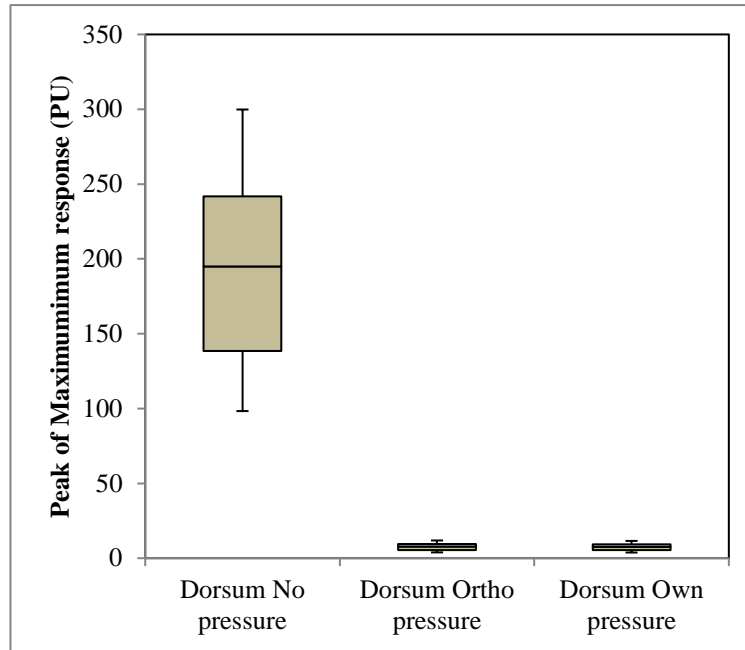


Figure 4.57 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of SNP on the plantar surface in control group

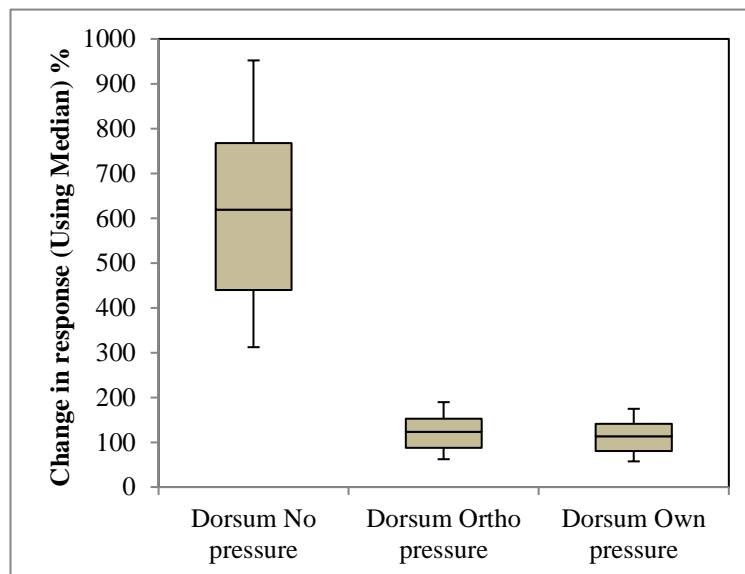


Figure 4.58 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of SNP on the plantar surface in control group

4.3.1.2 Blood Flow Changes in Diabetes Group

ACh iontophoresis showed significant differences ($p < 0.001$) between the no pressure condition and orthopaedic shoes as well as own shoes' pressure conditions in all flux parameters on both dorsal and plantar surfaces of the foot. However, no significant

differences were detected between the own shoes and orthopaedic shoes' pressure conditions in any of flux values, Peak of Maximum response or Change in response.

Alternatively, SNP iontophoresis revealed significant differences between the two tested shoes' pressures application in the change in response values on both dorsal and plantar surfaces ($p < 0.001$). Though, it maintained the same no significant difference relationship between the two shoes' pressures application as in response to ACh in flux values through the iontophoresis protocol and Peak of Maximum response as well as the significant differences detected between the no pressure condition and each shoes' pressure application.

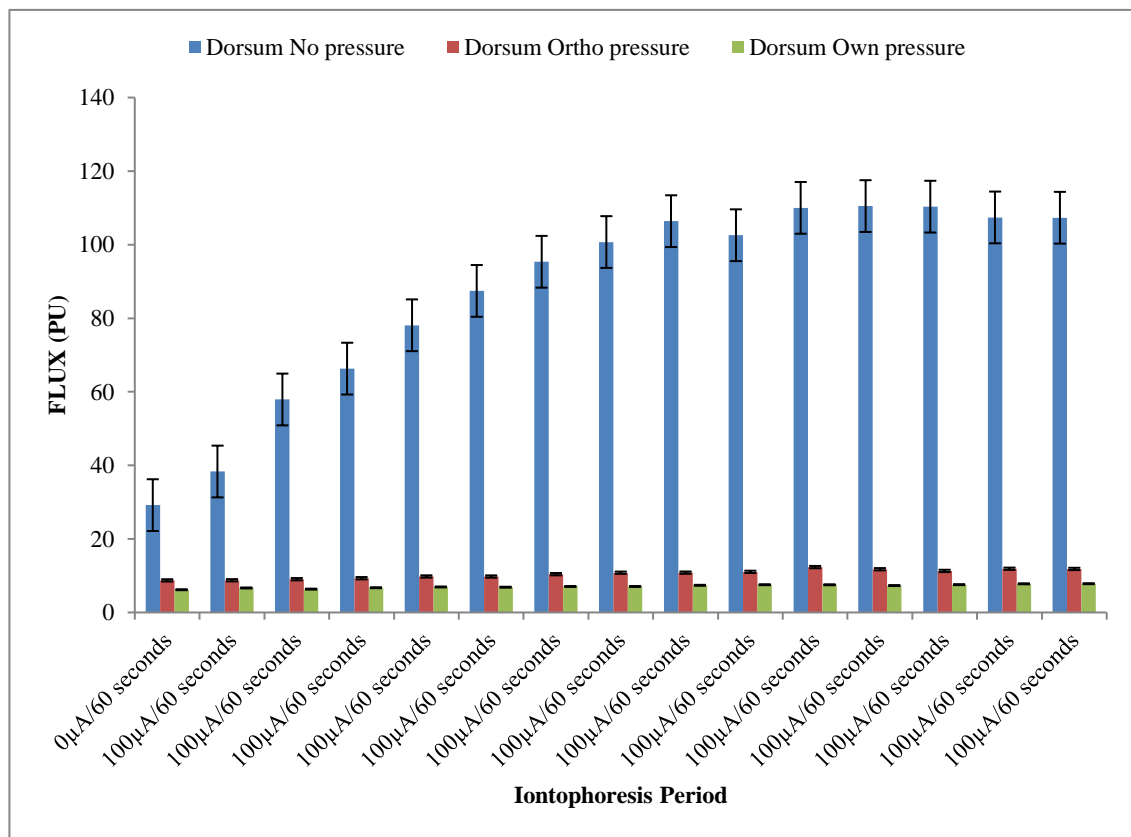


Figure 4.59 Means of flux median under the three tested pressure conditions in response to the iontophoresis of ACh on the dorsal surface in diabetes group

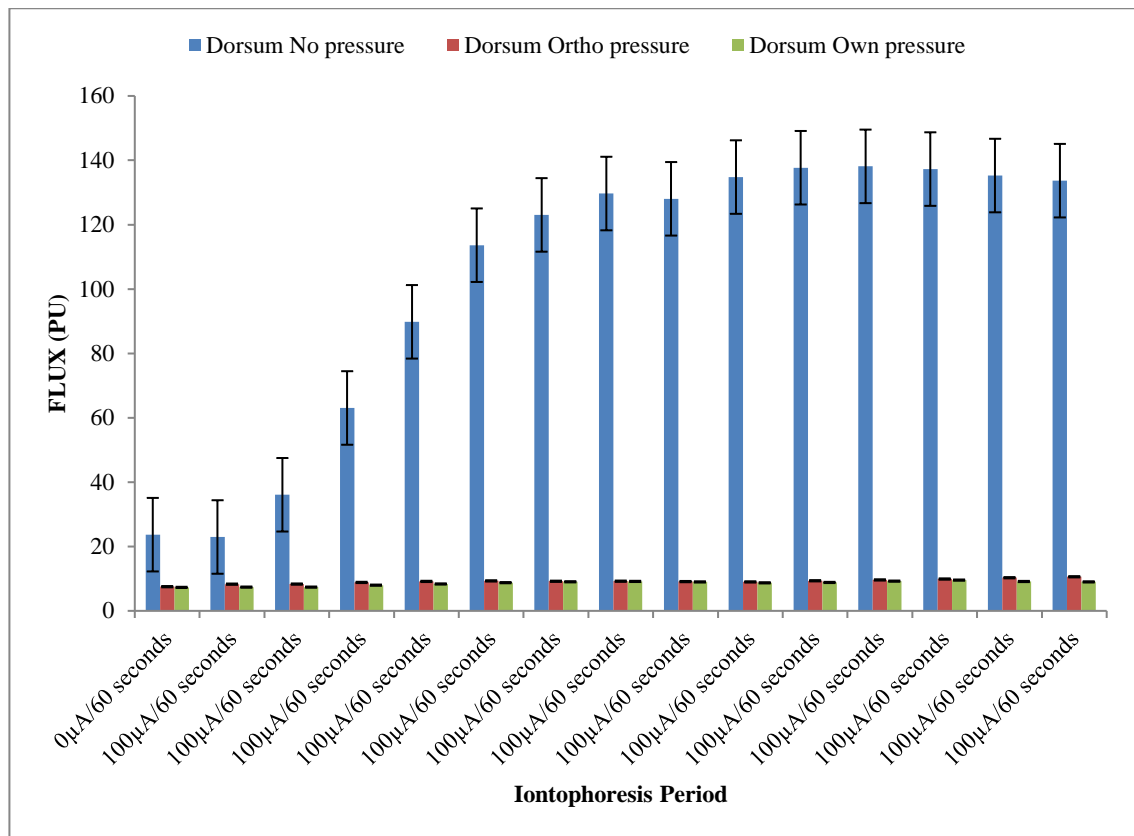


Figure 4.60 Means of flux median under the three tested pressure conditions in response to the iontophoresis of SNP on the dorsal surface in diabetes group

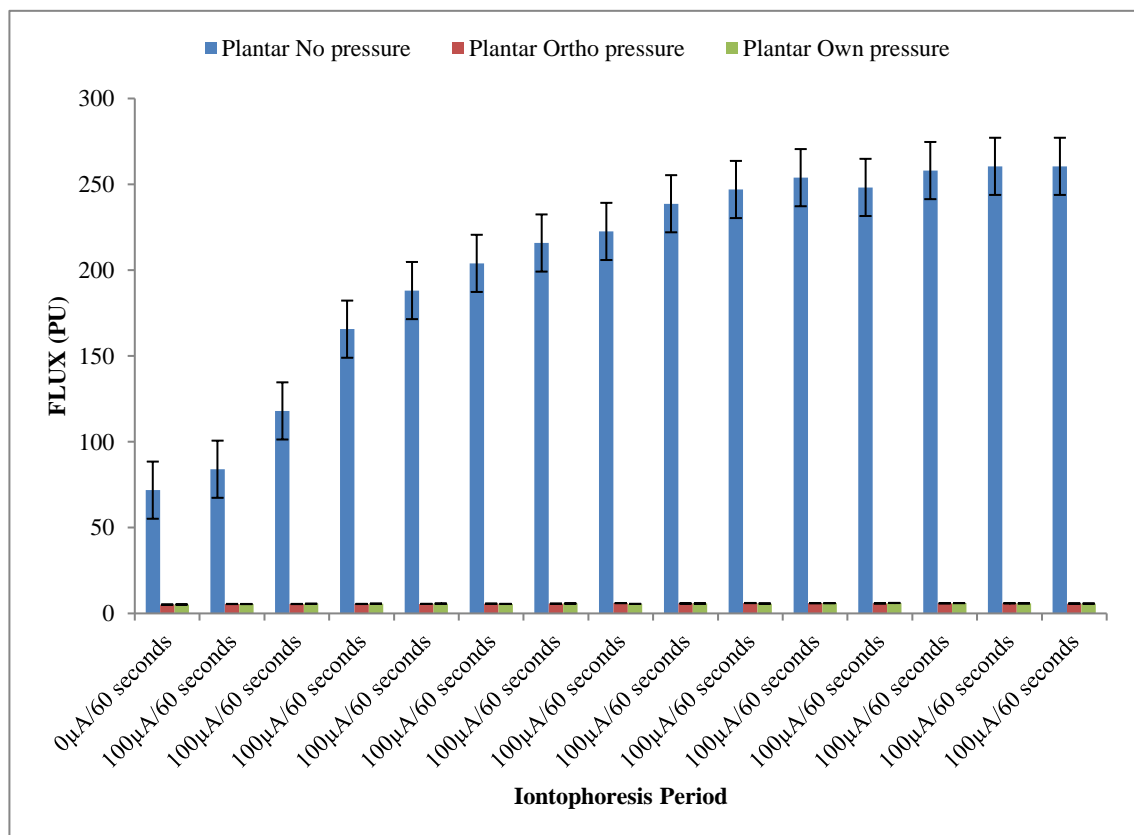


Figure 4.61 Means of flux median under the three tested pressure conditions in response to the iontophoresis of ACh on the plantar surface in diabetes group

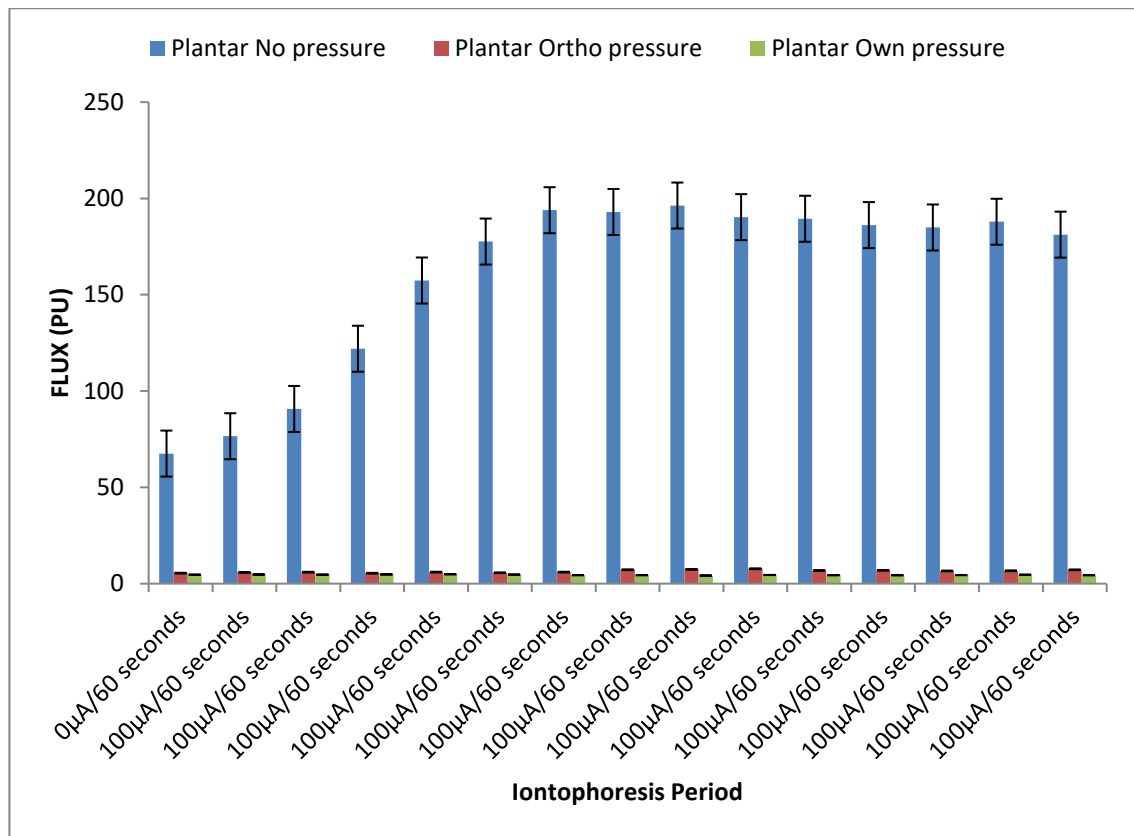


Figure 4.62 Means of flux median under the three tested pressure conditions in response to the iontophoresis of SNP on the plantar surface in diabetes group

Table 4.8 Comparison between the three tested pressure conditions changes in flux during the iontophoresis of ACh on the dorsal surface in diabetes group using: Kruskal Wallis test

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
Peak of Maximum response (PU)	Median	135.5	19.6a	13.9a	<0.001**
	IQR	82.5	7.7	5.1	
Change in response % (Using Flux Median)	Median	432.46	138.91a	130.57a	<0.001**
	IQR	306.74	74.53	52.89	
Change in response % Using Flux (Mean)	Median	416.30	128.38a	120.62a	<0.001**
	IQR	295.99	66.41	45.79	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

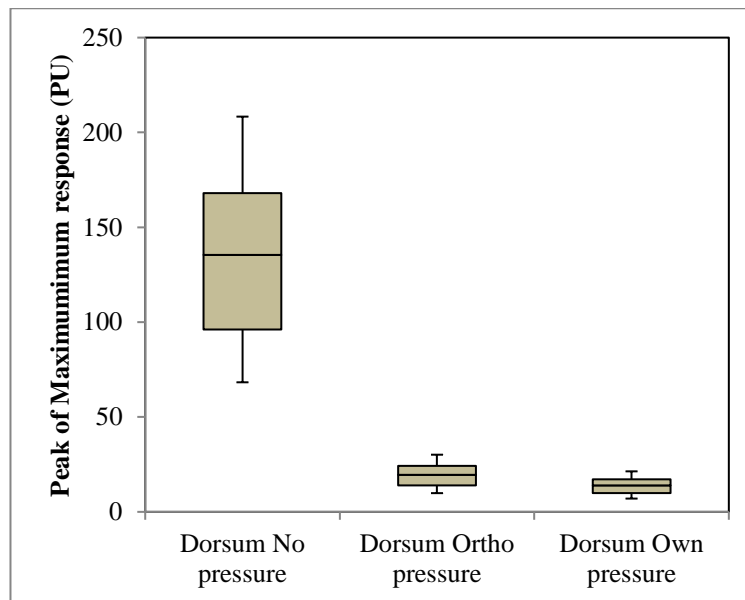


Figure 4.63 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group

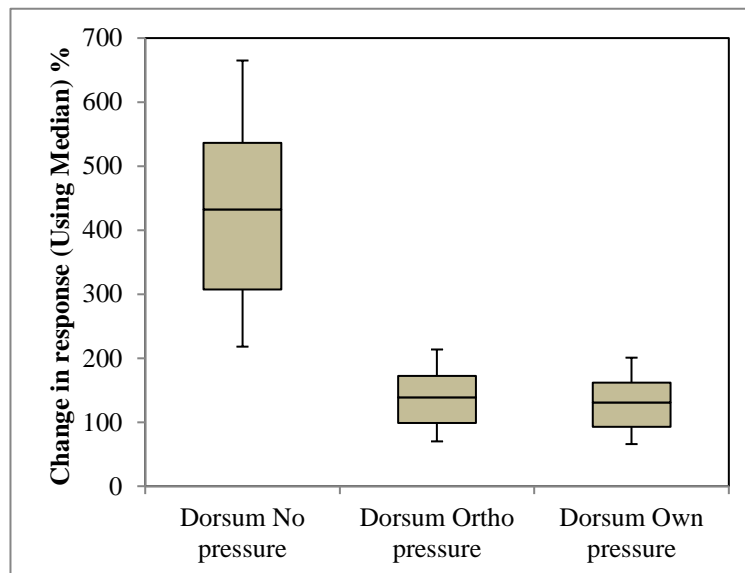


Figure 4.64 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of ACh on the dorsal surface in diabetes group

Table 4.9 Comparison between the three tested pressure conditions changes in flux during the iontophoresis of SNP on the dorsal surface in diabetes group using: Kruskal Wallis test

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
Peak of Maximum response (PU)	Median	158.2	17.8a	17.9a	<0.001**
	IQR	93.6	9.1	7.6	
Change in response % (Using Flux Median)	Median	646.21	139.55a	164.57ab	<0.001**
	IQR	450.85	53.12	81.43	
Change in response % (Using Flux Mean)	Median	618.07	122.62a	149.86ab	<0.001**
	IQR	430.25	43.33	73.40	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

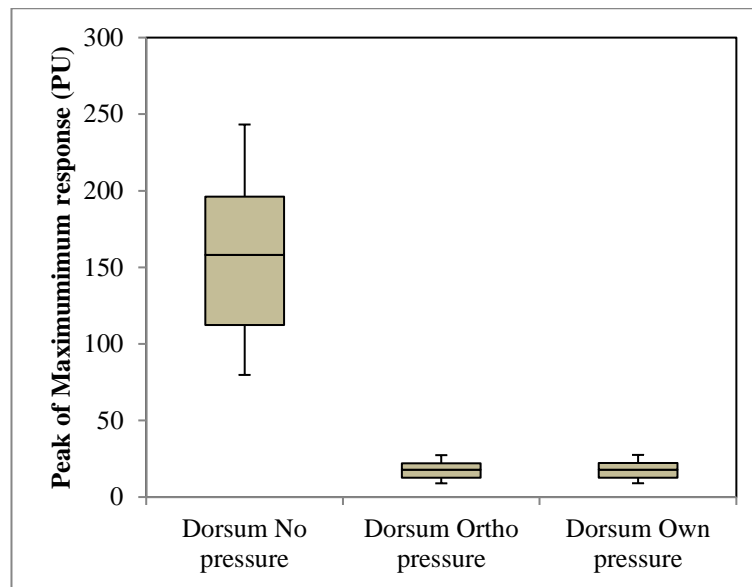


Figure 4.65 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group

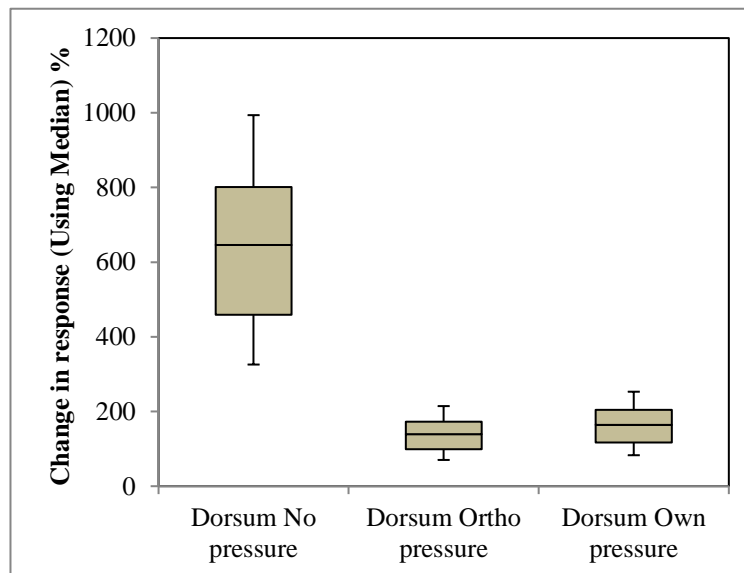


Figure 4.66 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of SNP on the dorsal surface in diabetes group

Table 4.10 Comparison between the three tested pressure conditions changes in flux during the iontophoresis of ACh on the plantar surface in diabetes group using: Kruskal Wallis test

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
Peak of Maximum response (PU)	Median	302.3	11.0a	11.1a	<0.001**
	IQR	160.5	4.3	4.0	
Change in response % (Using Flux Median)	Median	521.80	119.06a	125.08a	<0.001**
	IQR	562.09	51.10	58.00	
Change in response % (Using Flux Mean)	Median	495.48	107.53a	111.98a	<0.001**
	IQR	541.38	40.32	47.25	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

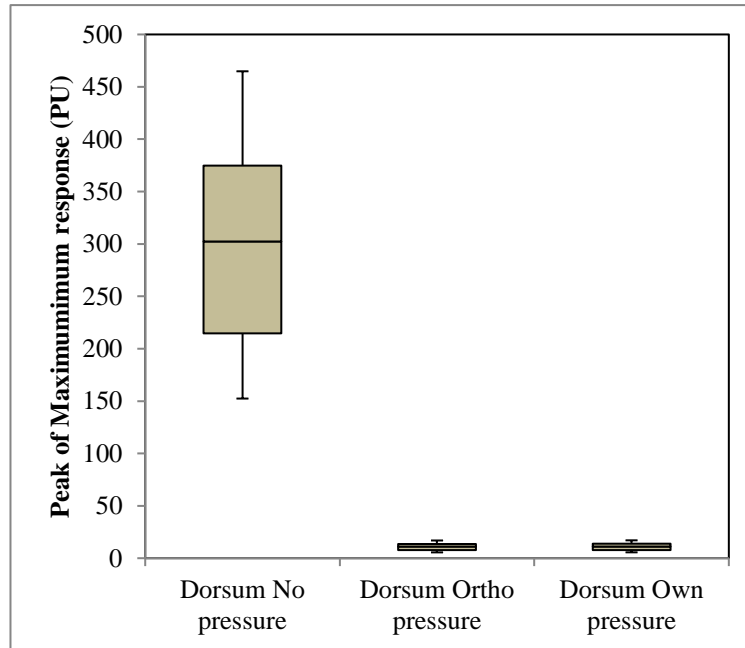


Figure 4.67 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of ACh on the plantar surface in diabetes group

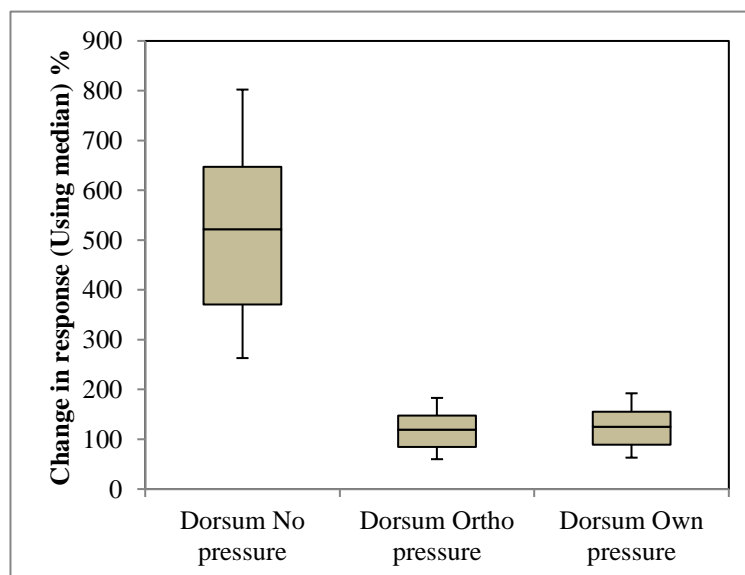


Figure 4.68 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of ACh on the plantar surface in diabetes group

Table 4.11 Comparison between the three tested pressure conditions changes in flux during the iontophoresis of SNP on the plantar surface in diabetes group using: Kruskal Wallis test

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
Peak of Maximum response (PU)	Median	237.8	14.3a	9.2a	<0.001**
	IQR	107.7	19.0	3.5	
Change in response % (Using Flux Median)	Median	319.17	136.23a	106.81ab	<0.001**
	IQR	140.20	85.07	45.60	
Change in response % (Using Flux Mean)	Median	297.90	119.60a	93.05ab	<0.001**
	IQR	134.05	65.19	37.43	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

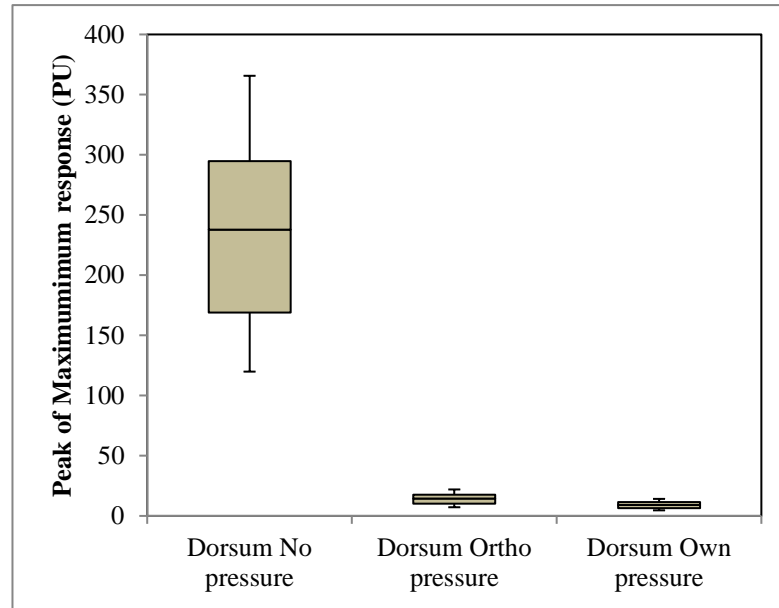


Figure 4.69 Comparison between the three tested pressure conditions' Peak of Maximum response (PU) during the iontophoresis of SNP on the plantar surface in diabetes group

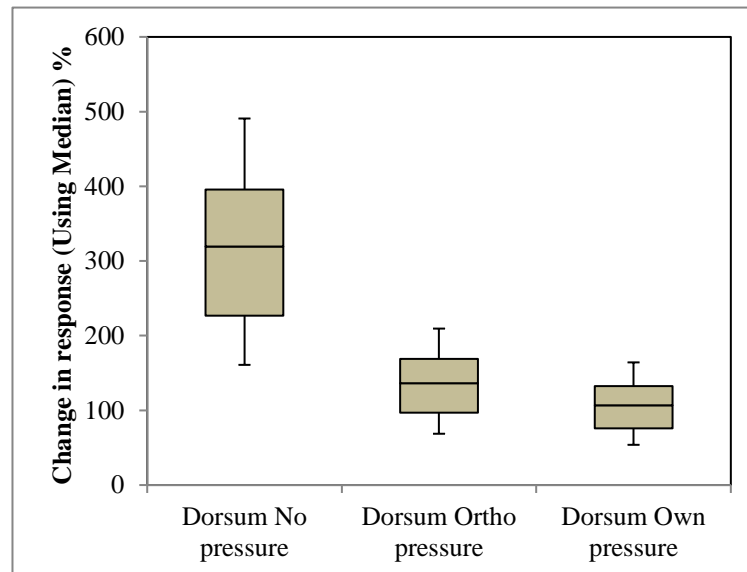


Figure 4.70 Comparison between the three tested pressure conditions' Change in response % (Using Flux Median) during the iontophoresis of SNP on the plantar surface in diabetes group

4.3.2 Comparison Between Control Group and Diabetes Group Flux Values

4.3.2.1 Changes on the Dorsal Surface

Blood flux in response to the iontophoresis of ACh under no pressure applied on the dorsum of the foot showed no significant differences between the two study groups in all iontophoresis periods and Peak of Maximum response. Although a significantly higher change from baseline ($p=0.037$) recorded in the control group (Flux median Change in response %; Mean \pm SD 756.05 \pm 467.04%) when compared to change in response in diabetes group (Flux median Change in response %; Mean \pm SD 450.48 \pm 319.52%). Iontophoresis of SNP under no pressure conditions on the foot dorsum showed no significant differences between study groups in all flux parameters including change in response from baseline blood flow.

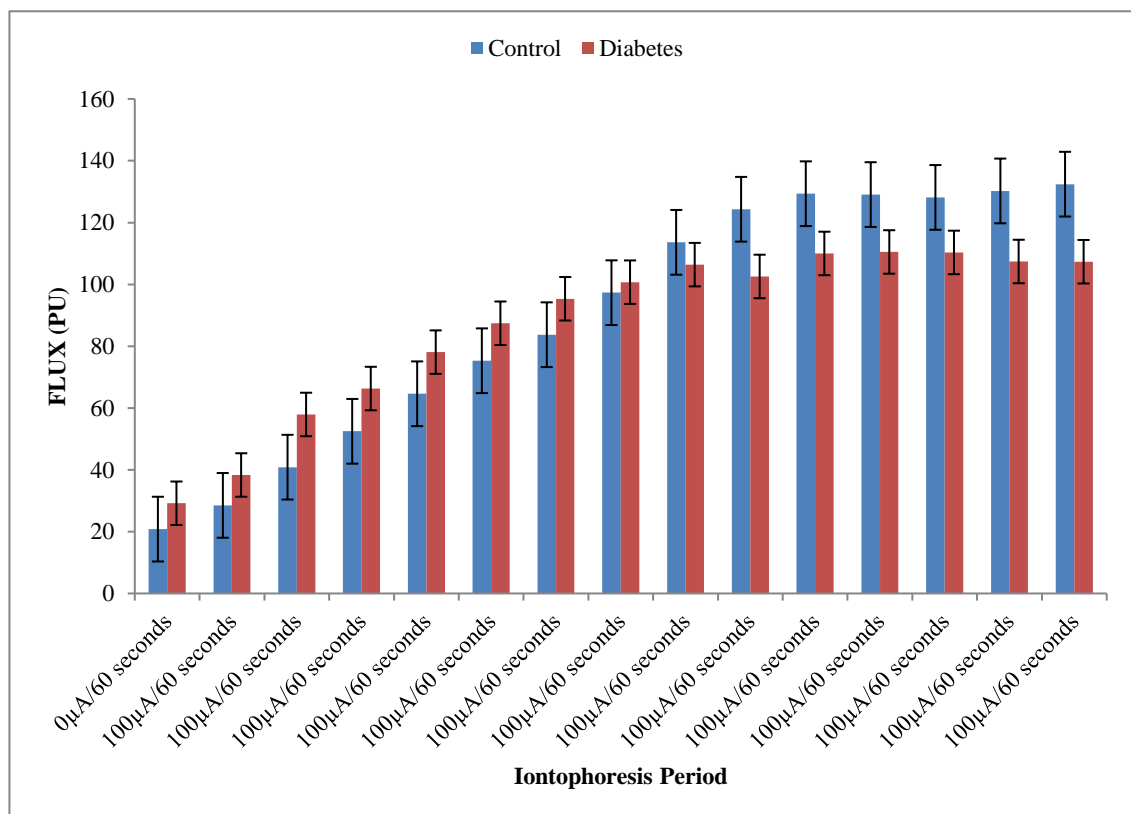


Figure 4.71 Control group and diabetes group means of flux median on the dorsum of the foot with no pressure applied during the iontophoresis of ACh

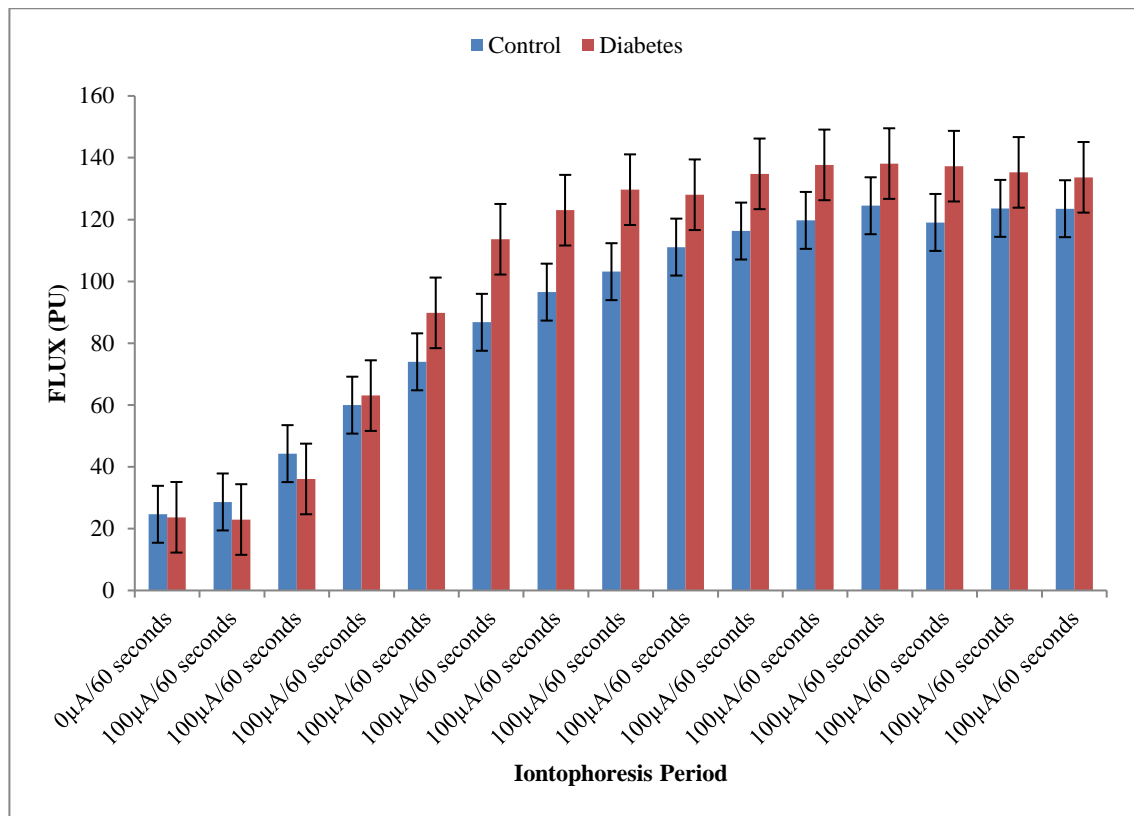


Figure 4.72 Control group and diabetes group means of flux median on the dorsum of the foot with no pressure applied during the iontophoresis of SNP

Table 4.12 Comparison between control group and diabetes group blood flux changes on the dorsum of the foot with no pressure applied during the iontophoresis of ACh using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	137.3	38.0	74.5	233.47	0.903
	Diabetes	135.5	82.5	40.1	369.01	
Change in response % (Using Flux Median)	Control	725.81	448.36	238.36	1905.94	0.041*
	Diabetes	432.46	306.74	104.75	1113.00	
Change in response % (Using Flux Mean)	Control	682.84	414.58	228.69	1771.97	0.047*
	Diabetes	416.30	295.99	102.61	1065.32	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

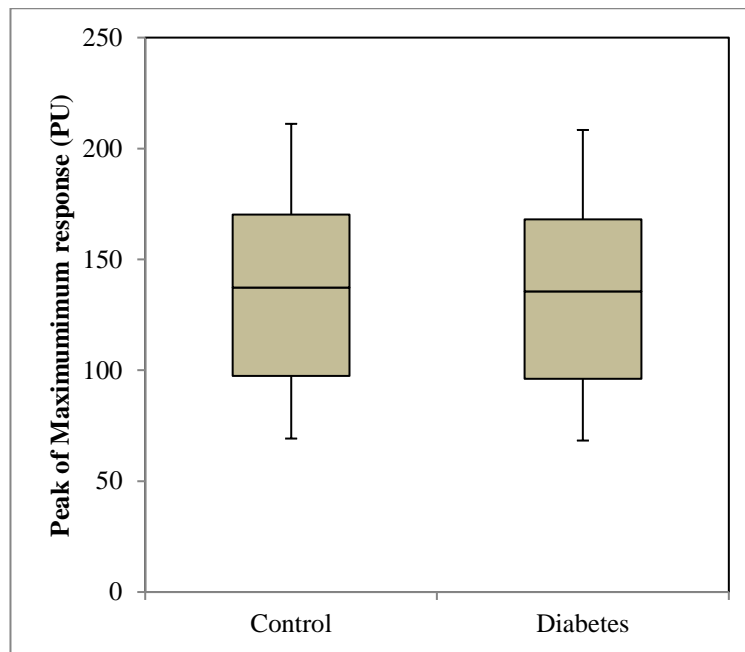


Figure 4.73 Comparison between control group and diabetes group Peak of Maximum response (PU) on the dorsum of the foot with no pressure applied during the iontophoresis of ACh

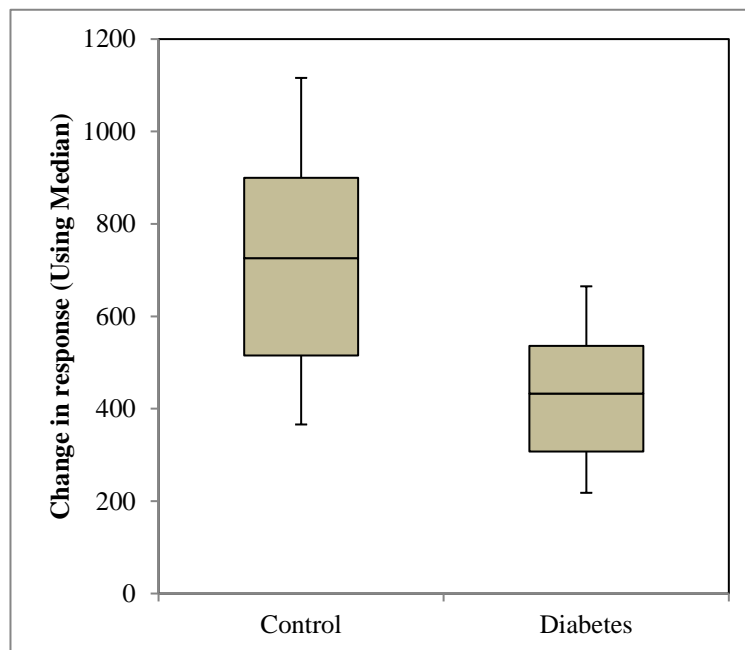


Figure 4.74 Comparison between control group and diabetes group Change in response % on the dorsum of the foot with no pressure applied during the iontophoresis of ACh

Table 4.13 Comparison between control group and diabetes group blood flux changes on the dorsum of the foot with no pressure applied during the iontophoresis of SNP using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	135.7	54.2	56.26	242.25	0.365
	Diabetes	158.2	93.6	67.04	413.13	
Change in response % (Using Flux Median)	Control	497.15	237.84	153.91	1087.50	0.280
	Diabetes	646.21	450.85	214.20	1607.15	
Change in response % (Using Flux Mean)	Control	482.65	234.56	157.04	1065.78	0.280
	Diabetes	618.07	430.25	212.22	1572.60	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

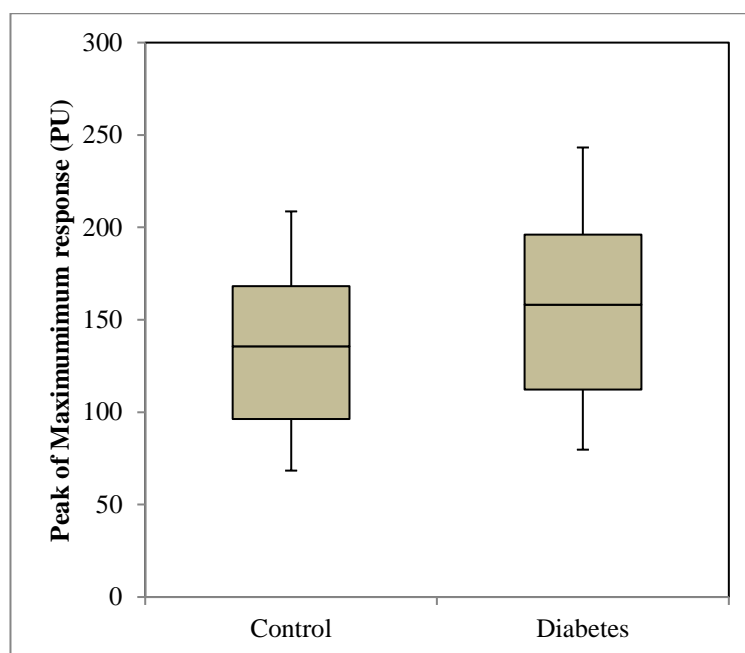


Figure 4.75 Comparison between control group and diabetes group Peak of Maximum response (PU) on the dorsum of the foot with no pressure applied during the iontophoresis of SNP

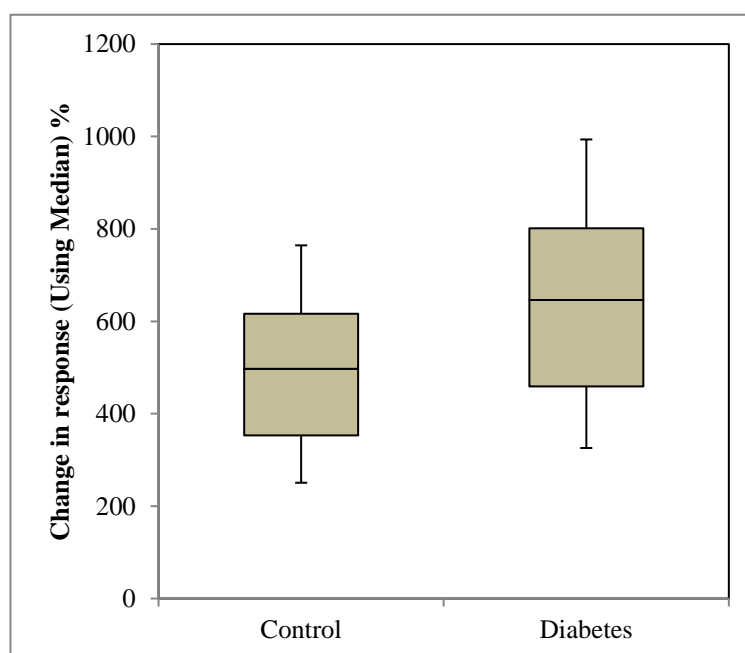


Figure 4.76 Comparison between control group and diabetes group Change in response % on the dorsum of the foot with no pressure applied during the iontophoresis of SNP

The dorsal surface showed a significant difference between groups at baseline period (0 μ A/60 seconds) when applying the orthopaedic shoes' pressure ($p=0.034$ with flux median and $p=0.045$ with flux mean). Yet only the first minute of ACh iontophoresis protocol when using flux median values maintained this significant difference ($p=0.035$). No other significant differences were then found between groups on the continuation of the iontophoresis protocol, in Peak of Maximum of response nor in the change in response percentage. The orthopaedic shoes' PP also, did not reveal any significant differences between groups in response to the SNP iontophoresis on the dorsal surface of the foot as seen with the no pressure condition.

Applying own shoes' PP on the dorsum of the foot did not exhibit any significant differences between groups in response to the iontophoresis of ACh nor SNP in any of the blood flux values, Peak of Maximum response or change in response from baseline.

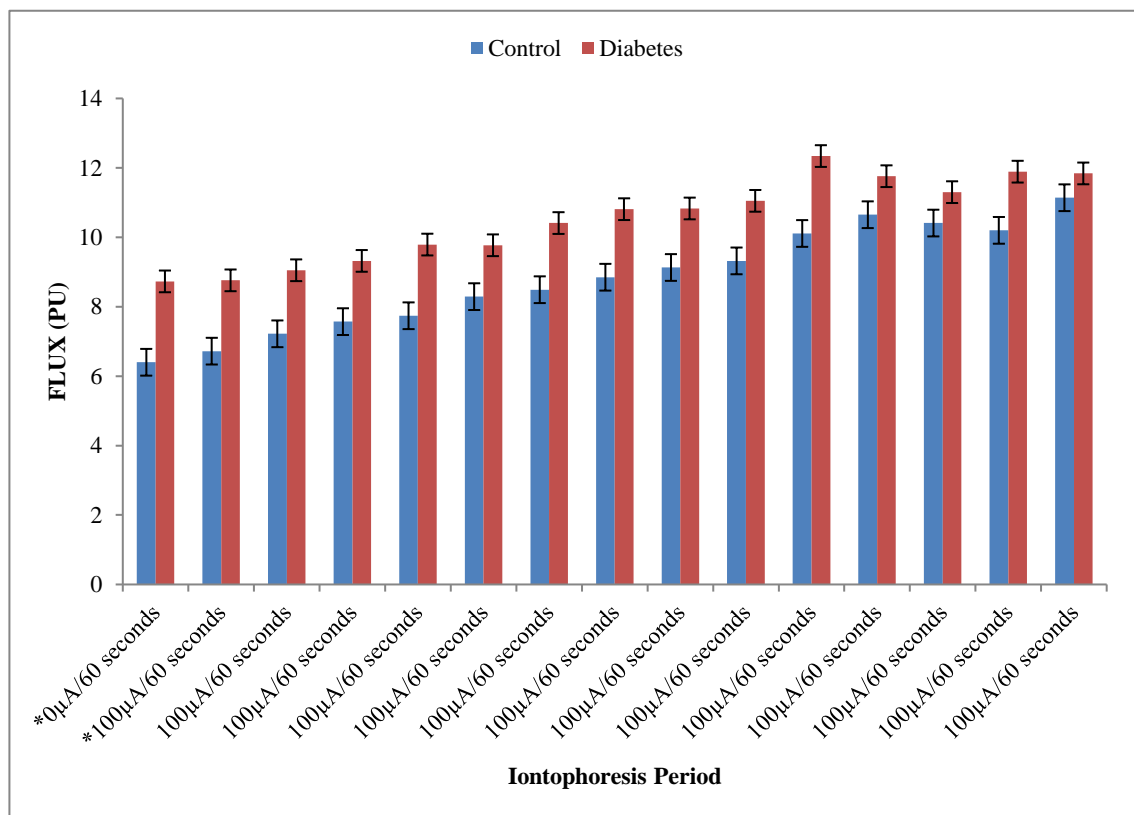


Figure 4.77 Control group and diabetes group means of flux median on the dorsum of the foot under PP in the orthopaedic shoes in response to the iontophoresis of ACh

*: $p < 0.05$ S

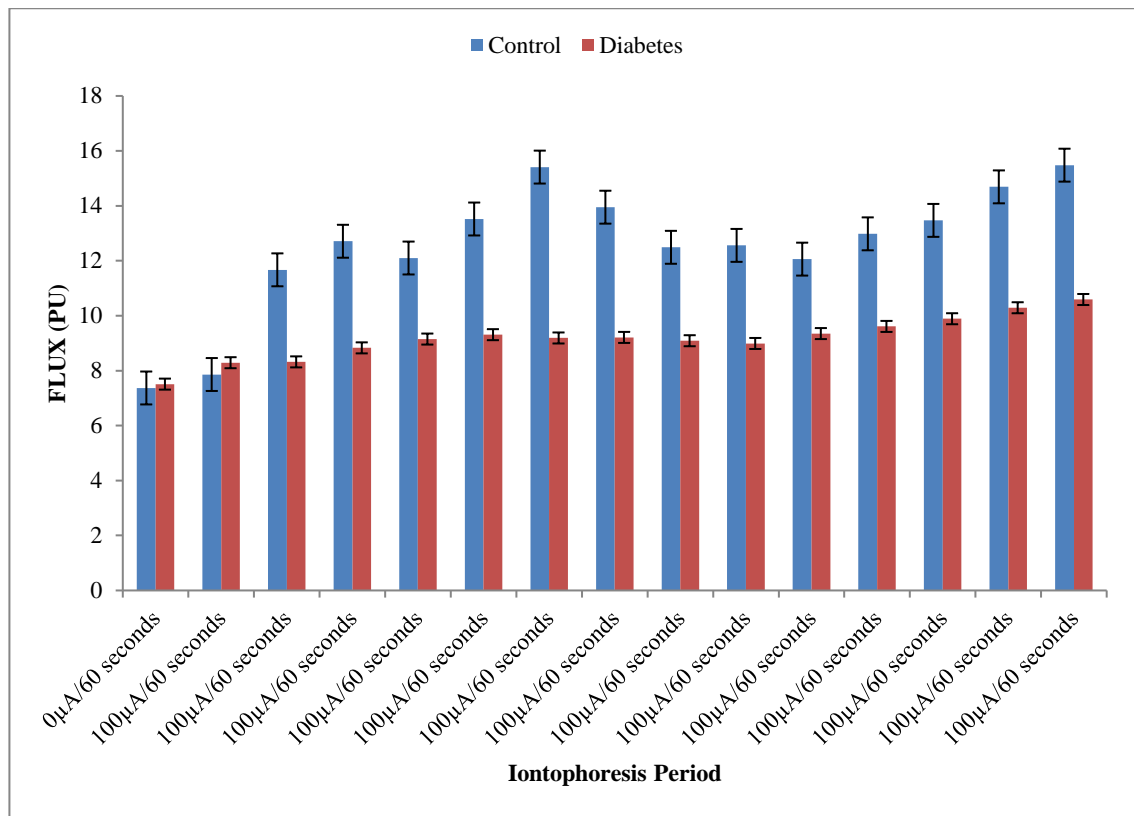


Figure 4.78 Control group and diabetes group means of flux median on the dorsum of the foot under PP in the orthopaedic shoes in response to the iontophoresis of SNP

Table 4.14 Comparison between control group and diabetes group blood flux changes on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	17.5	7.9	11.18	48.47	0.434
	Diabetes	19.6	7.7	8.39	37.53	
Change in response % (Using Flux Median)	Control	184.32	76.88	113.73	458.38	0.125
	Diabetes	138.91	74.53	40.97	307.95	
Change in response % (Using Flux Mean)	Control	165.96	72.72	104.14	446.39	0.134
	Diabetes	128.38	66.41	40.83	295.90	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

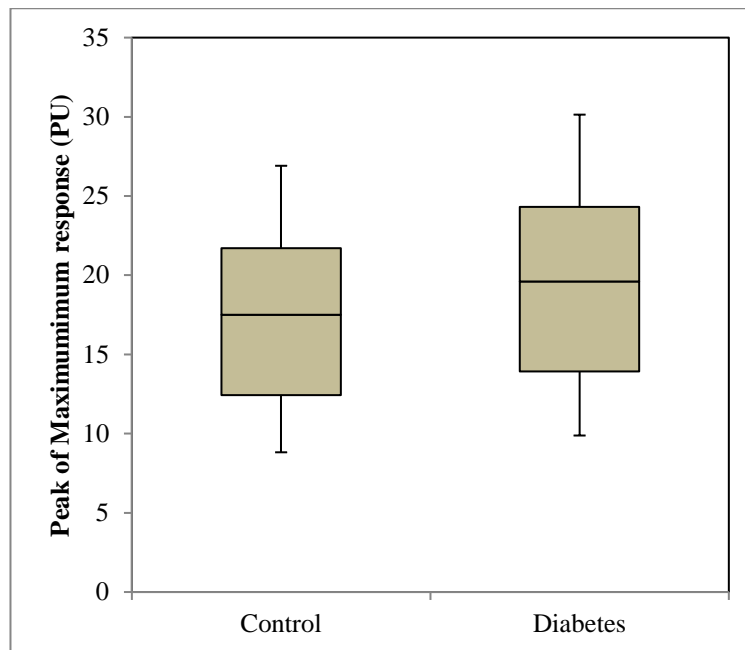


Figure 4.79 Comparison between control group and diabetes group Peak of Maximum response (PU) on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh

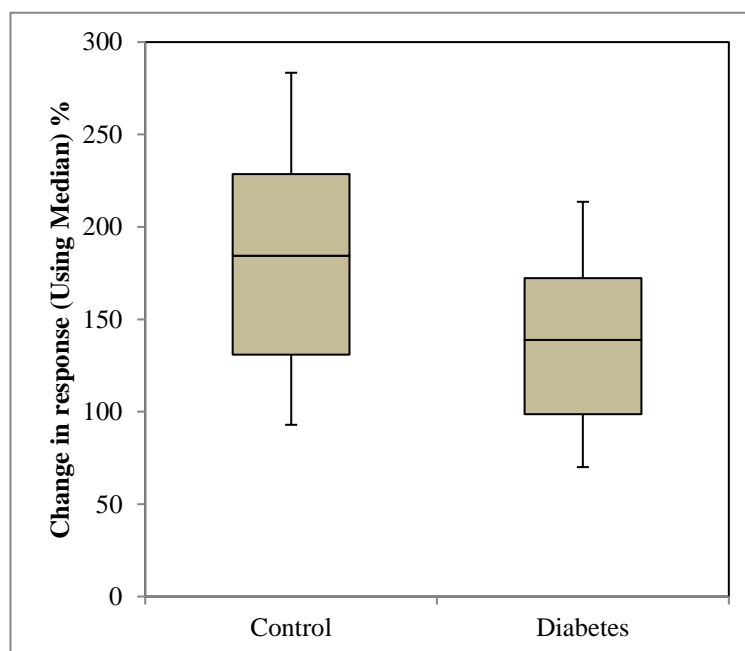


Figure 4.80 Comparison between control group and diabetes group Change in response % on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh

Table 4.15 Comparison between control group and diabetes group blood flux changes on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	23.0	26.7	6.64	137.13	0.467
	Diabetes	17.8	9.1	6.64	46.66	
Change in response % (Using Flux Median)	Control	191.68	119.32	45.29	633.29	0.111
	Diabetes	139.55	53.12	74.17	261.72	
Change in response % (Using Flux Mean)	Control	167.99	114.29	38.69	616.06	0.174
	Diabetes	122.62	43.33	74.17	216.61	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

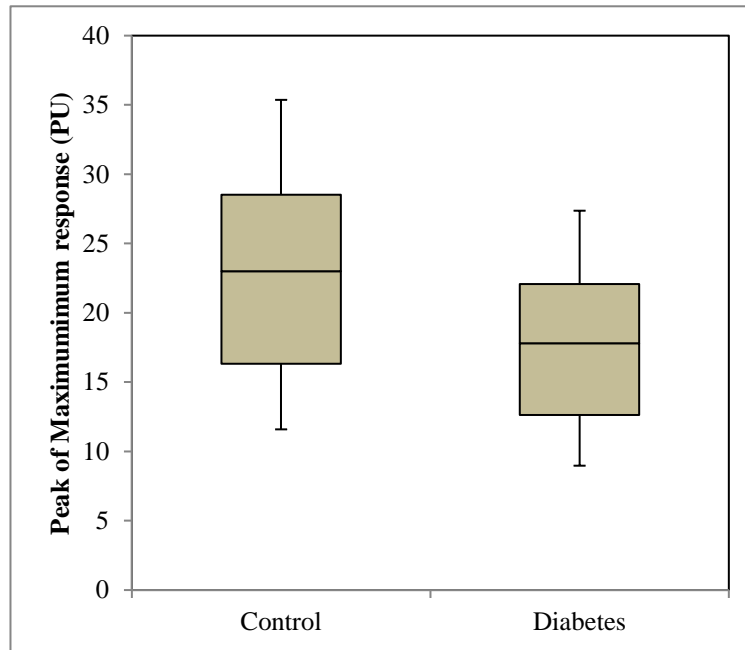


Figure 4.81 Comparison between control group and diabetes group Peak of Maximum response (PU) on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP

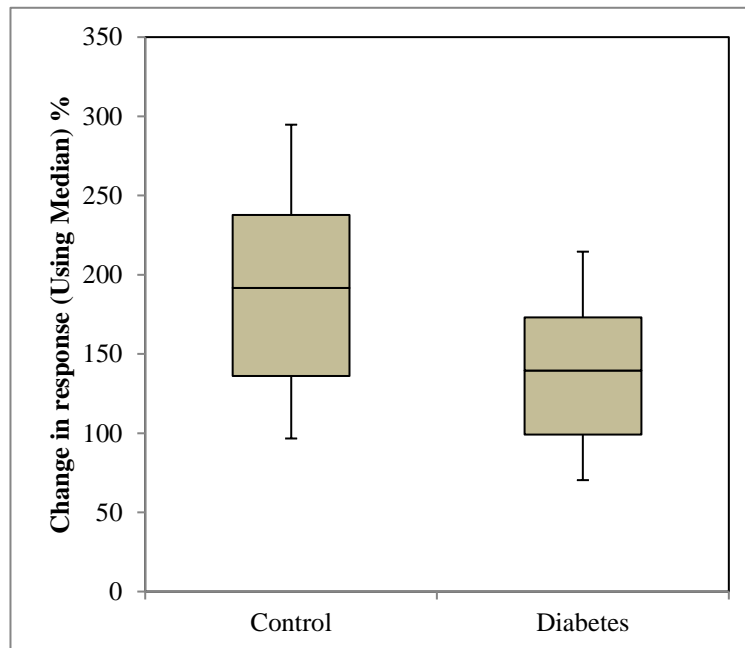


Figure 4.82 Comparison between control group and diabetes group Change in response % on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP

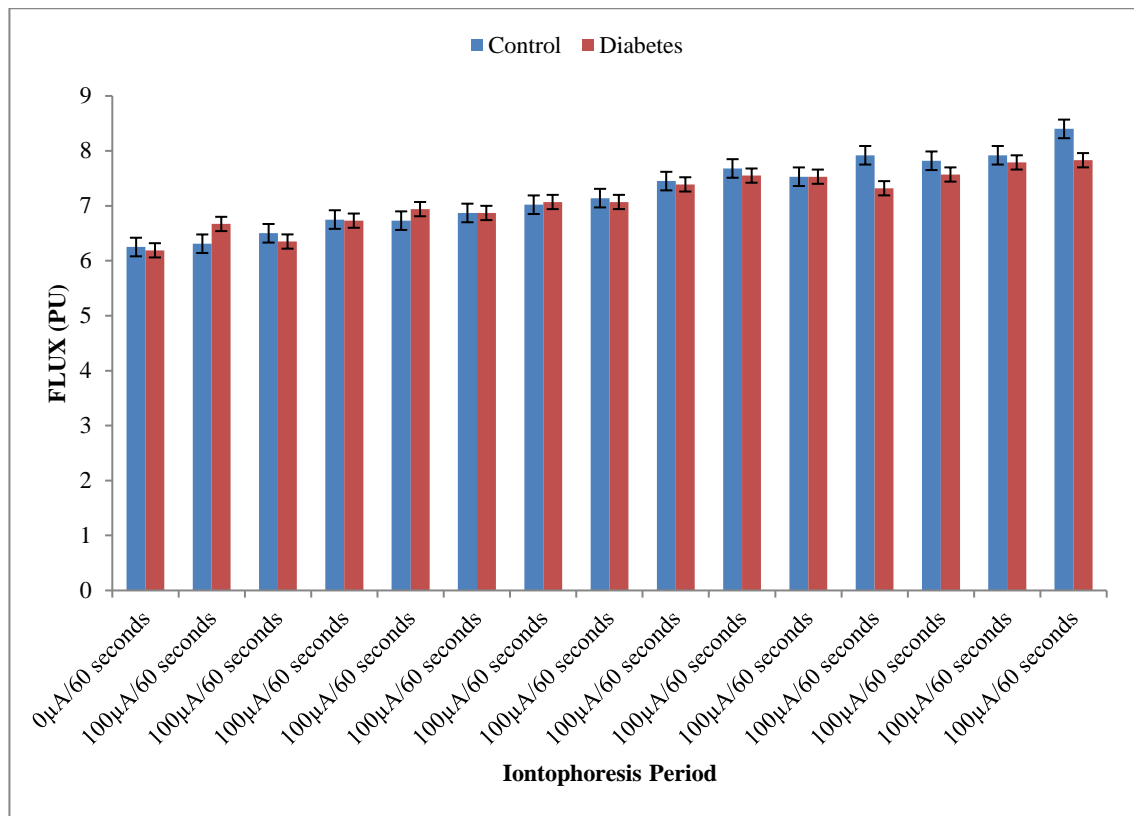


Figure 4.83 Control group and diabetes group means of flux median on the dorsum of the foot under PP in Own shoes in response to the iontophoresis of ACh

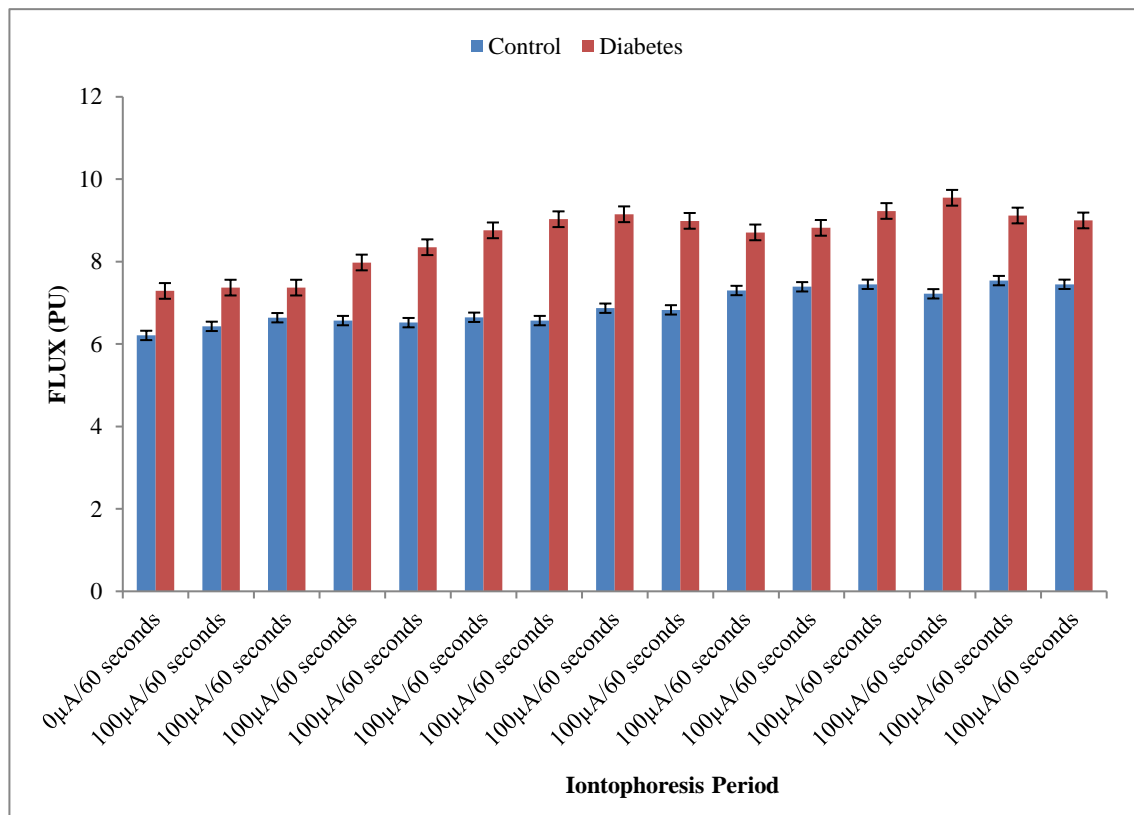


Figure 4.84 Control group and diabetes group means of flux median on the dorsum of the foot under PP in Own shoes in response to the iontophoresis of SNP

Table 4.16 Comparison between control group and diabetes group blood flux changes on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of ACh using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	14.3	3.5	8.94	23.5	0.491
	Diabetes	13.9	5.1	8.07	27.51	
Change in response % (Using Flux Median)	Control	143.66	64.19	29.87	274.80	0.588
	Diabetes	130.57	52.89	65.77	293.04	
Change in response % (Using Flux Mean)	Control	130.83	58.58	30.63	263.45	0.761
	Diabetes	120.62	45.79	64.24	255.00	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

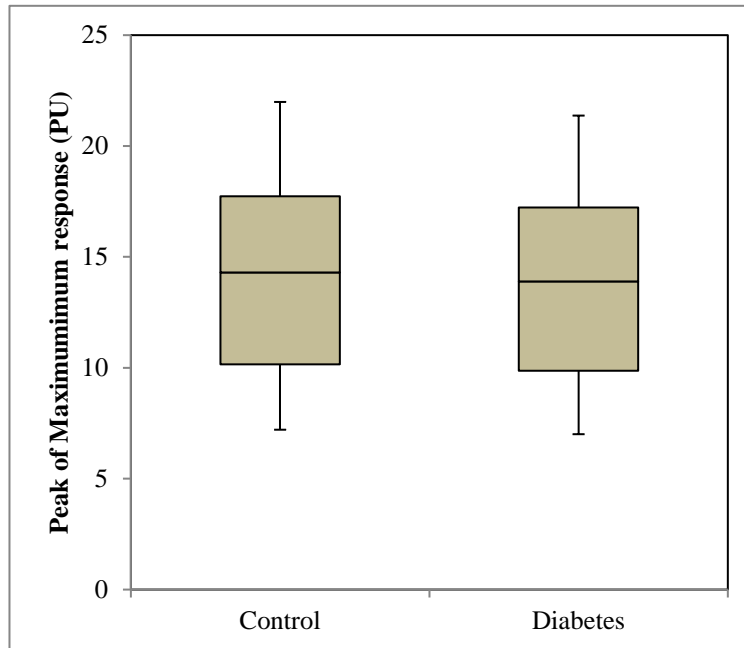


Figure 4.85 Comparison between control group and diabetes group Peak of Maximum response (PU) on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of ACh

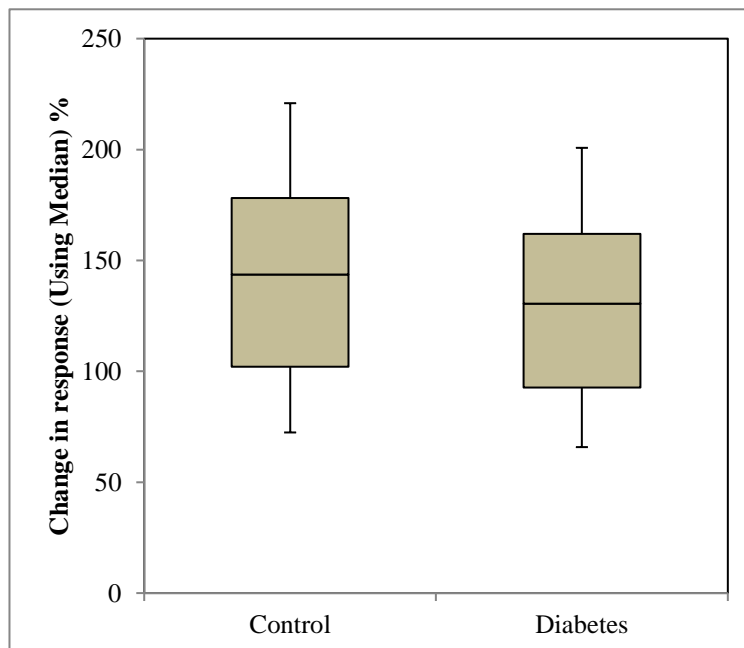


Figure 4.86 Comparison between control group and diabetes group Change in response % on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of ACh

Table 4.17 Comparison between control group and diabetes group blood flux changes on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of SNP using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	14.1	5.6	6.64	24.99	0.060
	Diabetes	17.9	7.6	9.37	36.44	
Change in response % (Using Flux Median)	Control	133.65	49.79	36.83	252.02	0.202
	Diabetes	164.57	81.43	84.80	340.80	
Change in response % (Using Flux Mean)	Control	119.89	42.17	37.44	215.18	0.193
	Diabetes	149.86	73.40	77.65	291.82	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

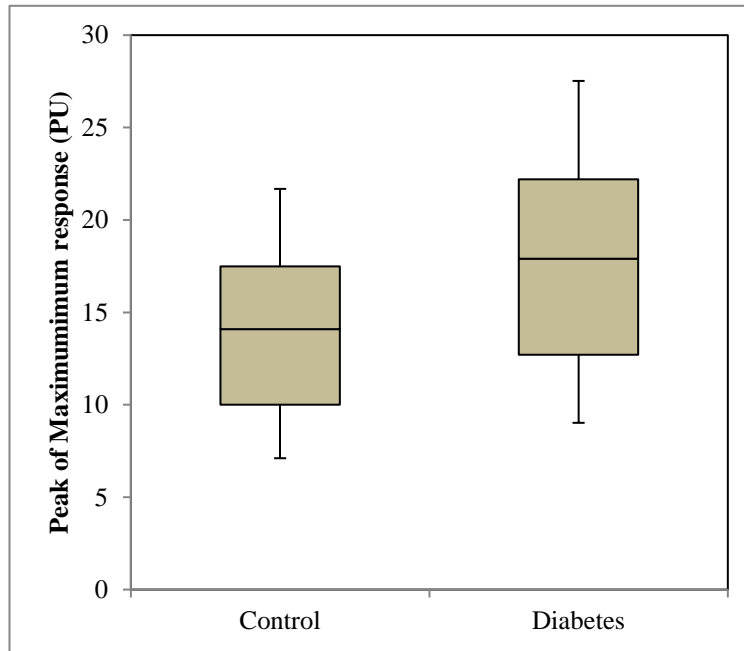


Figure 4.87 Comparison between control group and diabetes group Peak of Maximum response (PU) on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of SNP

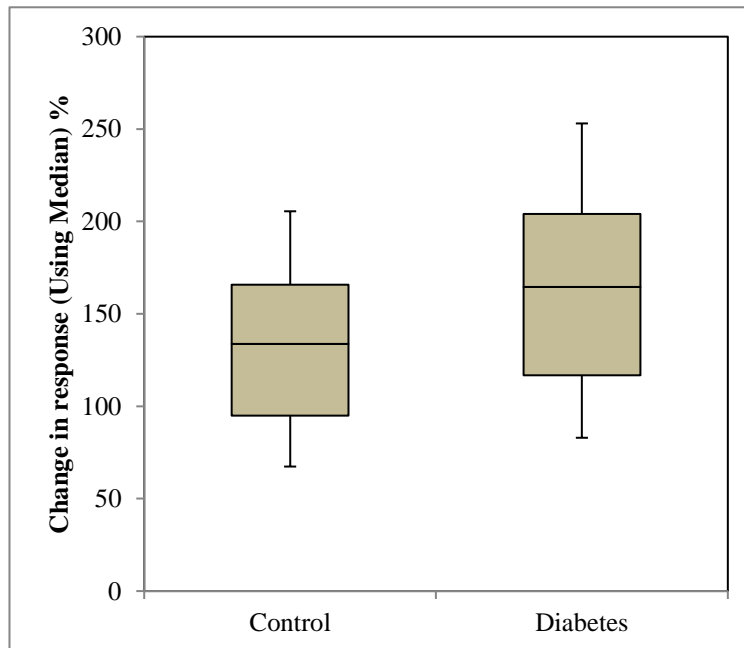


Figure 4.88 Comparison between control group and diabetes group Change in response % on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of SNP

4.3.2.2 Changes on the Plantar Surface

ACh iontophoresis at resting with no pressure applied on the plantar surface of participants' foot, showed significant differences between the study groups in the baseline (0 μ A current) blood flow ($p=0.046$), with higher means noted in the diabetes group (flux median Mean \pm SD 71.80 \pm 43.65 PU) compared to control (flux median Mean \pm SD 45.22 \pm 32.22 PU). This significant difference proceeded up to the 5th minute of the protocol in flux median data and 6th minute in flux means. No further significant differences were noted through the protocol, Peak of Maximum response or change in response percentage. Whereas iontophoresis of SNP under no pressure did not reveal any significant differences between groups in flux values, Peak of Maximum response or change in response on the plantar surface.

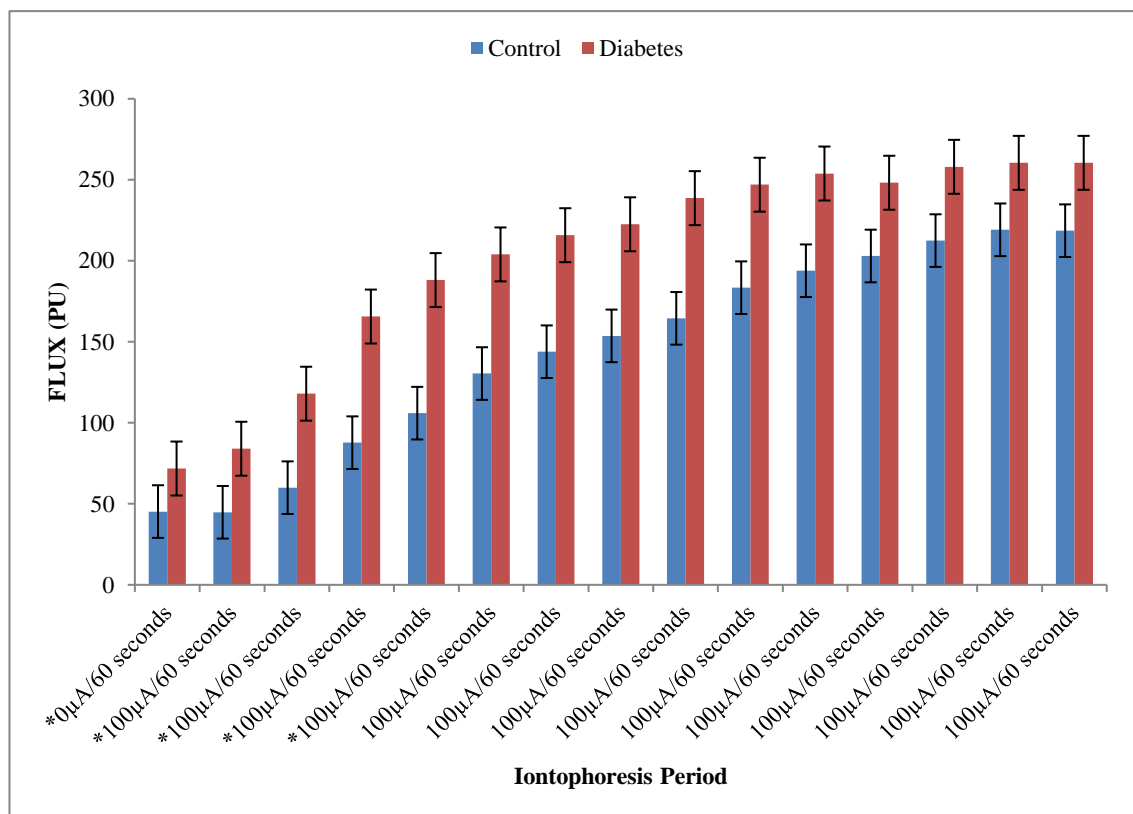


Figure 4.89 Control group and diabetes group means of flux median on the Plantar of the foot with no pressure applied during the iontophoresis of ACh

*: $p < 0.05$ S

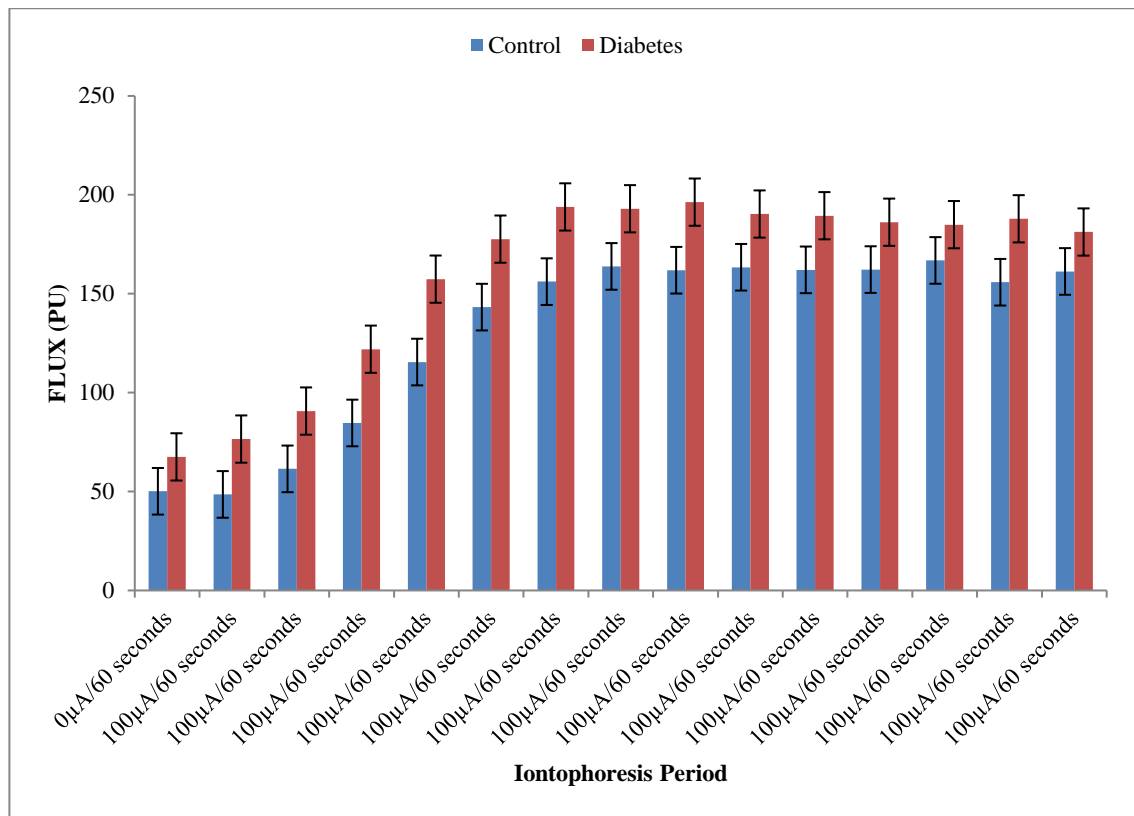


Figure 4.90 Control group and diabetes group means of flux median on the Plantar of the foot with no pressure applied during the iontophoresis of SNP

Table 4.18 Comparison between control group and diabetes group blood flux changes on the Plantar of the foot with no pressure applied during the iontophoresis of ACh using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	214.5	135.3	33.39	588.58	0.054
	Diabetes	302.3	160.5	47.67	662.91	
Change in response % (Using Flux Median)	Control	471.80	341.59	46.74	1272.23	0.791
	Diabetes	521.80	562.09	58.05	2298.87	
Change in response % (Using Flux Mean)	Control	442.23	330.58	51.80	1240.94	0.642
	Diabetes	495.48	541.38	58.37	2213.42	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

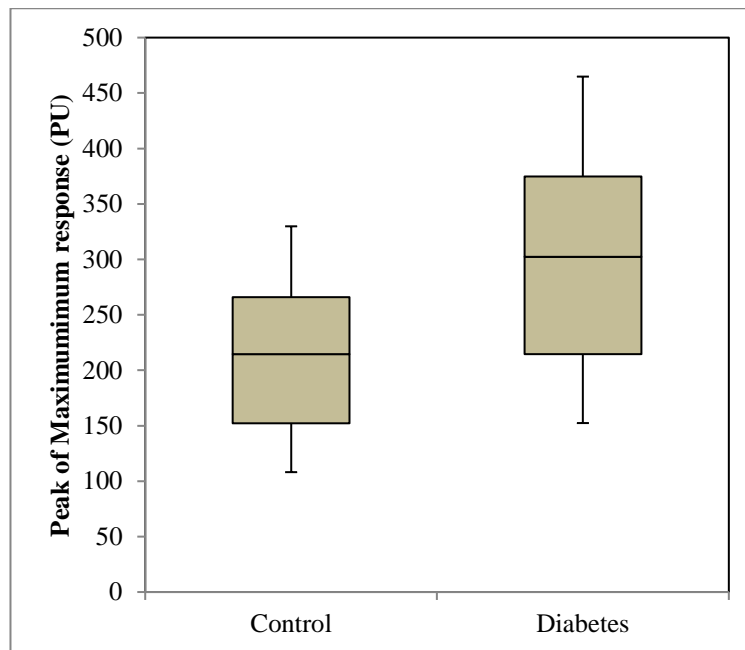


Figure 4.91 Comparison between control group and diabetes group Peak of Maximum response (PU) on the Plantar of the foot with no pressure applied during the iontophoresis of ACh

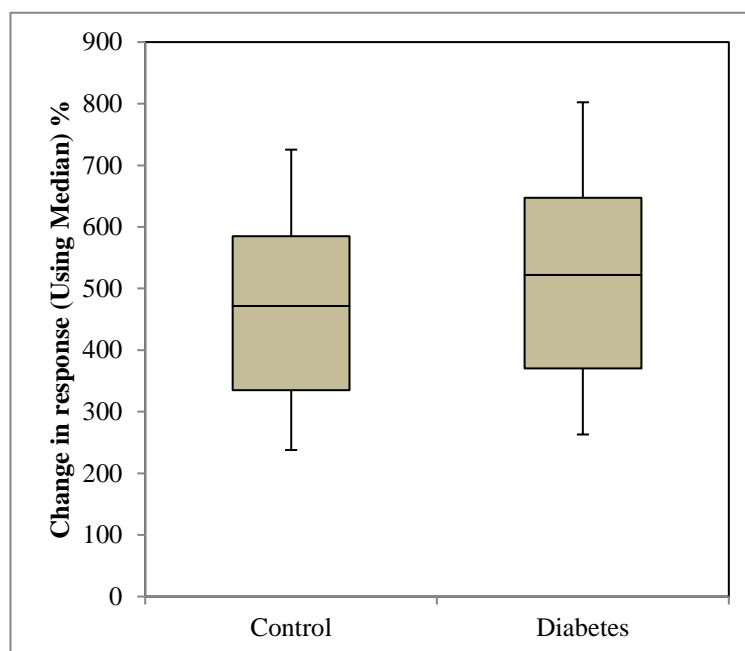


Figure 4.92 Comparison between control group and diabetes group Change in response % on the Plantar of the foot with no pressure applied during the iontophoresis of ACh

Table 4.19 Comparison between control group and diabetes group blood flux changes on the Plantar of the foot with no pressure applied during the iontophoresis of SNP using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	195.0	132.7	32.07	502.48	0.193
	Diabetes	237.8	107.7	110.78	485.71	
Change in response % (Using Median)	Control	619.20	693.98	16.21	2484.31	0.123
	Diabetes	319.17	140.20	66.77	611.09	
Change in response % (Using Mean)	Control	580.73	657.85	17.48	2348.29	0.125
	Diabetes	297.90	134.05	62.81	585.86	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

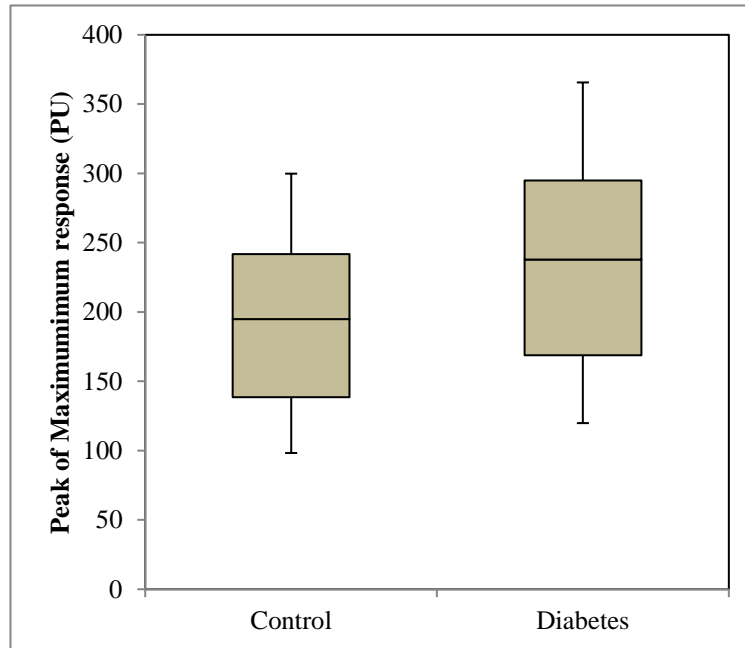


Figure 4.93 Comparison between control group and diabetes group Peak of Maximum response (PU) on the Plantar of the foot with no pressure applied during the iontophoresis of SNP

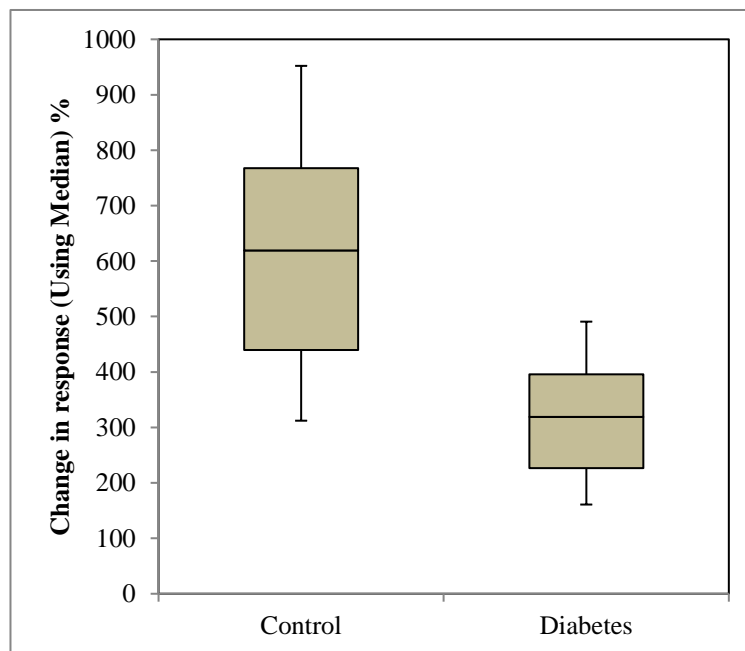


Figure 4.94 Comparison between control group and diabetes group Change in response % on the Plantar of the foot with no pressure applied during the iontophoresis of SNP

Significant differences were recorded between groups when applying the orthopaedic shoes' pressure during ACh iontophoresis on the plantar surface in flux median data; from baseline flow (0 μ A current) to 9th minute of the protocol and in 1st, 2nd, 6th, 7th and 8th minutes in the flux mean data. Yet, no significant differences were found in Peak of Maximum response or change in response percentage. The first minute of SNP drug delivery (2nd minute in the protocol) was significantly different between groups but no other significant differences were found in response to SNP iontophoresis under the orthopaedic pressure on the plantar surface.

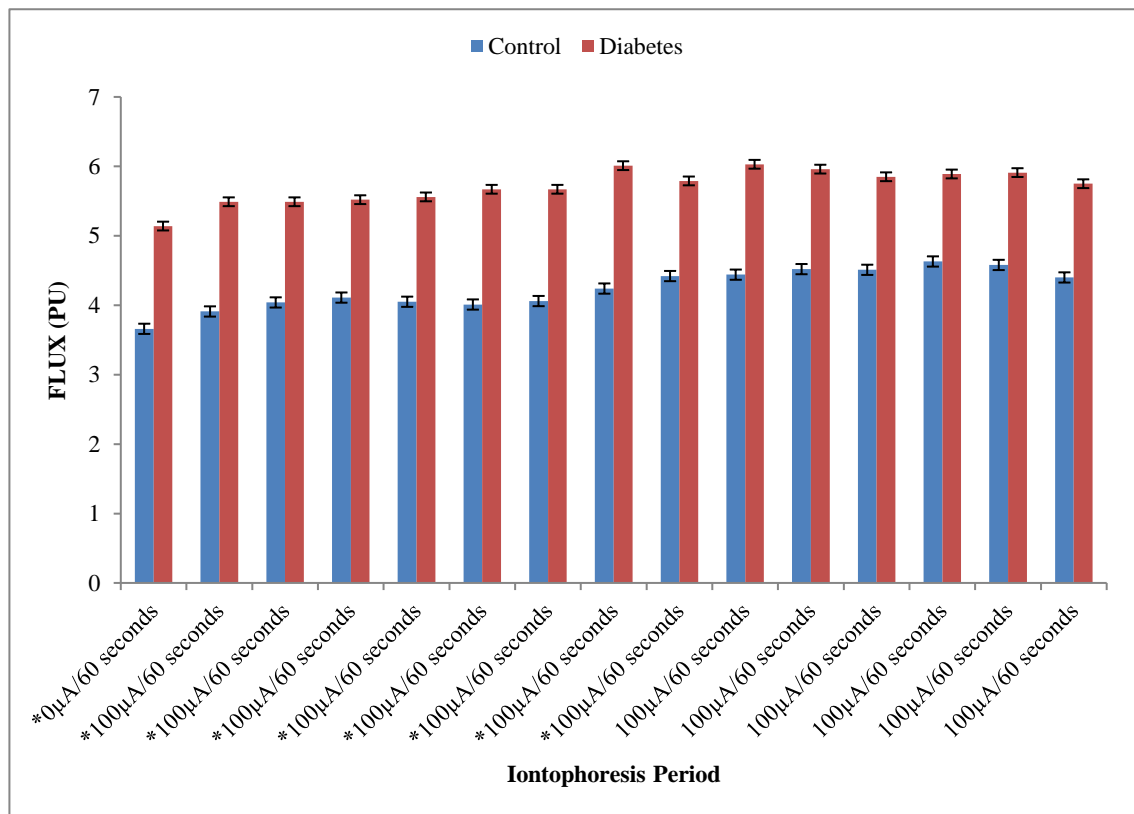


Figure 4.95 Control group and diabetes group means of flux median on the Plantar of the foot under PP in the orthopaedic shoes in response to the iontophoresis of ACh
*: $p < 0.05$ S

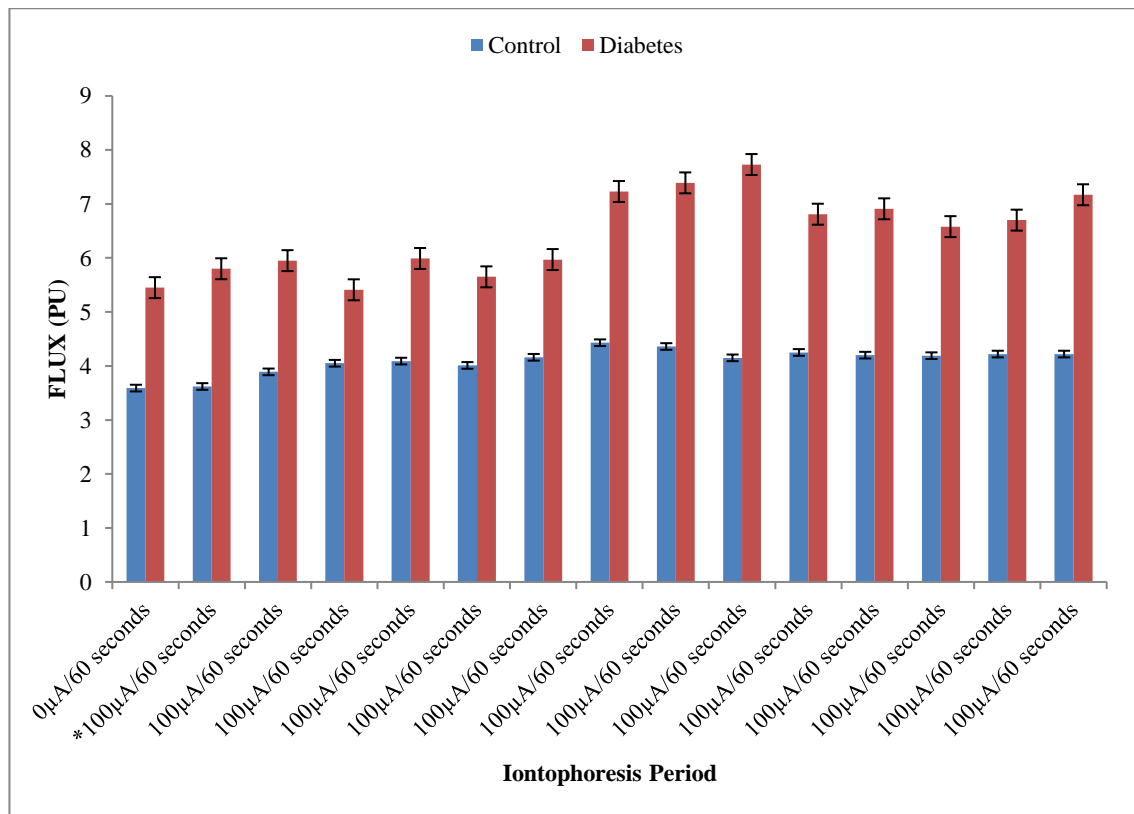


Figure 4.96 control group and diabetes group median of flux on the Plantar of the foot under PP in the orthopaedic shoes in response to the iontophoresis of SNP

*: $p < 0.05$ S

Table 4.20 Comparison between control group and diabetes group blood flux changes on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	8.6	4.0	4.22	21.98	0.061
	Diabetes	11.0	4.3	4.38	20.16	
Change in response % (Using Flux Median)	Control	140.35	61.32	78.91	322.58	0.372
	Diabetes	119.06	51.10	54.33	257.34	
Change in response % (Using Flux Mean)	Control	125.68	51.66	77.08	255.98	0.311
	Diabetes	107.53	40.32	48.11	198.32	

$p > 0.05$ NS; * $p < 0.05$ S; ** $p < 0.001$ HS.

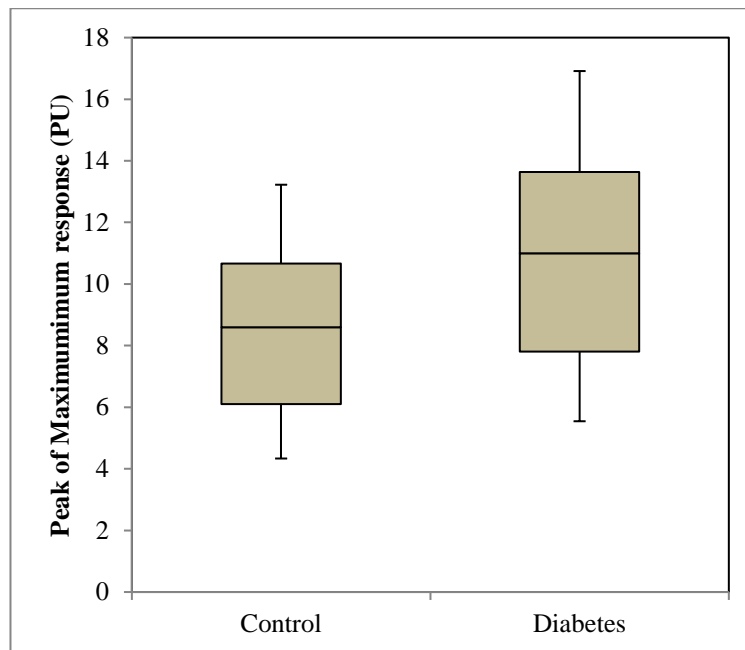


Figure 4.97 Comparison between control group and diabetes group Peak of Maximum response (PU) on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh

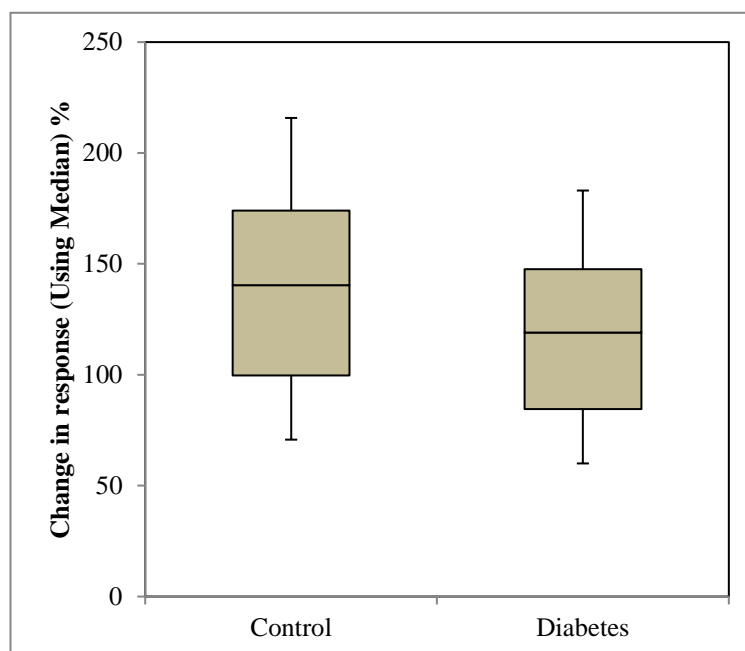


Figure 4.98 Comparison between control group and diabetes group Change in response % on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh

Table 4.21 Comparison between control group and diabetes group blood flux changes on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	7.7	3.4	3.71	17.02	0.084
	Diabetes	14.3	19.0	4.38	84.87	
Change in response % (Using Flux Median)	Control	123.24	83.48	65.78	401.81	0.922
	Diabetes	136.23	85.07	11.24	358.75	
Change in response % (Using Flux Mean)	Control	113.08	78.95	59.46	372.83	0.845
	Diabetes	119.60	65.19	12.92	270.93	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

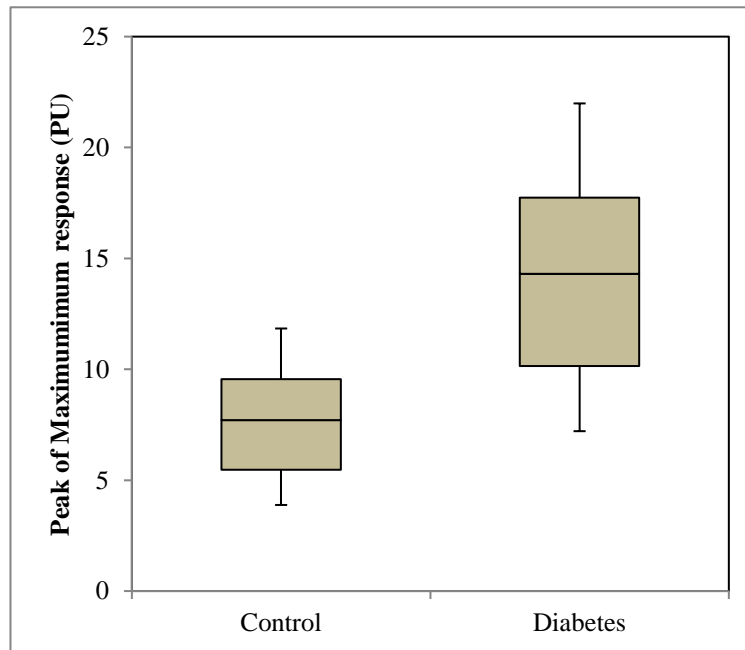


Figure 4.99 Comparison between control group and diabetes group Peak of Maximum response (PU) on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP

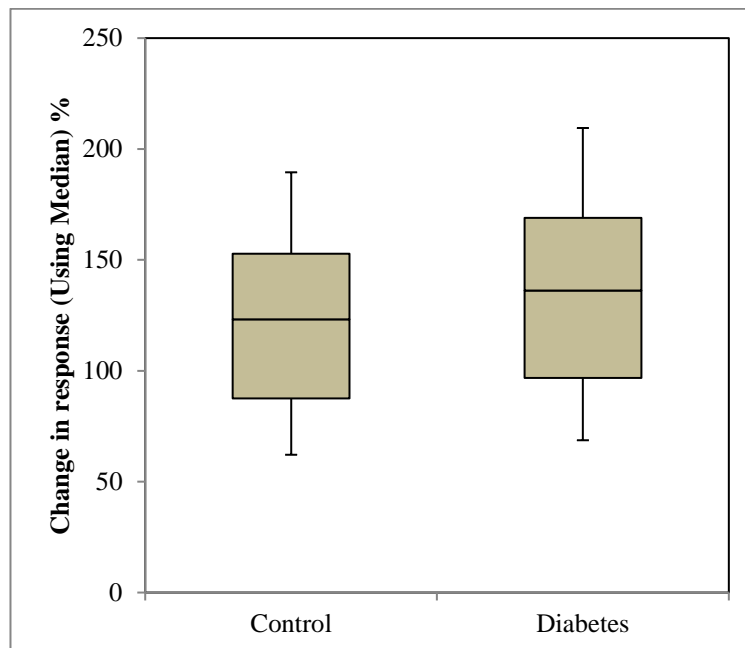


Figure 4.100 Comparison between control group and diabetes group Change in response % on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP

Although ACh iontophoresis under own shoes' pressure resulted in significantly higher flux values in diabetes group through the iontophoresis protocol periods and in Peak of Maximum response ($p < 0.05$), no significant differences were detected in the change in response in both median and mean flux data. SNP iontophoresis with own shoes' pressure had significant differences between groups with higher flux values in the diabetes group in the early 7 minutes of the protocol in median data and 1st, 2nd and 5th minutes in mean flux data. However, no significant difference was noted in Peak of Maximum response or change in responses percentage.

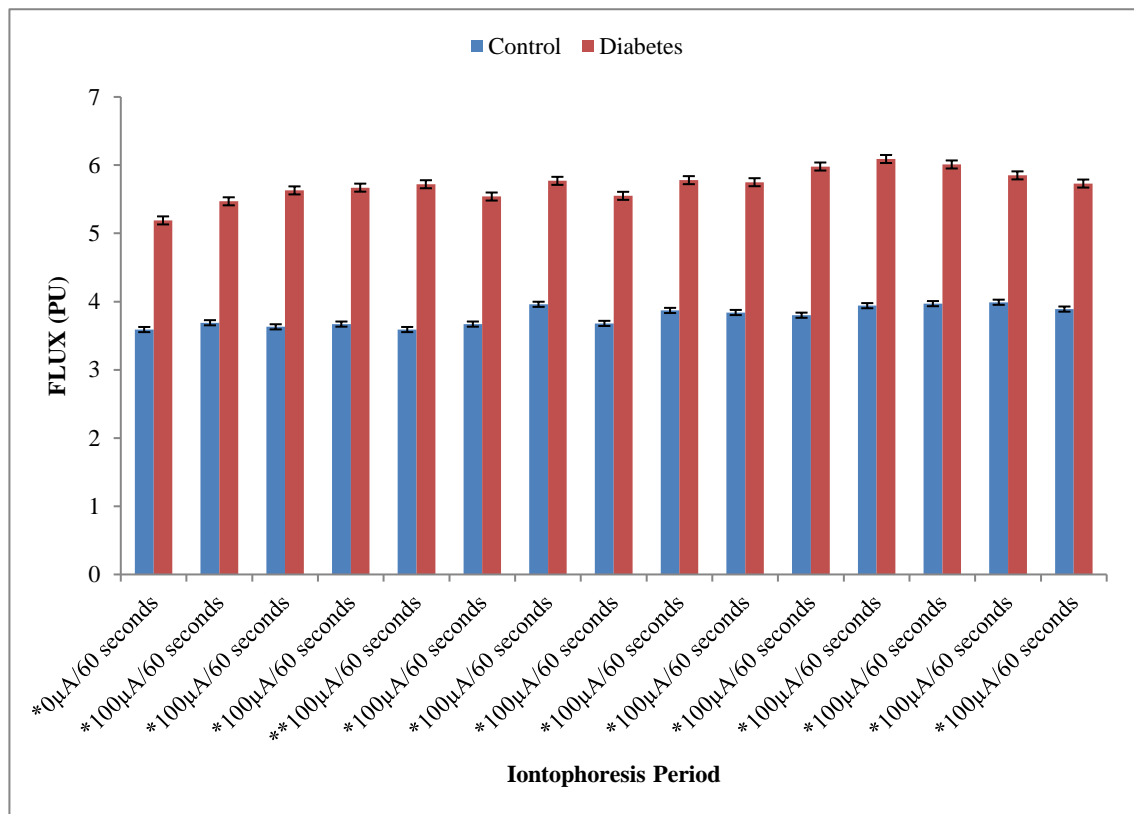


Figure 4.101 Control group and diabetes group means of flux median on the Plantar of the foot under PP in Own shoes in response to the iontophoresis of ACh
*: $p < 0.05$ S; **: $p < 0.001$ HS

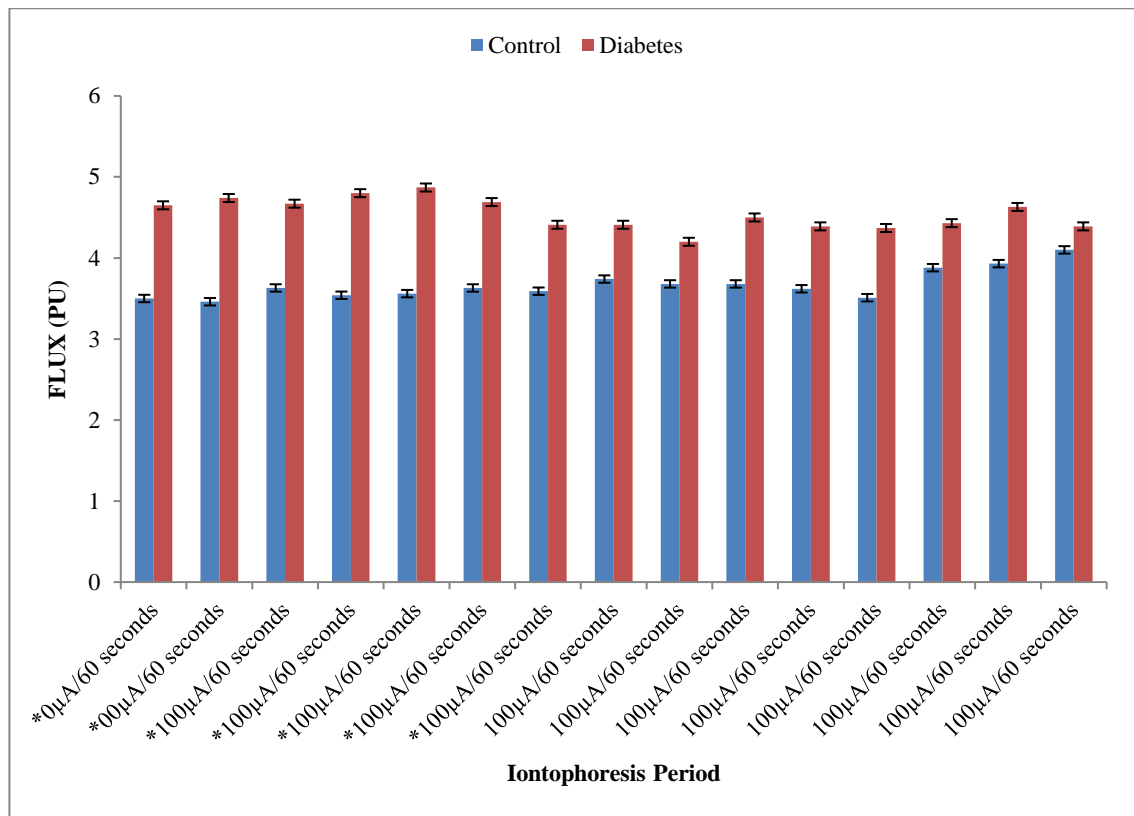


Figure 4.102 control group and diabetes group means of flux median on the Plantar of the foot under PP in Own shoes in response to the iontophoresis of SNP
*: $p < 0.05$ S

Table 4.22 Comparison between control group and diabetes group blood flux changes on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of ACh using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	7.8	3.3	3.8	16.92	0.008*
	Diabetes	11.1	4.0	4.34	16.9	
Change in response % (Using Flux Median)	Control	120.80	67.34	13.14	284.52	0.663
	Diabetes	125.08	58.00	50.67	257.34	
Change in response % (Using Flux Mean)	Control	111.71	61.98	14.61	267.80	0.659
	Diabetes	111.98	47.25	48.11	198.32	

$p > 0.05$ NS; * $p < 0.05$ S; ** $p < 0.001$ HS.

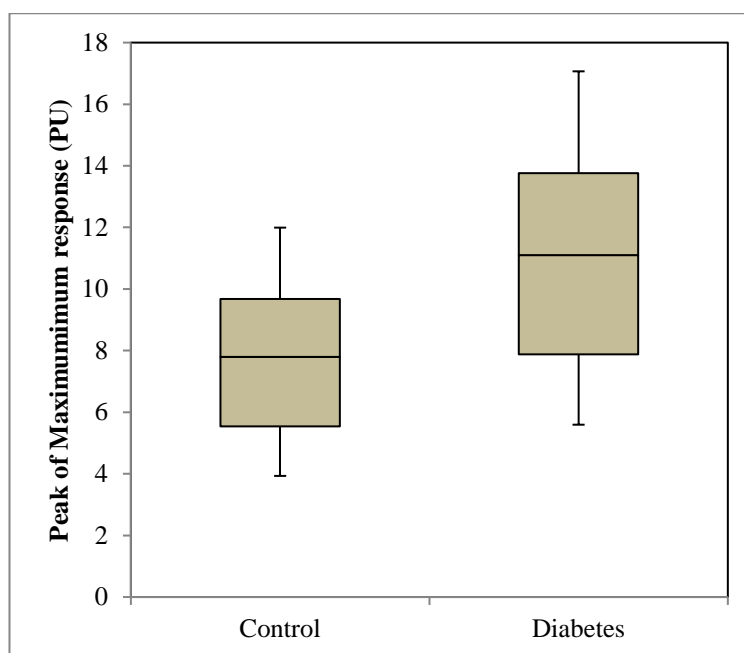


Figure 4.103 Comparison between control group and diabetes group Peak of Maximum response (PU) on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of ACh

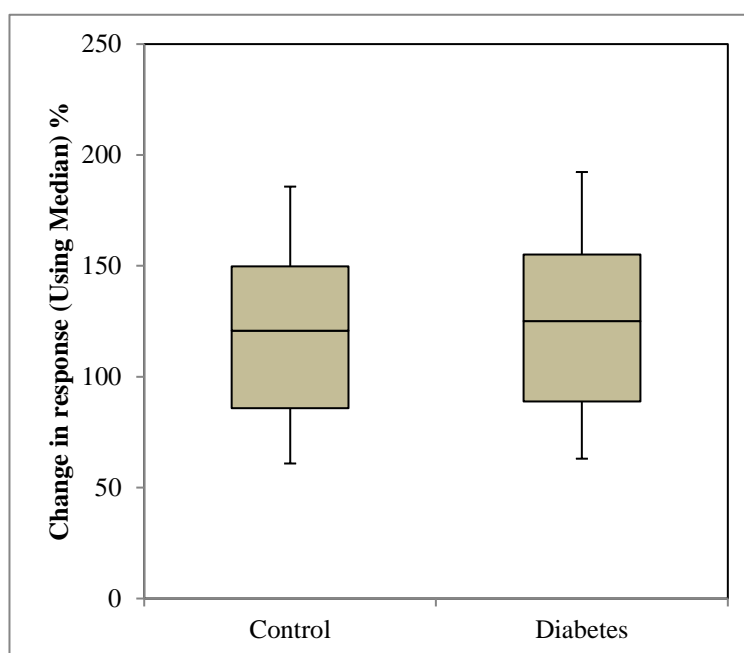


Figure 4.104 Comparison between control group and diabetes group Change in response % on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of ACh

Table 4.23 Comparison between control group and diabetes group blood flux changes on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of SNP using: Mann-Whitney test

Iontophoresis	Group	Median	IQR	Min.	Max.	p
Peak of Maximum response (PU)	Control	7.5	3.8	4.78	22.03	0.124
	Diabetes	9.2	3.5	4.45	17.42	
Change in response % (Using Median)	Control	113.57	56.70	61.21	286.51	0.793
	Diabetes	106.81	45.60	11.24	190.31	
Change in response % (Using Mean)	Control	99.44	40.63	57.96	215.52	0.834
	Diabetes	93.05	37.43	12.92	161.54	

p >0.05 NS; *p <0.05 S; **p <0.001 HS.

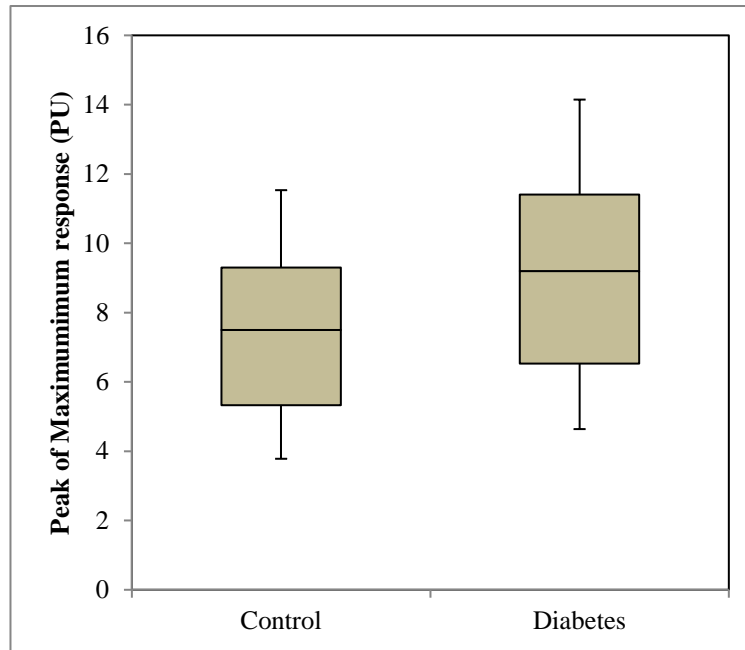


Figure 4.105 Comparison between control group and diabetes group Peak of Maximum response (PU) on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of SNP

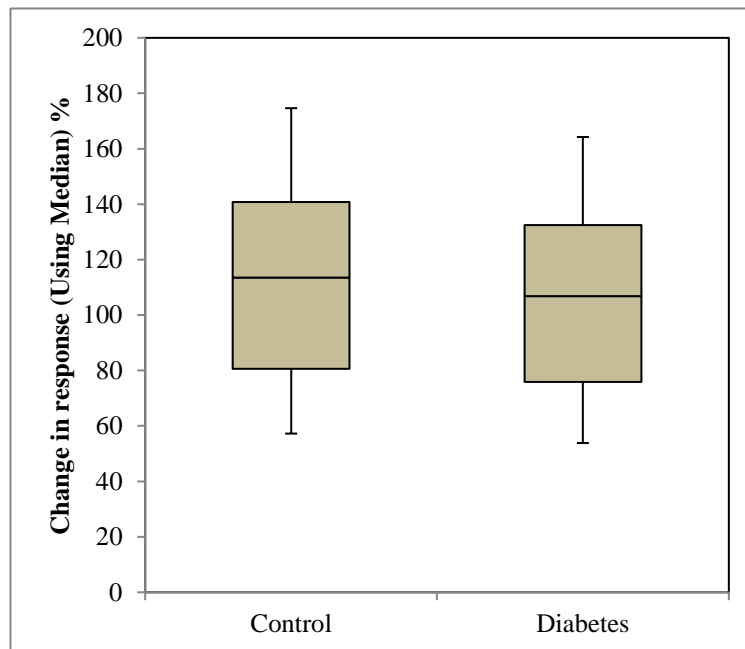


Figure 4.106 Comparison between control group and diabetes group Change in response % on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of SNP

4.3.3 Correlation of Peak Pressure with Blood Flux Values

Correlations were explored using Pearson correlation coefficient, between PP (KPa), on both foot surfaces within orthopaedic and own shoes, and the corresponding recorded flux (PU) at Baseline, prior to the delivery of any current (0µa/60 seconds), for flux median as well as flux mean values, Peak of Maximum response, Change in response for flux median and Change in response using flux mean. Two interactions with significant correlations were noted. In control group (Figure 4.107,4.108), dorsal PP in the orthopaedic shoes was significantly associated with the baseline blood flow at the start of ACh iontophoresis protocol with flux median ($r=0.465$ and $p=0.039$) as well as mean flux values ($r=0.495$ and $p=0.026$). In diabetes group, the only significant correlation detected (Figure 4.109), was between the dorsal PP in the orthopaedic shoes and Peak of Maximum response to SNP iontophoresis ($r=0.45$ and $p=0.036$).

However, on investigating these significant correlations, the effect of outliers was uncertain. Spearman correlation tends to be more robust against outliers (Schober *et al.*, 2018). Therefore, those significant associations were re-assessed with Spearman rank correlation test and no significance was detected.

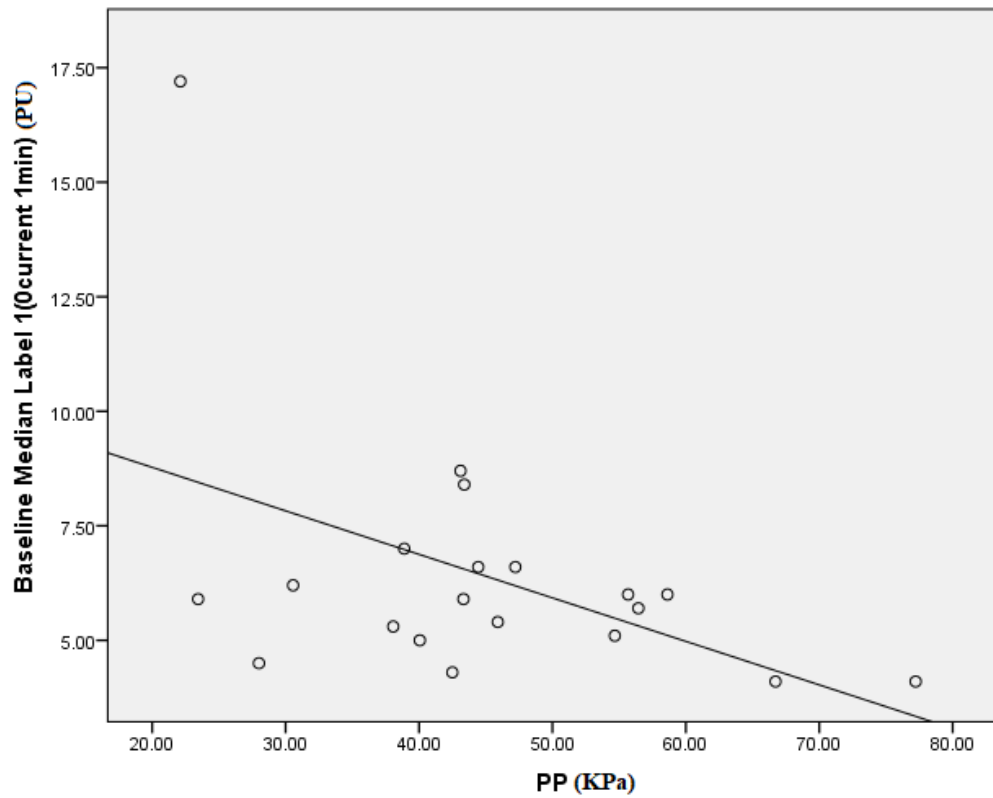


Figure 4.107 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Baseline Flux Median (PU) during the iontophoresis of ACh on the dorsal surface in control group

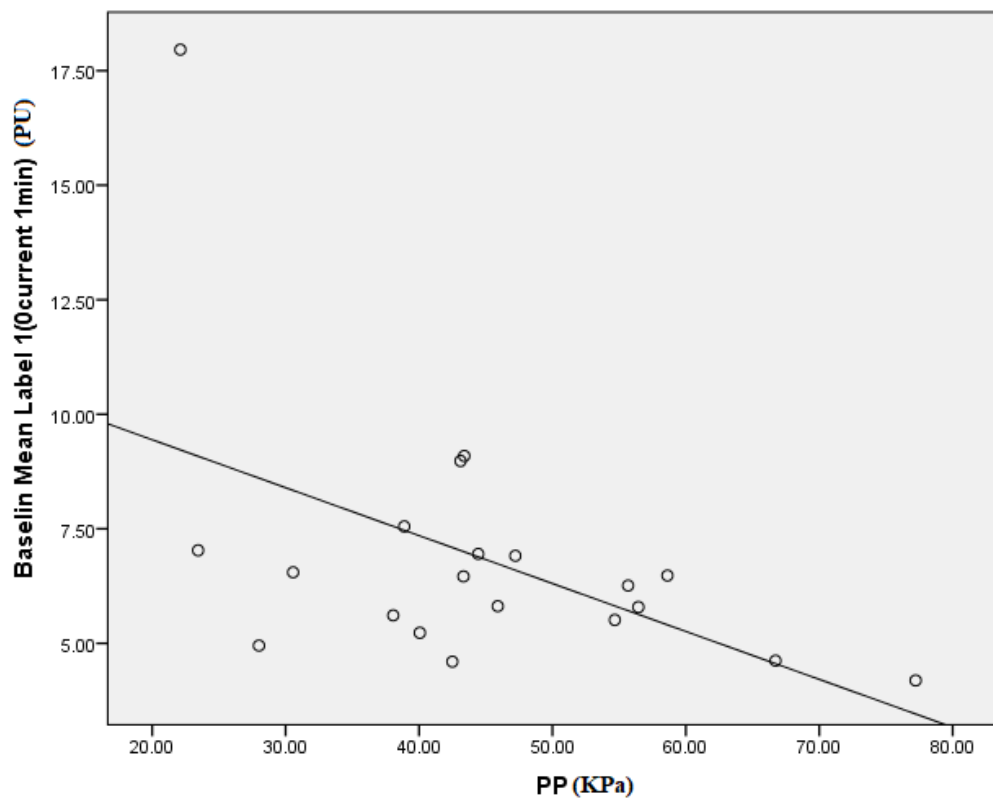


Figure 4.108 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Baseline Flux Mean (PU) during the iontophoresis of ACh on the dorsal surface in control group

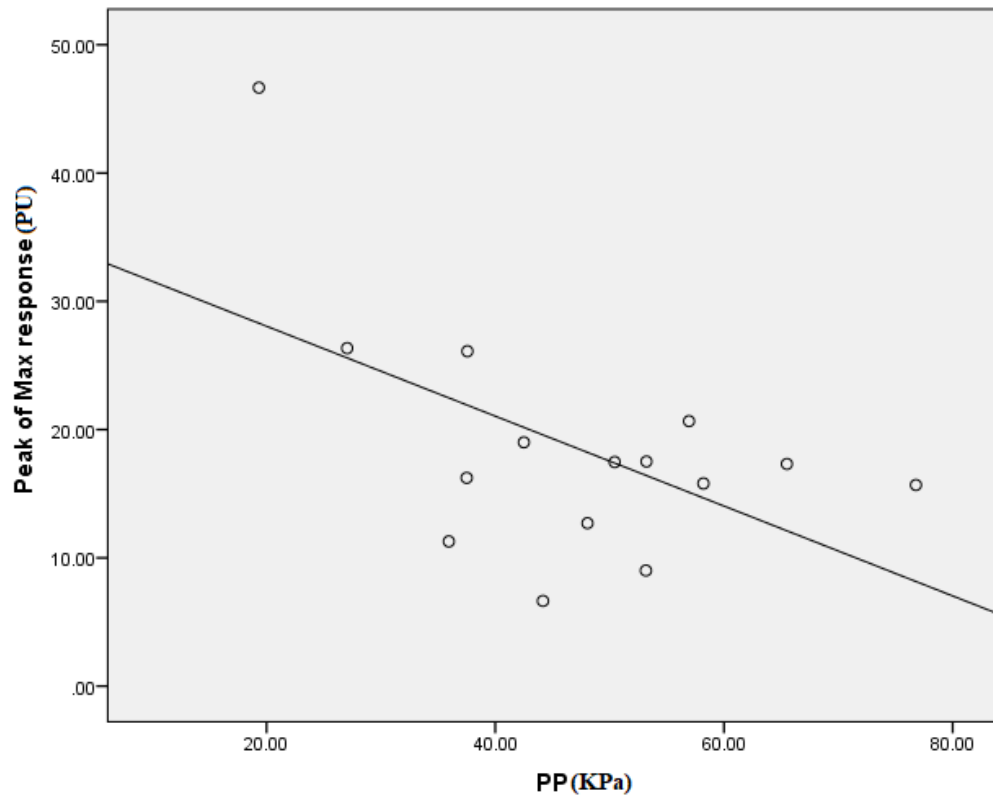


Figure 4.109 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Peak of Maximum response (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group

5.1 INTRODUCTION

Foot ulceration is a major complication of diabetes mellitus, known to result in enormous global morbidity, mortality and significant cost burden to healthcare systems. It is estimated that NHS England expenditure on the diabetic foot disease's care (almost 1% of the health service budget) accounts for more than the combined cost of breast, prostate and lung cancers with more than 90% of that expenditure related to ulceration (Kerr *et al.*, 2019). Yet, it is considered a preventable problem through evidence-based care (Lazzarini *et al.*, 2018). Hence, appropriate care and work on prevention are paramount to reduce the risks to patients and the resultant economic burden to society.

Various preventive interventions have been used in clinical practice and studied in the currently available literature. The International Working Group on the Diabetic Foot (IWGDF) has identified "Ensuring routine wearing of appropriate footwear" as one of the key preventive elements in its evidence-based 2019 guidelines on the prevention of foot ulcers in people with diabetes (Bus *et al.*, 2019b). Patients with diabetes who are at moderate or high risk of foot ulceration, due to loss of protective sensation, peripheral artery disease, foot deformity or combinations of them are advised to wear properly fitting footwear that protects and accommodates the shape of their feet. This includes adequate length, width and depth of the footwear, in order to prevent a first foot ulcer (Van Netten *et al.*, 2018).

High plantar pressure has been shown to be a significant independent risk factor for foot ulceration in diabetic foot (Waaijman *et al.*, 2014, Fernando *et al.*, 2016). Furthermore, the consistent use of therapeutic footwear with demonstrated plantar pressure relieving effect has been shown to significantly prevent the recurrence of plantar ulcers (Uccioli *et*

al., 1995, Armstrong *et al.*, 1998, Busch and Chantelau, 2003, Maciejewski *et al.*, 2004, Ulbrecht *et al.*, 2014, Fernando *et al.*, 2016).

Plantar pressure relieving effect needed for effective foot offloading was estimated to be a $\geq 30\%$ reduction in PP during walking, or a PP of $< 200\text{kPa}$ (if measured with a validated and calibrated pressure measuring system with a sensor size of 2 cm^2) (Bus *et al.*, 2016b). It is still relatively expensive to evaluate or design footwear using pressure measurement, however it should be considered a cost-effective procedure when it can reduce foot ulcers by approximately 50% in at-risk patients (Bus and Van Netten, 2016, Bus *et al.*, 2019a, Bus *et al.*, 2019b).

Currently, and to the best of our knowledge, there is no study that has investigated the effect of therapeutic footwear on the prevention of non-plantar lesions. However, ill-fitting footwear has been identified as an important cause of non-plantar ulcers. Therefore, it has been strongly recommended to consider properly fitting therapeutic shoes, custom-made insoles, or orthosis in case of foot deformity for ulcer's prevention. Properly fitting therapeutic footwear is particularly recommended for at-risk patients with diabetes who exhibit a pre-ulcerative sign, in order to change foot biomechanics and reduce pressure on vulnerable areas including previous ulcer location (Bus *et al.*, 2016a).

Our pilot study conducted with 13 non-diabetic volunteers, comparing PP in participants' own shoes with commercially available orthopaedic shoes, commonly prescribed to at-risk diabetic patients, demonstrated a significant difference in in-shoe PP on the dorsal surface ($p < 0.001$). However, no significant reduction in PP was detected on the plantar surface within the orthopaedic shoes, which would question one of the main purposes of this footwear. This also emphasises the significance of foot pressure measurement prior to any therapeutic footwear prescription in order to optimise pressure distribution and achieve the anticipated pressure reduction within these therapeutic interventions.

The current study examined the in-shoe foot pressure on the dorsal and plantar surfaces of the foot within participants' own shoes and one of the therapeutic footwear, commonly prescribed by the orthotic clinics for diabetic patients who are referred as having at-risk feet. Findings on both foot surfaces within the two shoes were compared between a group of subjects with Type 2 Diabetes and an age-matched control group of subjects without diabetes. The effect of pressure loading on endothelial function was also studied by monitoring the perfusion changes under pressure in response to the iontophoresis of two vasoactive agents, ACh, an endothelium-dependent vasodilator and SNP, an endothelium-independent vasodilator.

5.2 IN-SHOE FOOT PRESSURE ASSESSMENT

5.2.1 Dorsal Pressure

Based on professional opinion for the best shoe upper, it is the shoe upper with a design that can accommodate the shape of the foot even with deformities. Strategies to achieve the best shoe upper in diabetic patients with high-risk feet have involved the use of soft materials or leather that can be stretched, avoiding stitches and providing extra depth to accommodate deformities or orthosis. Also, lace-up shoes are preferred to secure snug closure, prevent sliding or misalignment and accommodate any change in the foot volume due to oedema (Miller *et al.*, 2000, Ulbrecht and Bus, 2020).

The current study examined the in-shoe dorsal pressure in one of the therapeutic footwear (referred to throughout as “orthopaedic shoes”) that claims to consider the previously described features to best accommodate high-risk feet in patients with diabetes. There were no significant differences between the two study groups in dorsal PP within the orthopaedic shoes as well as in participants' own shoes which they chose to bring to the testing session. However, both study groups had a significantly higher dorsal PP in

participants' own shoes when compared with dorsal PP in the orthopaedic shoes. The same relationship was noted in the total dorsal surface PTI and MF. Dorsal PP's findings match our pilot study outcomes and can be related to the deep toe-box and other characteristics considered in the materials and design of the orthopaedic shoes. Dorsal CA also showed no significant differences between groups in both tested shoe conditions. However, a significantly larger CA was recorded in the orthopaedic shoes in both study groups. The larger orthopaedic shoes' dorsal CA has helped in delivering a better pressure distribution which could explain the significantly lower dorsal PP recorded in the orthopaedic shoes. On examining individual foot areas, the first metatarsal head (DMH1) had the highest dorsal PP values in participants' own shoes. This foot area showed a significantly higher PP in own shoes than orthopaedic shoes in both groups and had significantly higher means in the diabetes group in both tested shoe conditions. Jordan and Bartlett (1995a) also observed the occurrence of the overall dorsal PP on the metatarsophalangeal joints and related this to being at the flex-line of the shoes during walking (Jordan and Bartlett, 1995a).

The highest dorsal PP in the orthopaedic shoes moved to the medial midfoot area (DM midfoot) in both groups. However, DM midfoot in the orthopaedic shoes was significantly lower than in the participants' own shoes and showed significantly higher values in the diabetes group in both tested shoe conditions. This may be related to the lace effect in the orthopaedic shoes. However, the diabetes group also experienced a significantly higher PP on DM midfoot in their own shoes where DM midfoot was the second-highest PP area after DMH1. Likewise, the highest dorsal PPs were previously reported on the medial side of the foot dorsum in running shoes independently of the lacing pattern (Hagen *et al.*, 2008, Hagen *et al.*, 2010). This may be related to the apical bony structure of the medial arch of the foot. Cheng and Hong (2010) recorded a greater PP on the lateral side of the foot dorsum, and Mei *et al.* (2014) argued that lateral

metatarsal region would be the best site to distinguish between different sports-feature-oriented footwears (Cheng and Hong, 2010, Mei *et al.*, 2014). However, Cheng and Hong (2010) used the flexible individual FSA sensors which are sensitive to folding causing measurement errors (Herbaut *et al.*, 2016). MF had the same highest values sites as PP, however, DM midfoot showed no significant differences in MF between the two shoes in the control group and DMH1 showed no significant differences between groups in their own shoes. Both tested shoe conditions had the highest PTI on DM midfoot that was significantly higher in own shoes in both study groups. Midfoot areas had the largest dorsal CA within both shoes in the two study groups. Significantly higher values were seen in the orthopaedic shoes on the two midfoot areas in the control group and area DL midfoot in the diabetes group, while DM midfoot within own shoes had the only significant differences between groups on the midfoot. These results confirm previous work that dorsal pressure data varies depending on the fit between the foot and the tested footwear at each anatomical point/foot region (Jordan *et al.*, 1997, Olaso *et al.*, 2007, Rupérez *et al.*, 2009). Therefore, the distinction between different footwear designs and attempts to improve them are possible through the study of the pressures caused by the footwear upper.

The shoe upper design and properties can affect how loads, such as the majority of anterior and lateral forces acting on the foot during gait, are applied to the foot. Therefore, the shoe upper plays a critical role in maintaining comfort and preventing foot injury (Greenhalgh *et al.*, 2012, Melvin, 2014). Yet foot pressure exerted by the shoe upper is one of the least studied (Olaso *et al.*, 2007). Comfort perception usually reflects an appropriate footwear fit which is essential in vulnerable feet as those in patients with diabetes. A sensation of discomfort forewarns of potentially harmful situations such as excessive pressure which can lead to tissue damage or ulceration. Unfortunately, patients with diabetic foot are usually lacking this protective signal due to the development of

peripheral neuropathy. Additionally, comfort perception and discomfort threshold are very subjective and difficult to define or quantify. Most literature which investigated dorsal foot pressure attempted to relate the exerted pressure to footwear comfort perception (Jordan and Bartlett, 1995, Hagen *et al.*, 2010, Herbaut *et al.*, 2016). Dorsal pressure was thought to provide an objective measurement for comfort perception as well as a validated tool for a good shoe fit, as traditional methods in measuring foot size are insufficient to determine good footwear fit which is particularly critical in high-risk feet such as diabetic foot (Cheng and Hong, 2010). Despite the fact that 37% to 59% of diabetic foot ulcers in patients suffering from multiple foot ulcerations were seen in dorsal areas (Eneroth *et al.*, 2004, Greenhalgh *et al.*, 2012), no known study has examined the impact of the shoe upper and dorsal pressure in diabetic foot and the best shoe upper design to reduce foot injury in this vulnerable population.

A comfortable fit in the orthopaedic shoes was perceived by subjects in both study groups which supports the significant negative correlations between dorsal PP and perceived comfort reported in earlier studies (Jordan *et al.*, 1997, Cheng and Hong, 2010, Hagen *et al.*, 2010, Herbaut *et al.*, 2016). Jordan *et al.* (1997) tested 10 shoes, classified in two groups; 5 models were considered comfortable shoes and 5 as uncomfortable. They recorded dorsal pressure on the flex line and the lace areas of the dorsum of the foot and noted significantly higher PP and MF in the uncomfortable shoe group. However dorsal CA was significantly lower in the comfortable shoe group which is different from our findings in the orthopaedic shoes although retain a lower dorsal PP (Jordan *et al.*, 1997). Alternatively, Jordan and Bartlett (1995b) noted that the decrease in comfort experienced with the shoe upper was accompanied by decreased forces and pressures, although no significant differences in dorsal PP and MF were found between the three shoes they tested (Jordan and Bartlett, 1995b). They attributed this relationship to the shoes upper's inflexibility which did not allow the shoes to crease and exert pressure on the foot, and it

was this that had been perceived as decreased comfort. However, they also highlighted the low sampling frequency of the dorsal pad they used could have caused actual PP and MF to be missed. Others found no significant correlation between perceived comfort and dorsal pressure data (Hagen *et al.*, 2008). However, a significant relationship was noted between dorsal PP and perceived stability which may have been the most valuable element that runners (tested population) would favour to prevent slipping within the shoe, hence reduce risk of injury.

The current study examined the timing of dorsal PP during the gait cycle which showed no significant differences between the two tested shoes in both study groups. Also, there was no significant difference between groups in both shoe conditions. The frequency of occurrence of dorsal PP showed a two peaks pattern with an increase at initial contact/loading response as well as pre-swing/initial swing phases. Similar timing for an increase in dorsal PP was noted by Takesue *et al.* (2019) who used FlexiForce[®] sensors to record dorsal pressure and a footswitch for synchronisation of dorsal pressure with the phases of the gait cycle (Takesue *et al.*, 2019). Earlier work of Jordan and Bartlett (1995a) described two dorsal force activities; a lower activity exerted just before the foot contacts the ground and a second higher force just prior to the foot leaving the ground (Jordan and Bartlett, 1995a). In the current study, the timing of dorsal MF peaks frequencies followed a similar pattern as PP. Shoe deformation with dorsiflexion of the foot in these phases of the gait cycle could be the reason for these two peaks. A significant difference in the time of dorsal PP between the two tested shoes was noted when using all participants' data as one group with more frequent peaks occurring within the orthopaedic shoes at a late point in the swing phase. These observations endorse the significance of dorsal pressure assessment, being also present during the non-contact phase of the gait cycle.

It is well-known that the choice of shoe upper can affect the comfort of a shoe, however, it is not known which of its properties has this effect. It could be the upper's shape, the

volume it creates for the foot inside the shoe or the properties of the composition material that affect pressure and comfort (Melvin, 2014). Currently, orthotists, clinicians, and other professionals' experience and judgment continue to be the mean for shoe upper's provision to accommodate the foot in patients who cannot give adequate feedback for a good shoe fit (Olaso *et al.*, 2007). However, dorsal pressure data would be more valuable in providing relevant information for footwear designers and manufacturers attempting to improve the comfort of their footwear. Identification of areas on the dorsum of the foot experiencing high PP can improve different features of footwear construction. For example, alterations based on pressure measurements in the material used in the shoe upper, the lacing system, or the protective footwear enhancements can assist in reducing the magnitude of dorsal pressure, and thus discomfort and injuries at various anatomical locations particularly in those with vulnerable feet.

5.2.2 Plantar Pressure

In-shoe plantar pressure measurement has been commonly used in both research and clinical settings, to effectively assess pressures experienced by patients who are at risk from a variety of foot problems including patients with diabetes mellitus (Cavanagh *et al.*, 1992, Guldemonnd *et al.*, 2007, Bacarin *et al.*, 2009, Owings *et al.*, 2009, Sacco *et al.*, 2009, Ledoux *et al.*, 2013). It offers the advantage of collecting continuous, real life pressure data in subjects with vulnerable feet such as diabetic neuropathy who are mostly advised to always wear their footwear during daily activities to better distribute loads and reduce the chance of external trauma to their feet. Therefore, the current study favoured in-shoe plantar pressure measurement as it offers a more indicative, valid and reliable mean for plantar pressure assessment.

The current study showed a significantly higher total in-shoe plantar PP within participants' own shoes in both study groups. However, the differences were significant

in one foot area (PT1) in the diabetes group and area PM midfoot had even a significantly higher PP within the orthopaedic shoes than own shoes in the control group. Also, heel areas, a major concern site in diabetic foot (Younes *et al.*, 2004), showed no significant differences between the two tested shoes in both study groups. Total plantar PTI showed no significant differences between the two shoes in the control group, although, medial plantar areas under toes, midfoot and heel were significantly higher in the orthopaedic shoes. The diabetes group, on the other hand, had a significantly higher total PTI within the orthopaedic shoes and the same relationship was seen across most of the foot areas except for the midfoot areas which were not significantly different between the two tested shoes in this study group. These minimal significant differences in PP seen in the diabetes group together with the significantly lower PTI in diabetes group' own shoes could be explained by patients' careful selection of their shoes due to their potential awareness of diabetic foot complications. Additionally, none of the participants in this study was known to have or show any signs of neuropathy which could have altered the perception of high loads.

Examining differences between groups in own shoe revealed, no significant difference in total PP, although, midfoot areas were significantly higher in diabetes groups and lateral areas under the toes and metatarsal heads were significantly higher in the control group. Orthopaedic shoes showed a significantly higher total PP in the diabetes group compared to the control group. The same significant differences were seen in most of the plantar foot areas in the orthopaedic shoes, except the toes areas which were significantly higher in the control group. Yet, diabetes group displayed no deformity or signs of neuropathy, which would reinforce the previous finding that abnormalities in plantar pressure may precede clinical signs of diabetic neuropathy (Pataky *et al.*, 2005, Tong *et al.*, 2011).

Similar to PP, no significant difference was detected in total PTI between groups in own shoes and the midfoot areas had significantly higher PTI in the diabetes group within own

shoes. The own shoes also showed a significantly higher PTI under the 1st metatarsal head in patients with diabetes while the 4th metatarsal area and all toes areas were significantly higher in the control group. The differences between groups in PTI within the orthopaedic shoes followed the same relationship as the PP, although the three medial metatarsal heads' areas were significantly higher in diabetes group than control group within the orthopaedic shoes. Plantar CA generally demonstrated no significant differences across different comparison setting, with the only significant difference found in the diabetes group within own shoes under the medial midfoot area. The reduction in plantar pressure under the toes areas in the diabetes group in this study has been a frequent observation when compared with non-diabetes controls (Stokes *et al.*, 1975, Ctercteko *et al.*, 1981, Veves *et al.*, 1991, Bacarin *et al.*, 2009). The diabetes group also continued to demonstrate a shift of PP from the big toe to the first metatarsal head, which is one of the most likely sites to ulcerate (Plank *et al.*, 2000, Bacarin *et al.*, 2009, Dayer. and Assal., 2009). Although PP under the 1st metatarsal head, a common location for plantar ulceration, showed no significant differences between groups in the two tested shoes, PTI was significantly higher within both shoes in the diabetes group. This emphasises the importance of evaluating not only PP but also its cumulative effect over time represented in PTI values as ulcer development can be influenced by the magnitude of pressure as well as the time of exposure to that pressure (Hsi *et al.*, 2002). In fact, the current study finding could indicate that PTI may be the first detectable sign for plantar pressure changes even before PP increase or peripheral neuropathy can be tested (Tong *et al.*, 2011). Alternatively, heel areas, another common site for ulceration, retained the highest PP and PTI within the orthopaedic shoes which were significantly higher in the diabetes group than the control. PTI is increasingly used in evaluating plantar loading with high interdependency noted between PTI and PP (Keijsers *et al.*, 2010, Waaijman and Bus, 2012). However, the added value of PTI reporting was debatable in the literature and

changes in PTI can be affected by walking speed which was not recorded or standardised in the current study (Bus and Waaijman, 2013).

Whole plantar surface MF showed no significant differences between the two tested shoes in the diabetes group, although midfoot areas were significantly higher within own shoes. The control group had a significantly higher total MF within the orthopaedic shoes that was only detected in the lateral heel area. The diabetes group recorded significantly higher MF than the control group in both tested shoe conditions. This was observed across different plantar areas except the toes which maintained a significantly lower MF. Highest MFs were recorded under the heel areas thus, even with significantly higher MF recorded in diabetes, MF distribution pattern was not yet different from the control group. Diabetic patients with neuropathy begin to exert extra force at the forefoot due to the subsequent impaired joint mobility, especially at the ankle joint, and intrinsic muscles atrophy (Srinivasan *et al.*, 2001, Rahman *et al.*, 2006).

The tested orthopaedic shoes were an off-the-shelf selection with no insole modifications which are usually added in orthotic clinics. Yet, it was not expected to show the significantly high overall plantar PP and PTI in the diabetes group where no significant differences were noted between the study groups within participants' own shoes. The diabetes group in this study, even with no neuropathy signs or symptoms, showed a reduction in pressure under the toes area. However, they continued to experience the same highest plantar pressure under the heel area as in the control group with no anterior displacement. This finding has frequently been described in earlier research works in particular with diabetic neuropathy (Caselli *et al.*, 2002, Grimm *et al.*, 2004, Pataky *et al.*, 2005, Bacarin *et al.*, 2009).

The findings of the current study confirm the essential need for pressure measurement as a reliable screening tool to achieve effective offloading and optimise the production of

footwear tailored for patients with diabetes to assist in foot ulcers prevention (Bus *et al.*, 2011, Bus *et al.*, 2016b, Jorgetto *et al.*, 2019, Chatwin *et al.*, 2020).

5.3 IMPACT OF PRESSURE ON ENDOTHELIAL RESPONSE

5.3.1 Introduction

Mechanical stress related to prolonged and/or high externally applied pressure, leading to local ischaemia and subsequent damage, has been an important contributing factor to skin breakdown. Likewise, impairment in the blood flow response to mechanical stimuli is a key risk factor in the development of ulcers. Therefore, daily activities such as the simple act of walking, which involves repetitive mechanical trauma, may further damage skin microcirculation, including the endothelium, thus increasing the risk of ulceration. Diabetic foot has a multifactorial pathology and both increased plantar pressures, as well as the alteration in the local microvascular reactivity, have been found to be associated with diabetic foot ulceration (Fromy *et al.*, 2002, Kořtka *et al.*, 2004, Patry *et al.*, 2013, Yih-Kuen *et al.*, 2013, Pu *et al.*, 2018).

Flynn (2014) investigated the relationship between walking barefoot plantar pressures across six areas of the forefoot with the baseline flux measured with LDF in each of these areas in a group of 60 subjects with and without Type 2 diabetes. No significant association was found. There were no significant differences in barefoot PP or baseline flux between Flynn's study groups and the author concluded that walking plantar pressure values did not influence the baseline resting perfusion, thus areas which experienced higher pressure during walking did not show altered perfusion when unloaded (Flynn, 2014). In the current study, negative correlations were generally noted between PP within the two tested shoes and blood flow parameters on dorsal and plantar surfaces of the foot in both study groups. However, the significant associations detected on the dorsal surface

may have been related to an outlier effect, and not a true reflection of the relation between PP and blood flow parameters. Therefore, Spearman rank correlation revealed no significant correlation between PP and changes in blood flow in response to acetylcholine and sodium nitroprusside under any of the testing conditions on dorsal or plantar surfaces in both study groups. This also agrees with the findings of Pu et al. (2018) who could not establish any significant correlation between in-shoe PP under the first metatarsal head and blood flow response to the accumulated pressure stimulus induced through walking on a treadmill in a group of 19 Type 2 diabetes patients with different peak plantar pressures. However, their study examined only one level of pressure stimulation and had limitations due to the large individual differences and small sample size (Pu *et al.*, 2018).

The current study simulated the premeasured dorsal and plantar in-shoe walking PPs to examine the influence of each foot surface's pressure on its microvascular response to the iontophoresis of ACh, an endothelium-dependent vasodilator and SNP, an endothelium-independent vasodilator.

5.3.2 Impact of Loading Pressure on Endothelial Function on Dorsal Surface

Both study groups showed a significant reduction in blood flow response to the iontophoresis of ACh and SNP when own shoes, as well as orthopaedic shoes pressure values, were applied on the dorsum of the foot. Although own shoes dorsal PP was significantly higher than orthopaedic shoes' PP in both study groups, the control group showed a significantly higher change in ACh and SNP response under the orthopaedic shoes' dorsal PP while the diabetes group only recorded a significant change in response with SNP use. The only significant difference between groups in changes in blood flow response under no pressure, own shoes PP and orthopaedic shoes PP on the dorsum of the foot was in flux change in response from baseline to the iontophoresis of ACh under no pressure, with a significantly higher change in response recorded in the control group.

There were no significant differences with ACh under own shoes' PP and orthopaedic shoes' PP, in addition to the no significant differences in response to SNP under any of the three pressure conditions. This goes with the no significant differences between groups in dorsal PP within the two tested shoes. However, these findings show an impairment in ACh endothelium-dependent vasodilation response on the foot dorsum in the diabetes group. This demonstrates that the diabetes group could not achieve the same reactivity as the control under the significantly lower orthopaedic PP alongside the reduced ACh response in the resting condition. Even with no known complications amongst the study subjects, diabetes mellitus has altered the ability of the endothelium to react to ACh.

Although no known study has investigated the simultaneous impact of dorsal PP application and blood flow response, there are a number of studies which agree with the current study findings that endothelium-dependent vasodilation response to iontophoresis of ACh was reduced in diabetic patients, particularly those without neuropathy, while the non-endothelium-dependent response to SNP was still preserved (Pitei *et al.*, 1997, Arora *et al.*, 1998, Hamdy *et al.*, 2001, Koitka *et al.*, 2004). This emphasises the possibility of the early endothelium functional impairment in diabetes that can precede any structural changes affecting the vascular smooth muscle cells function, tested in the response to the endothelium-independent vasoactive agent, SNP (Johnstone *et al.*, 1993, Vehkavaara *et al.*, 1999, Singh *et al.*, 2003, Schramm *et al.*, 2006). Other investigators evaluated skin microvascular function on the dorsum of the foot and found early reductions in endothelium-dependent and independent responses, prior to any clinical presentation of macrovascular or microvascular complications. (Veves *et al.*, 1998, Khan *et al.*, 2000).

Endothelium-dependent vasodilation is an important element in the inflammatory response involved in wound healing as well as the ability of the body to deal with local infection which can, in turn, lead to ulceration and gangrene. Therefore, this functional

ischaemia which has been expressed in the impaired ability of the microvasculature to efficiently produce endothelium-dependent vasodilation in response to mechanical stress, detected in the diabetes group, would play an important role in the predisposition of ulceration and the development of complications on the foot dorsum in patients with diabetes.

5.3.3 Impact of Loading Pressure on Endothelial Function on Plantar Surface

Both study groups showed a similar response on the plantar as that detected on the foot dorsum. When pressure values in both own shoes and orthopaedic shoes were applied there were a significant reduction from the resting (no pressure) blood flow response to the iontophoresis of ACh and SNP. Flynn (2014) noted the significant reduction in endothelial response to the iontophoresis of ACh and SNP started to occur even with the application of 50% of the barefoot plantar pressure. Further addition of the full (100%) walking pressure did not significantly reduce the blood flux from 50% pressure response (Flynn, 2014).

Plantar PP in the current study was significantly higher within own shoes than orthopaedic shoes in both study groups. However, the control group had no significant differences in blood flow changes in response between own shoes and orthopaedic shoes plantar PP application with ACh as well as SNP. The diabetes group maintained the same response on the plantar surface as they did on the dorsal surface. No significant differences found in the change in response from baseline in the diabetes group between the applications of the two shoes plantar PPs with ACh and a significantly higher change in response to SNP was noted under the orthopaedic shoes' PP than the own shoes' PP. However, Flynn (2014) noted a reduced capacity of the group with Type 2 diabetes to achieve

vasodilatation in response to SNP iontophoresis under plantar PP than the control group could do (Flynn, 2014).

The diabetes group in the current study showed significantly higher blood flux values than the control group on the plantar surface in response to ACh iontophoresis in resting/no pressure, under orthopaedic PP and own shoes PPs. However, no significant differences from the control group were detected in changes in response from baseline flux with the iontophoresis of ACh as well as SNP under all three pressure conditions. The significantly higher flux values in the diabetes group were more apparent under the own shoes plantar PP with ACh iontophoresis as well as SNP but to a lesser extent with SNP than ACh. Yet, own shoes plantar PP demonstrated no significant differences between the study groups and the diabetes group experiencing a significantly higher plantar PP than the control group within the orthopaedic shoes. Flynn (2014) recorded no significant differences in barefoot plantar PP between study groups, however, Type 2 diabetes group also maintained higher flux values than control throughout the delivery of pressure, at resting flux, 50% pressure and 100% pressure delivery with both vasoactive agents (Flynn, 2014). Current study's findings also agree with Newton et al. (2005) who conducted a pilot study to investigate the effect of local pressure on microvascular function in the diabetic foot. Subjects with diabetes in their study displayed higher plantar pressures than the control group, as demonstrated within the orthopaedic shoes in the current study, but no significant difference was found in ACh response (Newton et al., 2005).

Significantly higher blood flux values on the plantar surface in the diabetes group than control were previously recorded by Cobb and Claremont (2002). These substantially elevated levels of blood flux in the diabetes group may suggest an over-perfused plantar tissue that may not blanch to the same extent as in control subjects. This indicates the inability of the plantar surface microcirculation in diabetic patients to adapt to dynamic

changes taking place during normal daily loading as in walking (Cobb and Claremont, 2002). Impaired pressure-induced vasodilation and ineffective unloading response were previously reported in the foot of subjects with diabetes (Petrofsky *et al.*, 2009). Damage of sympathetic fibres, an early component of diabetic neuropathy that can precede any clinical presentation, could have impacted the plantar surface microvascular haemodynamics including increased arteriovenous shunt flow and nutritive capillary flow at rest (Tooke and Brash, 1996). That could have resulted in the current study observations of increased blood flow recorded with diabetes group in comparison to control.

Chronically raised plantar pressure areas in the diabetic foot can demonstrate an increase in the skin blood flow, compared with lower pressure areas on the same foot (Newton *et al.*, 2005). This was thought to be a physiological response to repeated tissue trauma from the high pressure which could lead to the development of local inflammatory response in diabetic foot (Flynn, 2014). In the current study, no significant differences were detected between groups in the blood flux change in response to ACh as well as SNP from baseline on the plantar surface in resting/under no pressure, under orthopaedic PP or own shoes PP. However, high-pressure plantar areas with increased blood flow have previously shown a reduced responsiveness of the endothelium-dependent vasodilatation by ACh iontophoresis when compared with low pressure sites on the foot of patients with diabetes. (Newton *et al.*, 2005). Therefore, further work is required to determine whether, and under what conditions, this additional hyperaemia in the diabetes group is protective or maladaptive.

5.4 CONTRIBUTION TO KNOWLEDGE

This study successfully investigated dorsal foot pressure and evaluated the impact of pressure application on the dorsal as well as the plantar surfaces of the foot in a group with diabetes and a control group of subjects with no diabetes.

Also, the results from the simultaneous investigation of endothelial function during pressure loading will add to the understanding of the interaction and the implications related to two of the major factors involved in the development of ulceration associated with diabetes: pressure alteration and endothelial dysfunction.

The findings from the investigation of dorsal foot pressure distribution and its impact will benefit the design of future therapeutic footwear in-order to better prevent diabetic foot ulceration.

5.5 LIMITATIONS OF THE STUDY

Recruitment of subjects with Type 2 Diabetes was facilitated by the Scottish Diabetes Research Network (SDRN). However, the older age of the recruited participants with diabetes resulted in difficulty in recruiting non-diabetic volunteers from the same age group, due to the prerequisite commitment to our lengthy protocol. Thus, the number of recruited subjects was limited because of the study time constraint.

It would have been preferred to collect all blood flow data on the same day to guarantee that all circumstances that can affect the blood flow response, such as food and caffeine intake, for each subject are identical for all the testing components. However, due to our lengthy iontophoresis protocol and the potential stress of long fasting associated with the study procedures particularly with diabetes group, the decision was made to collect data over two visits, yet participants still considered it a lengthy procedure. Therefore, possible

subject boredom and confined movement for a long duration may have caused some mental stimulation that may have affected the blood flux values.

Defining areas with high pressures on each foot surface then evaluating the blood flow response under different pressure conditions at each of these areas, would have offered better comparable findings. However, in order to test the same area on the foot surface with the three pressure conditions, a recovery interval would have been required for the vasoactive agent to clear away. That would have further lengthened the testing sessions. Therefore, a different area was used for each testing condition to avoid any residual effects on the next element of the assessment.

Carrying out measurements with subjects in the supine position may have not fully represented the blood flow as when the limb is dependent and loaded with the in-shoe walking pressure. However, this allowed the collection of data with minimal movement artefacts and to conduct the pressure loading simultaneously with the process of iontophoresis and blood flux measurement.

The foot was stabilised in a boot to limit any movement with the addition of pressure and subjects were instructed to lie still at all times. Yet small movements were still possible and may have caused some artefacts in the LDF recording during the assessment. Also, the actual pressure value transferred through the pressure delivery equipment could have been altered.

Every effort was taken to control the testing environment and clear written instructions were provided in which subjects were instructed to refrain from food and caffeine related drinks prior to assessment. However, there was no guarantee that participants followed the instructions or no evocation of mental stimulation or discomfort during the long testing period. All could have had an influence on the blood flow response and produced error in the flux readings obtained.

Although, one researcher conducted all testing procedures with prior training and every care was taken to limit possible errors, there is still a possible source of human error.

5.6 SUGGESTIONS FOR FUTURE RESEARCH

Including the low dorsal pressure, all PP values used in this study resulted in a significant reduction of blood flow response. It would be valuable to investigate different portions of PP measured on each foot surface in-order to detect the pressure threshold when these significant changes begin to occur. Also, further work could be undertaken to conduct a comparison between the microvascular response at foot areas with increased pressure and those experiencing lower pressures on both aspects of the foot.

The diabetes group involved in this study were free from complications. It would be beneficial to investigate a larger and more variable group of patients with diabetes including subjects who are known to present with neuropathy and/or previous ulceration. Also, a larger sample containing more subjects of each gender would help in studying the influence of gender on the study outcomes.

An interesting area of further study would be to investigate comfort in therapeutic footwear as most of the previous work was conducted on sport and casual footwear. Comfort is an important factor that influences diabetic patients' compliance and adherence to the use of such footwear, which is essential to achieve their purpose of pressure relief and ulcer prevention (Maciejewski *et al.*, 2004, Jorgetto *et al.*, 2019).

Finally, it would be interesting to conduct a prospective study on the effect of custom-made footwear, designed to take into consideration a pre-assessment of the subject's dorsal and plantar foot pressure distribution, on the development of the first ulcer and re-ulceration prevention.

CONCLUSION

The current study has investigated foot pressure experienced on both dorsal and plantar surfaces of the foot within participants' own comfortable shoes and when wearing orthopaedic footwear, commonly prescribed for at-risk patients with diabetes. Findings were compared between subjects with Type 2 Diabetes Mellitus with no foot complications and an age-matched control group of subjects with no diabetes.

There were no significant differences between the two study groups in dorsal PP within the orthopaedic shoes as well as in participants' own shoes. However, both study groups had a significantly higher dorsal PP in their own shoes when compared with the orthopaedic shoes. The same relationship was noted in dorsal surface PTI and MF. Dorsal CA also showed no significant differences between groups in both tested shoe conditions. However, a significantly larger CA was recorded in the orthopaedic shoes in both study groups.

Timing of the dorsal PP during the gait cycle showed no significant differences between the two tested shoes in both study groups as well as between groups in both shoe conditions. The frequency of occurrence of dorsal PP showed a two peaks pattern with an increase at initial contact/loading response then at pre-swing/initial swing phases. A significant difference in the time of dorsal PP between the two tested shoes was noted when combining all participants' data as one group. More frequent peaks occurred later in the swing phase within the orthopaedic shoes. Shoe deformation with dorsiflexion of the foot during these phases of the gait cycle could be the reason for these two peaks.

The own shoes showed no significant differences between groups in total planter PP, although, midfoot areas were significantly higher in diabetes groups and lateral areas under toes and metatarsal heads were significantly higher in the control group.

Orthopaedic shoes showed a significantly higher total plantar PP in the diabetes group compared to the control group.

A significantly higher total in-shoe plantar PP within participants' own shoes was noted in both study groups. However, this significant difference was apparent in one foot area in the diabetes group and the medial midfoot area had a significantly higher PP within the orthopaedic shoes in the control group.

Although orthopaedic footwear significantly reduced in-shoe pressure, pressure measurement would be a crucial prerequisite to adjust shoe design as well as insole requirements in-order to better distribute dorsal and plantar pressures, thus achieving effective offloading essential for ulcer prevention.

Blood perfusion changes under loading pressure have been studied in response to the iontophoresis of acetylcholine (ACh) an endothelium-dependent vasodilator and sodium nitroprusside (SNP) an endothelium-independent vasodilator. Negative correlations have been generally noted, however, no significant associations were detected between PP and changes in blood flow in response to ACh or SNP under any of the testing pressure conditions on dorsal or plantar surfaces in both study groups.

Both study groups have shown a significant reduction in blood flow response to the iontophoresis of ACh and SNP from resting/no pressure condition, under own shoes as well as orthopaedic shoes' PP values on the dorsal and plantar surfaces of the foot.

Although own shoes' dorsal PP was significantly higher than orthopaedic shoes' PP in both study groups, the control group showed a significantly higher change in response from baseline under the orthopaedic shoes' dorsal PP with ACh and SNP while diabetes group only recorded a significant change in response with SNP use. Comparing differences between groups in blood flow response under no pressure, own shoes' PP and

orthopaedic shoes' PP on the dorsum of the foot revealed the only significant difference to be in blood flux changes in response to the iontophoresis of ACh under no pressure. These findings indicate an impairment in ACh endothelium-dependent vasodilation response on the foot dorsum in the diabetes group that would play an important role in the predisposition of ulceration and the development of complications on the foot dorsum.

The diabetes group showed significantly higher blood flux values on the plantar surface in response to ACh iontophoresis in resting /no pressure, under orthopaedic PP and own shoes PPs. However, no significant differences from the control group were detected in changes in response from baseline flux with the iontophoresis of ACh as well as SNP on the plantar surface under any of the three pressure conditions. Early sympathetic neuropathy could have impacted the plantar surface microvascular haemodynamics and resulted in the increased blood flux values recorded in the diabetes group.

Although low PP values were recorded on the foot dorsum, a significant reduction in blood flow response was present on applying dorsal PP. Also, the dorsal foot surface was more sensitive to changes related to endothelial dysfunction in patients with diabetes. Therefore, dorsal pressure measurement and its impact investigation can offer a reliable, accessible tool for the assessment of diabetic foot and should be considered in the design and prescription of therapeutic footwear to reduce the risk of diabetic foot ulceration.

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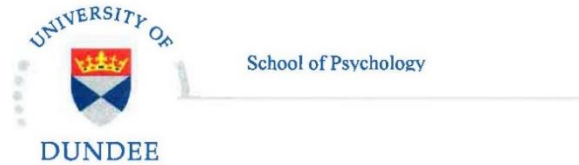
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APPENDIX 1



University of Dundee Research Ethics Committee

University of Dundee,
Dundee,
DD1 4HN.

6 November 2015

Dear Rania

Application Number: UREC 15161

Title: Assessment of the impact of loading pressure on endothelial function in diabetic foot

I am writing to you to advise you that your ethics application has been reviewed and approved by the University of Dundee Research Ethics Committee.

Approval is valid for three years from the date of this letter. Should your study continue beyond this point, please request a renewal of the approval.

Any changes to the approved documentation (e.g., study protocol, information sheet, consent form), must be approved by UREC.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'A. Schloerscheidt', on a light-colored rectangular background.

Dr Astrid Schloerscheidt
Chair, University of Dundee Research Ethics Committee

APPENDIX 2

Image © Warner Bros. Pictures The University of Dundee is a registered Scottish charity, No: SC015096



UNIVERSITY OF DUNDEE

Do you have HAPPY FEET?

VOLUNTEERS NEEDED FOR DIABETIC RESEARCH

Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

We are looking for volunteers to participate in research that we believe will contribute to the understanding of the development of foot ulcers in patients with diabetes mellitus. This will take place at the Institute of Motion Analysis and Research at Ninewells Hospital, Dundee. One visit lasting approximately two hours where in-shoe pressures and foot skin blood flow will be assessed. All information will be treated with the strictest confidence.

- Male or Female, aged 18 - 75
- In general good health
- Have no foot/leg/spinal problems

If you would like to volunteer or require further details please feel free to contact us at the contacts below.

This study has been reviewed and approved by the University of Dundee Research Ethics Committee




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01382 383520



imar@dundee.ac.uk
r.g.o.m.edris@dundee.ac.uk

APPENDIX 3

PARTICIPANT INFORMATION SHEET

Project title: Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

Invitation: You are being invited to take part in a research study, which we believe will contribute to a better understanding of the development of foot ulceration in patients with diabetes mellitus.

This study will be conducted by myself Rania Edris, a PhD candidate in the Department of Orthopaedic and Trauma Surgery at University of Dundee and under the supervision of Professor Rami Abboud and Dr Faisal Khan.

Before you decide whether or not you wish to participate, it is important to understand why the research is being carried out and what the study will involve. Please make sure you take the time to read the following provided information carefully and discuss it with others if you wish. If there is anything that is unclear or you would like more information, please do not hesitate to ask any questions. I will do my best to explain and provide any further information you may ask for now or later. You do not have to make an immediate decision. Take time to decide whether or not you wish to take part.

Purpose of the research study

Foot problems account for the most serious and costly complication of diabetes. The major adverse outcomes of diabetic foot are foot ulcers and amputations which are considered preventable as 85% of amputations are preceded by foot ulceration. Despite high rates of ulcers located on the top of diabetic foot most of the earlier studies and guidelines were focused on those on the sole of the foot. This study aims to investigate two of those factors which are thought to contribute to diabetic foot ulcers on the top as well as the sole of the foot. This will include changes which occur to small blood vessels found just under the skin surface, and the pressure placed on the foot by shoes. This study will investigate these two factors together trying to find out if there are differences in the way the small blood vessels respond under pressure with diabetes when compared to a group of people without diabetes. The activity of blood vessels will be assessed by placing a substance on the skin surface which when absorbed causes the small local vessels to enlarge temporarily and the amount of blood flowing through the vessels to increase. This increase in local blood flow can be measured on the skin surface by placing a probe against the skin.

The results of this project will add to the understanding of foot ulcers development and may help in designing better future therapeutic interventions to prevent diabetic foot ulceration.

What will happen if you decide to take part?

If you agree to participate you will be invited to the Institute of Motion Analysis and Research (IMAR) at Ninewells Hospital, Dundee for a single visit and asked to sign a consent form. **Note that you should eat breakfast or lunch as usual before travelling to the hospital, but avoid eating or drinking for 2 hours before the tests are carried out.** It is expected that this period of time will be taken up in the time it takes to travel to the hospital and in preparing you for the tests. In-shoe pressure measurement will be conducted into your own comfortable shoes and in a provided same size special shoes which is frequently prescribed to patients with diabetes. Two insoles will be used to perform the test. One will be placed on top of your foot inside socks which will be provided and another one under the sole

of your foot. Then you will be asked to walk at your normal pace 3 times along a 4 metre walkway. The insoles are connected wirelessly to a computer which is used for data analysis.

Then you will be asked to lie down on a couch. Equipment will be placed onto the top then the sole of your foot just behind your toes that will allow a jelly-like substance to be absorbed into your skin. A small pressure device will be placed against your skin and will place the skin under the same pressure measured in shoes when you were walking. A skin surface probe will be attached to your skin and will measure the amount of blood flowing through the surface blood vessels when they are under pressure. This process will be repeated using a second jelly-like substance also designed to expand your local small blood vessels. The process will take approximately 2 hours to complete where you will be asked to lie still. Time will be given between measurements for you to stretch or move around for your comfort.

We are planning to do this study on 2 phases. You will be contacted about the 2nd stage and asked if you wish to volunteer for it. If you decide to participate, an appointment will be assigned for you and you will be asked to sign a new consent form.

Risks

It is a safe procedure and there are no known risks or side effects with the equipment used in this study and all measurement systems are routinely used in clinical practice for patients. On occasion a mild tingling sensation can be felt at the start of the procedure, and if this should continue then the testing would be stopped.

Benefits

We cannot promise that this study will help you directly. However, your participation will be of great value in providing information that may help to prevent foot ulcers in the future for people with diabetes by providing a better understanding about why foot ulcers happen.

Expenses

Your participation in this study is entirely voluntary but any public transport travelling expenses for attending will be reimbursed.

Termination of participation

You are entirely free to choose if you want to participate or not. If you do not want to participate we respect your decision. During the project, if you decide to withdraw from the project, you are free to do so at any time without explanation or penalty and any collected data will be destroyed. A decision to withdraw at any time, or a decision not to take part, will not affect any future care you receive.

Confidentiality

All information about your participation in this study will be treated with strict confidentiality. The data collected will be coded and anonymous. No names will appear in any future publications or reports. All data will be stored on a secure server at the department of Orthopedics and Trauma Surgery. Only specific people, who are involved in the project, supervisory team to ensure that the study is being carried out correctly, will be able to access data. All individuals with access to data will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

For further information about this research study

If you have any issue or concerns regarding the project or you need any further information about the study and final results please do not hesitate to contact me at any time.

Rania Edris
TORT Centre, Level 6, Ninewells Hospital & Medical School, University of Dundee,
Dundee, DD1 9SY
E-mail: rgomedris@dundee.ac.uk
Tel: 01382383523

Supervisors' details:

Professor Rami Abboud PhD
Head of Department of Orthopaedic & Trauma Surgery
Director of Institute of Motion Analysis & Research (IMAR), University of Dundee
Email: r.j.abboud@dundee.ac.uk
Tel: 01382383502

Dr Faisel Khan PhD /Reader
Vascular & Inflammatory Diseases Research Unit
Institute of Cardiovascular Research
Ninewells Hospital & Medical School, University of Dundee
Email: f.khan@dundee.ac.uk
Tel: 01382383531

The University Research Ethics Committee of the University of Dundee has reviewed and approved this research study.

Thank you for considering taking part or taking time to read this information sheet. If you decide to take part in this study you will be given a copy of the information sheet and a signed consent form to keep.

APPENDIX 4

CONSENT FORM**Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot**

This study will investigate the effect of pressure on endothelial function in patients with diabetes in comparison with a normal control subjects.

By signing below you are indicating that you have read and understood the Participant Information Sheet and that you agree to take part in this research study.

Participant's signature

Date

Participant's name

Signature of person obtaining consent

Date

Name of person obtaining consent

"I agree to the use of anonymous extracts from this study
in conference papers and academic publications"

YES ☐ NO ☐

"I agree to video recording and digital photographs being
taken as part of this study"

YES ☐ NO ☐

"I agree to use of anonymous video and digital recordings
being used for teaching purposes and at academic meetings"

YES ☐ NO ☐

APPENDIX 5



Health Research Authority

London - Hampstead Research Ethics Committee

Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8009

18 February 2016

Prof Rami Abboud
Department of Orthopaedic and Trauma Surgery
Tayside Orthopaedic Rehabilitation Technology (TORT) Centre
Ninewells Hospital and Medical School, University of Dundee
DD1 9SY

Dear Prof Abboud

Study title: Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot
REC reference: 16/LO/0318
Protocol number: 2015DM18
IRAS project ID: 195922

Thank you for your letter of 11th February 2016. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 11 February 2016

Documents received

The documents received were as follows:

Document	Version	Date
Participant information sheet (PIS) [Participant Information Sheet]	1	10 February 2016
Participant information sheet (PIS) [Participant with Diabetes Information Sheet]	1	10 February 2016

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	Version	Date
Copies of advertisement materials for research participants [Poster]	1	01 February 2016
Covering letter on headed paper [Cover Letter]	1	02 February 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Sponsor letter]		
Letters of invitation to participant [Invitation Letter to patients]	1	02 February 2016
Other [Email Database Clarification]		
Other [Feedback Form]	1	02 February 2016
Participant consent form [Participant Consent]	1	02 February 2016
Participant information sheet (PIS) [Participant Information Sheet]	1	10 February 2016

Participant information sheet (PIS) [Participant with Diabetes Information Sheet]	1	10 February 2016
REC Application Form	5.2.1	21 January 2016
Research protocol or project proposal [Study protocol]	1	02 February 2016
Summary CV for Chief Investigator (CI) [Rami Abboud]		
Summary CV for student [Rania Edris]		
Summary CV for supervisor (student research) [Faisal Khan]		

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

16/LO/0318

Please quote this number on all correspondence

Yours sincerely



Amber Ecclestone
REC Assistant

E-mail: nrescommittee.london-hampstead@nhs.net

Copy to: *Mrs Natalie Smith*

APPENDIX 6



24 February 2016

Professor Rami Abboud
Head of Department of Orthopaedic & Trauma Surgery
Department of Orthopaedic & Trauma Surgery
TORT Centre
Ninewells Hospital and Medical School
Dundee
DD1 9SY

Dear Professor Abboud,

R&D MANAGEMENT APPROVAL – TAYSIDE

Title: Assessment of the impact of loading pressure on endothelial function in diabetic foot.

Chief Investigator: Professor Rami Abboud

Principal Investigator/Local Collaborator: Miss Rania Edris

Tayside Ref: 2015DM18 NRS Ref: N/A

REC Ref: 16/LO/0318

Sponsors: University of Dundee and NHS Tayside

Funder: Unfunded

Many thanks for your application to carry out the above project here in NHS Tayside. I am pleased to confirm that the project documentation (as outlined below) has been reviewed, registered and Management Approval has been granted for the study to proceed locally in Tayside.

Approval is granted on the following conditions:-

- ALL Research must be carried out in compliance with the Research Governance Framework for Health & Community Care, Health & Safety Regulations, data protection principles, statutory legislation and in accordance with Good Clinical Practice (GCP).
- All amendments to be notified to TASC R&D Office via the correct amendment pathway. Either direct to the R&D Office or via the Lead Co-ordinating Centre depending on how the study is set up (<http://www.hra.nhs.uk/nhshsc-rd-uk-process-management-amendments/>).
- All local researchers must hold either a Substantive Contract, Honorary Research Contract, Honorary Clinical Contract or Letter of Access with NHS Tayside where required (<http://www.nihr.ac.uk/policy-and-standards/research-passports.htm>).
- TASC R&D Office to be informed of change in Principal Investigator, Chief Investigator or any additional research personnel locally.
- Notification to TASC R&D Office of any change in funding.

- As custodian of the information collated during this research project you are responsible for ensuring the security of all personal information collected in line with NHS Scotland IT Security Policies, until destruction of this data.
- All eligible and adopted studies will be added to the UKCRN Portfolio database <http://public.ukcrn.org.uk/>. Recruitment figures for eligible and adopted studies must be recorded onto the Portfolio every month. This is the responsibility of the lead UK site. If you are the lead, or only UK site, we can provide help or advice with this. For information, contact Sarah Kennedy (01382 383882 or sarah.kennedy17@nhs.net) or Margaret Marshall (01382 383091 or margaret.marshall7@nhs.net).
- Annual reports are required to be submitted to TASC R&D Office with the first report due 12 months from date of issue of this management approval letter and at yearly intervals until completion of the study.
- Notification of early termination within 15 days or End of Trial within 90 days followed by End of Trial Report within 1 year to TASC R&D Office.
- You may be required to assist with and provide information in regard to audit and monitoring of study.

Please note you are required to adhere to the conditions, if not, NHS management approval may be withdrawn for the study.

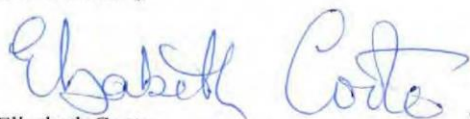
Approved Documents

Document	Version	Date
Protocol	1	02/02/16
Participant Information Sheet (No Diabetes)	1	10/02/16
Participant Information Sheet (Diabetes)	1	10/02/16
Consent Form	1	02/02/16
Invitation Letter	1	02/02/16
Study Participant Feedback Form	1	02/02/16
Poster	1	01/02/16
REC – Favourable Ethical Opinion Letter		10/02/16
REC – Evidence of Compliance Letter		18/02/16

May I take this opportunity to wish you every success with your project.

Please do not hesitate to contact TASC R&D Office should you require further assistance.

Yours sincerely



Elizabeth Coote
Head of Non-Commercial Research Services

Tayside medical Science Centre (TASC)
Ninewells Hospital & Medical School
TASC Research & Development Office
Residency Block, Level 3
George Pirie Way
Dundee DD1 9SY
Email: liz.coote@nhs.net
Tel: 01382 383876 Fax: 01382 740122

c.c. Miss Rania Edris
Ms Nikki Gribben
Ms Margaret Marshall
TASC Feasibility Team

APPENDIX 7



Health Research Authority

London - Hampstead Research Ethics Committee

Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Tel: 02071048127

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

24 March 2017

Prof Rami Abboud
Department of Orthopaedic and Trauma Surgery
Tayside Orthopaedic Rehabilitation Technology (TORT) Centre
Ninewells Hospital and Medical School, University of Dundee
DD1 9SY

Dear Prof Abboud

Study title:	Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot
REC reference:	16/LO/0318
Protocol number:	2015DM18
Amendment number:	01
Amendment date:	07 March 2017
IRAS project ID:	195922

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The Committee requested clarification from you that you sought to increase the study visits from 1 visit at 2 hours in length, to 2 visits at 3 hours in length (per visit). The Committee requested this be spelled out in the supporting information.

You confirmed and complied with this request. The Committee were happy to accept your revised documents.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants [Volunteer Recruitment Poster]	2	24 March 2017
Letters of invitation to participant [Invitation Letter]	2	24 March 2017
Notice of Substantial Amendment (non-CTIMP) [Amendment Form]		07 March 2017
Participant information sheet (PIS) [Participant Information Sheet]	2	24 March 2017
Participant information sheet (PIS) [Participant with Diabetes Information Sheet]	2	24 March 2017

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

16/LO/0318:	Please quote this number on all correspondence
--------------------	-------------------------------------------------------

Yours sincerely



Signed on behalf of
Miss Stephanie Ellis, BEM
Chair

E-mail: nrescommittee.london-hampstead@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Natalie Smith*

London - Hampstead Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 13 March 2017

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Miss Stephanie Ellis, BEM	Former Civil Servant	Yes	
Dr Alicia Isabel Etchegoyen Holiday	Psychiatrist	Yes	

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Miss Nafeesa Khanam	REC Assistant
Mr Matt Rogerson	REC Manager

APPENDIX 8



28 March 2017

Professor Rami J Abboud
 Honorary Clinical Scientist
 Institute of Motion Analysis & Research (IMAR)
 Department of Orthopaedic and Trauma Surgery
 TORT Centre
 Dundee
 DD1 9SY

Dear Professor Abboud,

ACCEPTANCE OF AMENDMENT LETTER

Title: Assessment of the impact of loading pressure on endothelial function in diabetic foot.

Chief Investigator: Professor Rami J Abboud

Principal Investigator/Local Collaborator: Miss Rania Edris

Tayside Ref: 2015DM18

REC Ref: 16/LO/0318

Amendment Number: 01 Amendment Date: 07 March 2017

Thank you for submitting the above amendment for review by the R&D Office here in NHS Tayside.

Following my assessment of the proposed changes I am pleased to confirm that NHS Tayside has no objection to these being implemented locally.

Approved Documents

Document	Version	Date
Copies of advertisement materials for research participants [Volunteer Recruitment Poster]	2	24 March 2017
Letter of invitation to participant [Invitation Letter]	2	24 March 2017
Notice of Substantial Amendment (non-CTIMP) [Amendment Form]		07 March 2017
Participant information sheet (PIS) [Participant Information Sheet]	2	24 March 2017
Participant information sheet (PIS) [Participant with Diabetes Information Sheet]	2	24 March 2017

Version 2.0 dated 06/01/15

Non-NRS Study Amendment Approval (Ethical-Regulatory Approvals in Place)

I thank you for keeping the R&D Office informed of the study progress.

Yours Sincerely



Elizabeth Coote
Head of Non-Commercial Research Services

Tayside medical Science Centre (TASC)
Ninewells Hospital & Medical School
TASC Research & Development Office
Residency Block, Level 3
George Pirie Way
Dundee DD1 9SY
Email: liz.coote@nhs.net
Tel: 01382 383876 Fax: 013812 740122

c.c. Rania Edris
Nikki Gribben

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UNIVERSITY OF DUNDEE

Do you have HAPPY FEET?

VOLUNTEERS NEEDED FOR DIABETIC RESEARCH

Assessment of the Impact of Loading Pressure
on Endothelial Function in Diabetic Foot

We are looking for volunteers to participate in research that we believe will contribute to the understanding of the development of foot ulcers in patients with diabetes mellitus. This will take place at the Institute of Motion Analysis and Research at Ninewells Hospital, Dundee. Two visits lasting approximately three hours each (6 hours in total) where in-shoe pressures and foot skin blood flow will be assessed. All information will be treated with the strictest confidence.

- Male or Female, aged 55 - 75
- In general good health and no heart problems
- Have no foot/leg/spinal problems

If you would like to volunteer or require further details please feel free to contact us at the contacts below.

This study has been reviewed and approved by the University of Dundee Research Ethics Committee and by the Tayside Committee on Medical Research Ethics

  **01382 383500**
01382 383520  **imar@dundee.ac.uk**
r.g.o.m.edris@dundee.ac.uk

V2 24/03/2017

APPENDIX 10

**Department of Public Health****Division of Population Health Sciences***Head of Section*
Professor I K Crombie

TO: Rania Edris
FROM: Professor Iain Crombie
DATE: 23 November 2015
TEL: Ext 83745 (01382 383745)
SUBJECT: Caldicott Guardian Approval

I have now considered your application for Caldicott Guardian Approval and I am very pleased to say that I am happy to grant this. You should retain a copy of this email for your project/portfolio as evidence of approval.

Yours sincerely

A handwritten signature in blue ink, appearing to read 'Iain Crombie'.

Professor Iain K Crombie

APPENDIX 11



TORT Centre, Level 6,
Ninewells Hospital & Medical School,
University of Dundee,
Dundee, DD1 9SY

Patient's Name
Patient's Address 1
Patient's Address 2
Patient's Town/City
Patient's Postcode

DATE

Dear

**Scottish Diabetes Research Network – Invitation to participate in a clinical research study:
Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot**

As someone who has registered with the SDRN Research Register, and kindly agreed to allow us to contact you if a suitable study arose, I would like to offer you the opportunity to participate in the above study. The study has received a favourable ethical opinion by London - Hampstead Research Ethics Committee and been approved by NHS Tayside Research and Development Department as well as being supported by the network. Please find enclosed invitation letter telling you about the study.

If you would like to take part, or would like to find out more about this study, or you do not wish to take part in the study we would be grateful if you could complete the attached cut off slip and return it to us in the enclosed stamped addressed envelope. If you prefer to speak with the researcher, Miss Rania Edris can be contacted on 01382383523.

Thank you for taking the time to read this letter and we look forward to hearing from you.

Yours sincerely

Professor R J Abboud
BEng, MSc, PhD, ILTM, SMIEEE, Hon FRCS (Eng.)
Head of Department, Orthopaedic & Trauma Surgery



PLEASE NOTE - COMPLETING THIS SLIP DOES NOT COMMIT YOU TO TAKING PART IN THE STUDY.

YES I would like to take part / find out more about the study.
(Please circle the appropriate answer.)

NO I do not wish to take part in the study.

NAME:

ADDRESS:

TEL. NUMBER:

MOBILE TEL NUMBER:

E-MAIL ADDRESS:

Signature.....

Date.....

APPENDIX 12



Department of Orthopaedic and Trauma Surgery

SCHOOL OF MEDICINE

March 24, 2017

[Click [here](#) and type recipient's address]

Dear Sir/Madam

As you will be aware patients with diabetes can sometimes develop problems with their feet. Foot problems in patients with diabetes can occur at differing rates and severity and can be related to circulation. We are contacting you to ask if you would consider volunteering to help us with our current project which will be looking at subtle changes in the blood flow through the skin when different in-shoe pressures are applied to the foot. A separate sheet containing full details regarding this project will be send to you if consider participation.

This is a non-invasive study which has no known side effects or problems. We hope our work will provide some valuable information about how small blood vessels function in diabetes. It will involve two visits to our laboratory in Ninewells Hospital lasting approximately three hours each (6 hours in total) and **travel costs will be refunded for the visit**. All you have to do at this stage is to fill in the return slip and send it in the Freepost addresses envelope provided.

Should you decide to volunteer, your cooperation and support for this study would be very much appreciated and would be a great contribution to ongoing research related to the lower limb and foot problems which can be associated with diabetes.

Yours sincerely

Professor R J Abboud BEng, MSc, PhD, ILTM, SMIEEE, Hon FRCS (Eng.)
Associate Dean for Learning & Teaching and Head of Postgraduate Division
Head of Department, Orthopaedic & Trauma Surgery
Director, Institute of Motion Analysis & Research (IMAR)
Tel: ++44-1382-425746
Fax: ++44-1382-496200
Email: r.j.abboud@dundee.ac.uk

V2 24/03/2017

Department of Orthopaedic and Trauma Surgery

Tayside Orthopaedic and Rehabilitation Technology (TORT) Centre, Postgraduate Division, School of Medicine
Ninewells Hospital and Medical School, University of Dundee, DD1 9SY, Scotland, United Kingdom

tel ++44 (0)1382 383500 email ortho@dundee.ac.uk fax ++44 (0)1382 383501
www.orthopaedics.dundee.ac.uk • www.facebook.com/ortho.dundee

The University of Dundee is a Scottish Registered Charity, No. SC015096



Department of Orthopaedic and Trauma Surgery

SCHOOL OF MEDICINE

RETURN SUP

I am happy to participate in the research project entitled

Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

Full Name	
Telephone Number	
Mobile number	
Email address	
Postal address	

Please return to:

Mrs Sheila MacDonald
 Institute of Motion Analysis and Research (IMAR)
 University Department of Orthopaedics and Trauma Surgery
 Tayside Orthopaedic and Rehabilitation Technology (TORT) Centre
 Ninewells Hospital and Medical School
 Dundee DD1 9SY
 Email: s.a.z.macdonald@dundee.ac.uk

V2 24/03/2017

Department of Orthopaedic and Trauma Surgery

Tayside Orthopaedic and Rehabilitation Technology (TORT) Centre, Postgraduate Division, School of Medicine
 Ninewells Hospital and Medical School, University of Dundee, DD1 9SY, Scotland, United Kingdom

tel ++44 (0)1382 383500 email ortho@dundee.ac.uk fax ++44 (0)1382 383501
www.orthopaedics.dundee.ac.uk • www.facebook.com/ortho.dundee

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APPENDIX 13



PARTICIPANT INFORMATION SHEET

Project title: Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

Invitation: You have been invited as someone who does not have diabetes to take part in this research study, which we believe will contribute to a better understanding of the development of foot ulceration in patients with diabetes mellitus.

This study will be conducted by myself Rania Edris, a PhD candidate in the Department of Orthopaedic and Trauma Surgery at University of Dundee and under the supervision of Professor Rami Abboud and Dr Faisal Khan.

Before you decide whether or not you wish to participate, it is important to understand why the research is being carried out and what the study will involve. Please make sure you take the time to read the following provided information carefully and discuss it with others if you wish. If there is anything that is unclear or you would like more information, please do not hesitate to ask any questions. I will do my best to explain and provide any further information you may ask for now or later. You do not have to make an immediate decision. Take time to decide whether or not you wish to take part.

Purpose of the research study

Foot problems account for the most serious and costly complication of diabetes. The major adverse outcomes of diabetic foot are foot ulcers and amputations which are considered preventable as 85% of amputations are preceded by foot ulceration. Despite high rates of ulcers located on the top of diabetic foot most of the earlier studies and guidelines were focused on those on the sole of the foot. This study aims to investigate two of those factors which are thought to contribute to diabetic foot ulcers on the top as well as the sole of the foot. This will include changes which occur to small blood vessels found just under the skin surface, and the pressure placed on the foot by shoes. This study will investigate these two factors together trying to find out if there are differences in the way the small blood vessels respond under pressure with diabetes when compared to a group of people without diabetes. The activity of blood vessels will be assessed by placing a substance on the skin surface which when absorbed causes the small local vessels to enlarge temporarily and the amount of blood flowing through the vessels to increase. This increase in local blood flow can be measured on the skin surface by placing a probe against the skin.

The results of this project will add to the understanding of foot ulcers development and may help in designing better future therapeutic interventions to prevent diabetic foot ulceration.

What will happen if you decide to take part?

If you agree to participate you will be invited to the Institute of Motion Analysis and Research (IMAR) at Ninewells Hospital, Dundee for two visits and asked to sign a consent form. **Note that you should have breakfast or lunch as usual before travelling to hospital, but please avoid eating any food or drinking caffeine containing drinks or other beverages during the 2 hours prior to testing. Please note that it is only ordinary water can be ingested in the 2 hours before testing.** It is expected that this period of time will be taken up in the time it takes to travel to the hospital and in preparing you for the tests and refreshments will be offered after.



First visit will include In-shoe pressure measurement into your own comfortable shoes and in a provided same size special shoes which is frequently prescribed to patients with diabetes. Two insoles will be used to perform the test. One will be placed on top of your foot inside socks which will be provided and another one under the sole of your foot. Then you will be asked to walk at your normal pace 5 times along a 4 metre walkway. The insoles are connected wirelessly to a computer which is used for data analysis.

Then you will be asked to lie down on a couch. Equipment will be placed onto the top of your right foot just behind your toes that will allow a jelly-like substance to be absorbed into your skin. A small pressure device will be placed against your skin and will place the skin under the same pressure measured in shoes when you were walking. A skin surface probe will be attached to your skin and will measure the amount of blood flowing through the surface blood vessels when they are under pressure. This process will be repeated using a second jelly-like substance also designed to expand your local small blood vessels. At your second visit same process will be repeated on the sole of your foot. The procedure will take approximately **3 hours per session (6 hours in total)** to be completed where you will be asked to lie still. Time will be given between measurements for you to stretch or move around for your comfort.

Risks

It is a safe procedure and there are no known risks or side effects with the equipment used in this study and all measurement systems are routinely used in clinical practice for patients. On occasion a mild tingling sensation can be felt at the start of the procedure, and if this should continue then the testing would be stopped.

If something wrong happen or you have a concern about any aspect of this study, you should ask the chief researcher Rania Edris who will do her best to answer your questions. If you remain unhappy need any further information or wish to complain formally, you can contact Prof Rami Abboud, Director of the Institute of Motion Analysis and Research Ninewells Hospital Dundee DD1 9SY Tel no. 01382383502.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against University of Dundee but you may have to pay your legal costs.

Confidentiality

All information about your participation in this study will be treated with strict confidentiality. The data collected will be coded and anonymous. No names will appear in any future publications or reports. All data will be stored on a secure server at the department of Orthopedics and Trauma Surgery. Only specific people, who are involved in the project, supervisory team to ensure that the study is being carried out correctly, will be able to access data. All individuals with access to data will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

Benefits

We cannot promise that this study will help you directly. However, your participation will be of great value in providing information that may help to prevent foot ulcers in the future for people with diabetes by providing a better understanding about why foot ulcers happen.



Expenses

Your participation in this study is entirely voluntary but any public transport travelling expenses for attending will be reimbursed.

Termination of participation

You are entirely free to choose if you want to participate or not. If you do not want to participate we respect your decision. During the project, if you decide to withdraw from the project, you are free to do so at any time without explanation or penalty and any collected data will be destroyed. A decision to withdraw at any time, or a decision not to take part, will not affect any future care you receive.

For further information about this research study

If you have any issue or concerns regarding the project or you need any further information about the study and final results please do not hesitate to contact me at any time.

Rania Edris
TORT Centre, Level 6, Ninewells Hospital & Medical School, University of Dundee,
Dundee, DD1 9SY
E-mail: rgomedris@dundee.ac.uk
Tel: 01382383523

Supervisors' details:

Professor Rami Abboud PhD
Head of Department of Orthopaedic & Trauma Surgery
Director of Institute of Motion Analysis & Research (IMAR), University of Dundee
Email: r.j.abboud@dundee.ac.uk
Tel: 01382383502

Dr Faisal Khan PhD /Reader
Vascular & Inflammatory Diseases Research Unit
Institute of Cardiovascular Research
Ninewells Hospital & Medical School, University of Dundee
Email: f.khan@dundee.ac.uk
Tel: 01382383531

University of Dundee Research Ethics Committee has reviewed and approved this research study. Tayside Committee on Medical Research Ethics, which is responsible for scrutinizing proposals for clinical research has examined this proposal and has raised no objections from the point of view of medical ethics. It is a requirement that the research records are made available to monitors from NHS Tayside whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.

Thank you for considering taking part or taking time to read this information sheet. If you decide to take part in this study you will be given a copy of the information sheet and a signed consent form to keep.

APPENDIX 14



PARTICIPANT INFORMATION SHEET

Project title: Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

Invitation: You have been invited as someone who have diabetes to take part in this research study, which we believe will contribute to a better understanding of the development of foot ulceration in patients with diabetes mellitus.

This study will be conducted by myself Rania Edris, a PhD candidate in the Department of Orthopaedic and Trauma Surgery at University of Dundee and under the supervision of Professor Rami Abboud and Dr Faisal Khan.

Before you decide whether or not you wish to participate, it is important to understand why the research is being carried out and what the study will involve. Please make sure you take the time to read the following provided information carefully and discuss it with others if you wish. If there is anything that is unclear or you would like more information, please do not hesitate to ask any questions. I will do my best to explain and provide any further information you may ask for now or later. You do not have to make an immediate decision. Take time to decide whether or not you wish to take part.

Purpose of the research study

Foot problems account for the most serious and costly complication of diabetes. The major adverse outcomes of diabetic foot are foot ulcers and amputations which are considered preventable as 85% of amputations are preceded by foot ulceration. Despite high rates of ulcers located on the top of diabetic foot most of the earlier studies and guidelines were focused on those on the sole of the foot. This study aims to investigate two of those factors which are thought to contribute to diabetic foot ulcers on the top as well as the sole of the foot. This will include changes which occur to small blood vessels found just under the skin surface, and the pressure placed on the foot by shoes. This study will investigate these two factors together trying to find out if there are differences in the way the small blood vessels respond under pressure with diabetes when compared to a group of people without diabetes. The activity of blood vessels will be assessed by placing a substance on the skin surface which when absorbed causes the small local vessels to enlarge temporarily and the amount of blood flowing through the vessels to increase. This increase in local blood flow can be measured on the skin surface by placing a probe against the skin.

The results of this project will add to the understanding of foot ulcers development and may help in designing better future therapeutic interventions to prevent diabetic foot ulceration.

What will happen if you decide to take part?

If you agree to participate you will be invited to the Institute of Motion Analysis and Research (IMAR) at Ninewells Hospital, Dundee for two visits and asked to sign a consent form. **Note that you should have breakfast or lunch as usual before travelling to hospital, but please avoid eating any food or drinking caffeine containing drinks or other beverages during the 2 hours prior to testing. Please note that it is only ordinary water can be ingested in the 2 hours before testing.** It is expected that this period of time will be taken up in the time it takes to travel to the hospital and in preparing you for the tests and refreshments will be offered after.



First visit will include In-shoe pressure measurement into your own comfortable shoes and in a provided same size special shoes which is frequently prescribed to patients with diabetes. Two insoles will be used to perform the test. One will be placed on top of your foot inside socks which will be provided and another one under the sole of your foot. Then you will be asked to walk at your normal pace 5 times along a 4 metre walkway. The insoles are connected wirelessly to a computer which is used for data analysis.

Then you will be asked to lie down on a couch. Equipment will be placed onto the top of your right foot just behind your toes that will allow a jelly-like substance to be absorbed into your skin. A small pressure device will be placed against your skin and will place the skin under the same pressure measured in shoes when you were walking. A skin surface probe will be attached to your skin and will measure the amount of blood flowing through the surface blood vessels when they are under pressure. This process will be repeated using a second jelly-like substance also designed to expand your local small blood vessels. At your second visit same process will be repeated on the sole of your foot. The procedure will take approximately **3 hours per session (6 hours in total)** to be complete where you will be asked to lie still. Time will be given between measurements for you to stretch or move around for your comfort.

Please review your GP/diabetician about your participation and the needed fasting period (approx. 5 hours), any diet and diabetes medications changes/arrangement you should follow before and after the study session prior to your visit. In case you feel unwell or have a low blood sugar we would reschedule the appointment. For your safety your blood sugars will be checked before leaving the department by a qualified nurse from our team.

Risks

It is a safe procedure and there are no known risks or side effects with the equipment used in this study and all measurement systems are routinely used in clinical practice for patients. On occasion a mild tingling sensation can be felt at the start of the procedure, and if this should continue then the testing would be stopped.

If something wrong happen or you have a concern about any aspect of this study, you should ask the chief researcher Rania Edris who will do her best to answer your questions. If you remain unhappy need any further information or wish to complain formally, you can contact Prof Rami Abboud, Director of the Institute of Motion Analysis and Research Ninewells Hospital Dundee DD1 9SY Tel no. 01382383502.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against University of Dundee but you may have to pay your legal costs.

Confidentiality

All information about your participation in this study will be treated with strict confidentiality. The data collected will be coded and anonymous. No names will appear in any future publications or reports. All data will be stored on a secure server at the department of Orthopedics and Trauma Surgery. Only specific people, who are involved in the project, supervisory team to ensure that the study is being carried out correctly, will be able to access data. All individuals with access to data will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.



Benefits

We cannot promise that this study will help you directly. However, your participation will be of great value in providing information that may help to prevent foot ulcers in the future for people with diabetes by providing a better understanding about why foot ulcers happen.

Expenses

Your participation in this study is entirely voluntary but any public transport travelling expenses for attending will be reimbursed.

Termination of participation

You are entirely free to choose if you want to participate or not. If you do not want to participate we respect your decision. During the project, if you decide to withdraw from the project, you are free to do so at any time without explanation or penalty and any collected data will be destroyed. A decision to withdraw at any time, or a decision not to take part, will not affect any future care you receive.

For further information about this research study

If you have any issue or concerns regarding the project or you need any further information about the study and final results please do not hesitate to contact me at any time.

Rania Edris

TORT Centre, Level 6, Ninewells Hospital & Medical School, University of Dundee,
Dundee, DD1 9SY

E-mail: rgomedris@dundee.ac.uk

Tel: 01382383523

Supervisors' details:

Professor Rami Abboud PhD

Head of Department of Orthopaedic & Trauma Surgery

Director of Institute of Motion Analysis & Research (IMAR), University of Dundee

Email: r.j.abboud@dundee.ac.uk

Tel: 01382383502

Dr Faisal Khan PhD /Reader

Vascular & Inflammatory Diseases Research Unit

Institute of Cardiovascular Research

Ninewells Hospital & Medical School, University of Dundee

Email: f.khan@dundee.ac.uk

Tel: 01382383531

University of Dundee Research Ethics Committee has reviewed and approved this research study. Tayside Committee on Medical Research Ethics, which is responsible for scrutinizing proposals for clinical research has examined this proposal and has raised no objections from the point of view of medical ethics. It is a requirement that the research records are made available to monitors from NHS Tayside whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.

Thank you for considering taking part or taking time to read this information sheet. If you decide to take part in this study you will be given a copy of the information sheet and a signed consent form to keep.

APPENDIX 15



Participant Identification Number for this study:



CONSENT FORM

Assessment of the Impact of Loading Pressure on Endothelial Function in Diabetic Foot

Name of Researcher: Rania Edris

Please initial box

1. I confirm that I have read the information sheet dated..... (Version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from Institute of Motion Analysis and Research (IMAR), where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
5. I agree to the use of anonymous extracts from this study in conference papers and academic publications. ☐
6. I agree to video recording and digital photographs being taken as part of this study. ☐
7. I agree to use of anonymous video and digital recordings being used for teaching purposes and at academic meetings. ☐
8. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature

_____	_____	_____
Name of Person taking consent	Date	Signature

V1: 02/02/2016

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

APPENDIX 16

Tables for results of the study protocol application**4.2 In-Shoe Foot Pressure Assessment**

- 4.2.1 Peak Pressure: Table 1-4
- 4.2.2 Pressure Time Integral: Tables 5-8
- 4.2.3 Contact Area: Tables 9-12
- 4.2.4 Maximum Force: Tables 13-16
- 4.2.5 Time for Peak Pressure: Tables 17-19

4.3 Assessment of the Impact of Pressure on Endothelial Function

- 4.3.1 Comparison of Flux Values Under the Three Tested Pressure Conditions Within the Study Groups
 - 4.3.1.1 Blood Flow Changes in Control Group: Tables 20-27
 - 4.3.1.2 Blood Flow Changes in Diabetes Group: Tables 28-35
- 4.3.2 Comparison Between Control Group and Diabetes Group Flux Values
 - 4.3.2.1 Changes on the Dorsal Surface: Tables 36-47
 - 4.3.2.2 Changes on the Plantar Surface: Tables 48-59
- 4.3.3 Correlation of Peak Pressure with Blood Flux Values: Tables 60-91

Table 1 Comparison between Orthopaedic Shoes and Own Shoes PP (KPa) in control group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	21.21	12.94	4.17	50	<0.001**
	Own Shoes	31.63	23.54	4.17	88.33	
DT Lat/M2	Orthopaedic Shoes	32.33	14.49	5.83	81.67	<0.001**
	Own Shoes	46.76	22.60	10.83	96.67	
DMH1/M3	Orthopaedic Shoes	30.27	13.87	5.83	63.33	<0.001**
	Own Shoes	55.51	27.94	9.17	133.33	
DMH2/M4	Orthopaedic Shoes	21.48	12.40	4.17	61.67	<0.001**
	Own Shoes	40.25	27.65	5.83	121.67	
DMH3/M5	Orthopaedic Shoes	23.12	15.65	4.17	83.33	<0.001**
	Own Shoes	32.75	19.70	4.17	86.67	
DMH4/M6	Orthopaedic Shoes	28.94	14.76	4.17	75.83	<0.001**
	Own Shoes	36.24	19.12	5.83	92.5	
DMH5/M7	Orthopaedic Shoes	23.62	13.36	6.67	58.33	<0.001**
	Own Shoes	33.44	20.73	5	78.33	
DM midfoot/M8	Orthopaedic Shoes	36.76	16.04	10	77.5	0.023*
	Own Shoes	42.14	21.78	8.33	103.33	
DL midfoot/M9	Orthopaedic Shoes	24.34	14.04	8.33	64.17	0.009*
	Own Shoes	30.27	21.80	5.83	87.5	
PT1/ M10	Orthopaedic Shoes	222.23	53.07	130	360	0.164
	Own Shoes	232.48	65.52	132.5	432.5	
PT2/M11	Orthopaedic Shoes	150.72	36.81	57.5	255	0.043*
	Own Shoes	161.80	50.74	75	352.5	
PT3/M12	Orthopaedic Shoes	130.38	30.83	57.5	197.5	0.004*
	Own Shoes	142.44	36.47	67.5	250	
PMH1/M13	Orthopaedic Shoes	218.69	49.28	130	352.5	0.007*
	Own Shoes	241.55	82.79	97.5	542.5	
PMH2/M14	Orthopaedic Shoes	205.95	45.68	130	310	<0.001**
	Own Shoes	232.69	64.53	117.5	432.5	
PMH3/M15	Orthopaedic Shoes	182.22	41.35	100	287.5	<0.001**
	Own Shoes	224.34	70.80	102.5	432.5	
PMH4/M16	Orthopaedic Shoes	149.58	39.38	82.5	262.5	<0.001**
	Own Shoes	188.60	63.30	92.5	337.5	
PMH5/M17	Orthopaedic Shoes	130.98	48.43	42.5	320	<0.001**
	Own Shoes	160.40	68.79	55	405	
PM midfoot/M18	Orthopaedic Shoes	63.69	28.95	7.5	145	0.002*
	Own Shoes	53.47	24.91	10	117.5	
PL midfoot/M19	Orthopaedic Shoes	98.48	31.28	20	187.5	0.310
	Own Shoes	104.19	56.27	17.5	310	
PM heel/M20	Orthopaedic Shoes	233.94	48.77	147.5	377.5	0.814
	Own Shoes	235.61	65.30	117.5	512.5	
PL heel/M21	Orthopaedic Shoes	235.78	46.22	140	380	0.660
	Own Shoes	238.86	66.11	130	435	
Whole Dorsal surface PP (KPa)	Orthopaedic Shoes	49.22	14.05	20	83.33	<0.001**
	Own Shoes	70.65	23.96	25.00	133.33	
Whole Plantar surface PP (KPa)	Orthopaedic Shoes	275.25	44.05	190	380	<0.001**
	Own Shoes	307.77	72.32	202.5	542.5	

p >0.05 Not Significant (NS); *p <0.05 Significant (S); **p <0.001 Highly Significant (HS)

Table 2 Comparison between Orthopaedic Shoes and Own Shoes PP (KPa) in diabetes group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	21.59	14.49	1.67	64.17	0.222
	Own Shoes	24.65	21.59	7.5	146.67	
DT Lat/M2	Orthopaedic Shoes	28.62	13.72	10.83	64.17	0.009*
	Own Shoes	33.33	12.78	9.17	61.67	
DMH1/M3	Orthopaedic Shoes	37.57	14.40	12.5	64.17	<0.001**
	Own Shoes	65.43	32.86	25.83	162.5	
DMH2/M4	Orthopaedic Shoes	26.99	13.60	8.33	58.33	0.004*
	Own Shoes	33.86	20.08	5.83	100	
DMH3/M5	Orthopaedic Shoes	21.63	8.81	4.17	47.5	<0.001**
	Own Shoes	27.34	16.11	4.17	62.5	
DMH4/M6	Orthopaedic Shoes	25.77	9.62	5.83	45	<0.001**
	Own Shoes	32.49	16.04	0	74.17	
DMH5/M7	Orthopaedic Shoes	16.59	6.61	5.83	37.5	<0.001**
	Own Shoes	27.31	15.19	7.5	56.67	
DM midfoot/M8	Orthopaedic Shoes	41.01	13.46	12.5	66.67	<0.001**
	Own Shoes	52.10	24.84	20	131.67	
DL midfoot/M9	Orthopaedic Shoes	23.94	10.27	5.83	45	<0.001**
	Own Shoes	31.37	12.89	9.17	50.83	
PT1/M10	Orthopaedic Shoes	195.53	58.12	87.5	355	0.040*
	Own Shoes	215.97	84.85	47.5	412.5	
PT2/M11	Orthopaedic Shoes	130.37	43.61	55	212.5	0.186
	Own Shoes	139.40	55.68	47.5	307.5	
PT3/M12	Orthopaedic Shoes	97.06	33.20	40	197.5	0.673
	Own Shoes	95.09	35.15	25	177.5	
PMH1/M13	Orthopaedic Shoes	232.89	66.90	117.5	402.5	0.067
	Own Shoes	253.56	95.54	115	555	
PMH2/M14	Orthopaedic Shoes	221.41	44.68	150	347.5	0.140
	Own Shoes	231.23	52.35	157.5	362.5	
PMH3/M15	Orthopaedic Shoes	189.35	44.08	110	282.5	0.207
	Own Shoes	197.22	47.24	112.5	302.5	
PMH4/M16	Orthopaedic Shoes	162.25	55.02	72.5	332.5	0.477
	Own Shoes	157.38	44.73	80	292.5	
PMH5/M17	Orthopaedic Shoes	132.06	64.56	42.5	365	0.250
	Own Shoes	141.39	53.94	37.5	265	
PM midfoot/M18	Orthopaedic Shoes	74.26	33.14	32.5	230	0.444
	Own Shoes	71.00	29.35	22.5	145	
PL midfoot/M19	Orthopaedic Shoes	116.92	52.65	52.5	332.5	0.195
	Own Shoes	125.81	47.81	47.5	295	
PM heel/M20	Orthopaedic Shoes	249.26	40.56	172.5	345	0.295
	Own Shoes	242.29	55.80	155	357.5	
PL heel/M21	Orthopaedic Shoes	257.99	45.13	167.5	467.5	0.162
	Own Shoes	246.94	68.15	150	542.5	
Whole Dorsal surface PP (KPa)	Orthopaedic Shoes	47.80	12.10	16.67	66.67	<0.001**
	Own Shoes	73.84	33.27	28.33	162.50	
Whole Plantar surface PP (KPa)	Orthopaedic Shoes	290.32	49.57	195.00	467.50	0.006*
	Own Shoes	316.64	85.54	180.00	555.00	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 3 Comparison between control group and diabetes group PP (KPa) in Own Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	31.63	23.54	4.17	88.33	0.019*
	Diabetes	24.65	21.59	7.5	146.67	
DT Lat/M2	Control	46.76	22.60	10.83	96.67	<0.001**
	Diabetes	33.33	12.78	9.17	61.67	
DMH1/M3	Control	55.51	27.94	9.17	133.33	0.012*
	Diabetes	65.43	32.86	25.83	162.5	
DMH2/M4	Control	40.25	27.65	5.83	121.67	0.046*
	Diabetes	33.86	20.08	5.83	100	
DMH3/M5	Control	32.75	19.70	4.17	86.67	0.023*
	Diabetes	27.34	16.11	4.17	62.5	
DMH4/M6	Control	36.24	19.12	5.83	92.5	0.106
	Diabetes	32.49	16.04	0	74.17	
DMH5/M7	Control	33.44	20.73	5	78.33	0.011*
	Diabetes	27.31	15.19	7.5	56.67	
DM midfoot/M8	Control	42.14	21.78	8.33	103.33	<0.001**
	Diabetes	52.10	24.84	20	131.67	
DL midfoot/M9	Control	30.27	21.80	5.83	87.5	0.642
	Diabetes	31.37	12.89	9.17	50.83	
PT1/M10	Control	232.48	65.52	132.5	432.5	0.090
	Diabetes	215.97	84.85	47.5	412.5	
PT2/M11	Control	161.80	50.74	75	352.5	<0.001**
	Diabetes	139.40	55.68	47.5	307.5	
PT3/M12	Control	142.44	36.47	67.5	250	<0.001**
	Diabetes	95.09	35.15	25	177.5	
PMH1/M13	Control	241.55	82.79	97.5	542.5	0.298
	Diabetes	253.56	95.54	115	555	
PMH2/M14	Control	232.69	64.53	117.5	432.5	0.850
	Diabetes	231.23	52.35	157.5	362.5	
PMH3/M15	Control	224.34	70.80	102.5	432.5	<0.001**
	Diabetes	197.22	47.24	112.5	302.5	
PMH4/M16	Control	188.60	63.30	92.5	337.5	<0.001**
	Diabetes	157.38	44.73	80	292.5	
PMH5/M17	Control	160.40	68.79	55	405	0.019*
	Diabetes	141.39	53.94	37.5	265	
PM midfoot/M18	Control	53.47	24.91	10	117.5	<0.001**
	Diabetes	71.00	29.35	22.5	145	
PL midfoot/M19	Control	104.19	56.27	17.5	310	0.002*
	Diabetes	125.81	47.81	47.5	295	
PM heel/M20	Control	235.61	65.30	117.5	512.5	0.401
	Diabetes	242.29	55.80	155	357.5	
PL heel/M21	Control	238.86	66.11	130	435	0.354
	Diabetes	246.94	68.15	150	542.5	
Whole Dorsal surface PP (KPa)	Control	70.65	23.96	25.00	133.33	0.389
	Diabetes	73.84	33.27	28.33	162.50	
Whole Plantar surface PP (KPa)	Control	307.77	72.32	202.5	542.5	0.384
	Diabetes	316.64	85.54	180	555	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 4 Comparison between control group and diabetes group PP (KPa) in Orthopaedic Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	21.21	12.94	4.17	50	0.832
	Diabetes	21.59	14.49	1.67	64.17	
DT Lat/M2	Control	32.33	14.49	5.83	81.67	0.044*
	Diabetes	28.62	13.72	10.83	64.17	
DMH1/M3	Control	30.27	13.87	5.83	63.33	<0.001**
	Diabetes	37.57	14.40	12.5	64.17	
DMH2/M4	Control	21.48	12.40	4.17	61.67	<0.001**
	Diabetes	26.99	13.60	8.33	58.33	
DMH3/M5	Control	23.12	15.65	4.17	83.33	0.379
	Diabetes	21.63	8.81	4.17	47.5	
DMH4/M6	Control	28.94	14.76	4.17	75.83	0.056
	Diabetes	25.77	9.62	5.83	45	
DMH5/M7	Control	23.62	13.36	6.67	58.33	<0.001**
	Diabetes	16.59	6.61	5.83	37.5	
DM midfoot/M8	Control	36.76	16.04	10	77.5	0.029*
	Diabetes	41.01	13.46	12.5	66.67	
DL midfoot/M9	Control	24.34	14.04	8.33	64.17	0.804
	Diabetes	23.94	10.27	5.83	45	
PT1/M10	Control	222.23	53.07	130	360	<0.001**
	Diabetes	195.53	58.12	87.5	355	
PT2/M11	Control	150.72	36.81	57.5	255	<0.001**
	Diabetes	130.37	43.61	55	212.5	
PT3/M12	Control	130.38	30.83	57.5	197.5	<0.001**
	Diabetes	97.06	33.20	40	197.5	
PMH1/M13	Control	218.69	49.28	130	352.5	0.060
	Diabetes	232.89	66.90	117.5	402.5	
PMH2/M14	Control	205.95	45.68	130	310	0.009*
	Diabetes	221.41	44.68	150	347.5	
PMH3/M15	Control	182.22	41.35	100	287.5	0.198
	Diabetes	189.35	44.08	110	282.5	
PMH4/M16	Control	149.58	39.38	82.5	262.5	0.039*
	Diabetes	162.25	55.02	72.5	332.5	
PMH5/M17	Control	130.98	48.43	42.5	320	0.883
	Diabetes	132.06	64.56	42.5	365	
PM midfoot/M18	Control	63.69	28.95	7.5	145	0.009*
	Diabetes	74.26	33.14	32.5	230	
PL midfoot/M19	Control	98.48	31.28	20	187.5	<0.001**
	Diabetes	116.92	52.65	52.5	332.5	
PM heel/M20	Control	233.94	48.77	147.5	377.5	0.011*
	Diabetes	249.26	40.56	172.5	345	
PL heel/M21	Control	235.78	46.22	140	380	<0.001**
	Diabetes	257.99	45.13	167.5	467.5	
Whole Dorsal surface PP (KPa)	Control	49.22	14.05	20.00	83.33	0.409
	Diabetes	47.80	12.10	16.67	66.67	
Whole Plantar surface PP (KPa)	Control	275.25	44.05	190	380	0.013*
	Diabetes	290.32	49.57	195	467.5	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 5 Comparison between Orthopaedic Shoes and Own Shoes in PTI (KPa.s) in control group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	25.34	14.19	4.52	68.23	0.108
	Own Shoes	29.75	28.12	3.75	132.15	
DT Lat/M2	Orthopaedic Shoes	56.47	35.63	7.65	145.92	0.047*
	Own Shoes	70.37	71.65	4.68	369.9	
DMH1/M3	Orthopaedic Shoes	31.54	15.44	1.38	80.05	<0.001**
	Own Shoes	78.10	73.32	10.95	303.02	
DMH2/M4	Orthopaedic Shoes	25.95	13.70	6.73	72.52	<0.001**
	Own Shoes	37.29	27.65	5.67	160.62	
DMH3/M5	Orthopaedic Shoes	21.33	20.29	0.38	109.83	<0.001**
	Own Shoes	34.20	34.31	3.95	199.38	
DMH4/M6	Orthopaedic Shoes	38.32	23.69	4.65	113.15	0.380
	Own Shoes	41.67	36.84	3.32	218.4	
DMH5/M7	Orthopaedic Shoes	39.19	25.57	5.9	113.15	0.094
	Own Shoes	47.76	52.84	1.75	296.82	
DM midfoot/M8	Orthopaedic Shoes	79.39	43.87	14.38	207.78	0.032*
	Own Shoes	95.59	74.50	17.95	321.2	
DL midfoot/M9	Orthopaedic Shoes	49.33	29.72	9.73	149.63	0.284
	Own Shoes	54.23	43.18	10.92	194.72	
PT1/ M10	Orthopaedic Shoes	243.13	92.40	87.85	543.9	<0.001**
	Own Shoes	204.12	99.87	57.35	629.05	
PT2/M11	Orthopaedic Shoes	178.29	70.72	69	413.5	0.021*
	Own Shoes	159.14	62.73	45.15	378.85	
PT3/M12	Orthopaedic Shoes	176.18	58.33	66.05	356.6	0.072
	Own Shoes	163.23	58.22	48.65	332.5	
PMH1/M13	Orthopaedic Shoes	265.03	78.56	117.35	595.4	0.176
	Own Shoes	248.59	114.75	97.1	661.05	
PMH2/M14	Orthopaedic Shoes	280.88	76.78	113.05	449.25	0.077
	Own Shoes	262.57	90.09	114	536.8	
PMH3/M15	Orthopaedic Shoes	260.99	72.47	118.95	430.15	0.282
	Own Shoes	250.50	85.09	114.25	487.6	
PMH4/M16	Orthopaedic Shoes	219.05	77.01	93.1	388.65	0.172
	Own Shoes	232.58	83.41	121.85	460.5	
PMH5/M17	Orthopaedic Shoes	217.53	81.37	57.65	425.25	0.364
	Own Shoes	227.73	99.77	64.3	592.4	
PM midfoot/M18	Orthopaedic Shoes	92.31	49.77	11.7	238.9	<0.001**
	Own Shoes	69.19	36.06	4.9	168.4	
PL midfoot/M19	Orthopaedic Shoes	166.24	60.40	41.2	309.2	0.235
	Own Shoes	156.37	73.62	23.3	410.4	
PM heel/M20	Orthopaedic Shoes	323.13	79.97	180.25	517.55	0.016*
	Own Shoes	295.24	105.71	123.15	706.4	
PL heel/M21	Orthopaedic Shoes	331.87	81.71	182.3	514.85	0.956
	Own Shoes	332.62	133.80	144.7	754.55	
Whole Dorsal surface PTI (KPa.s)	Orthopaedic Shoes	100.29	34.64	27.28	207.78	<0.001**
	Own Shoes	144.27	83.78	33.37	369.90	
Whole Plantar surface PTI (KPa.s)	Orthopaedic Shoes	367.29	74.54	216.25	595.40	0.507
	Own Shoes	375.74	125.98	161.65	754.55	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 6 Comparison between Orthopaedic Shoes and Own Shoes in PTI (KPa.s) in diabetes group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	30.84	28.68	1.27	185.73	0.015*
	Own Shoes	22.40	21.32	2.07	120.77	
DT Lat/M2	Orthopaedic Shoes	39.41	20.49	12.08	120.87	0.054
	Own Shoes	45.96	28.54	14.68	130.07	
DMH1/M3	Orthopaedic Shoes	38.02	14.84	14.65	76.97	<0.001**
	Own Shoes	72.23	55.12	12.2	230.27	
DMH2/M4	Orthopaedic Shoes	29.48	18.65	4.47	88.82	0.328
	Own Shoes	26.92	19.66	1.32	92.53	
DMH3/M5	Orthopaedic Shoes	20.46	10.08	2.82	44.32	0.868
	Own Shoes	20.17	14.64	1	60.08	
DMH4/M6	Orthopaedic Shoes	30.70	16.83	5.15	82.93	0.975
	Own Shoes	30.78	20.09	0	91.2	
DMH5/M7	Orthopaedic Shoes	24.96	13.19	5.05	74.98	0.002*
	Own Shoes	33.13	23.10	5.97	99.93	
DM midfoot/M8	Orthopaedic Shoes	106.91	61.94	18.38	246.9	<0.001**
	Own Shoes	145.62	101.63	25.67	521.12	
DL midfoot/M9	Orthopaedic Shoes	53.75	27.90	8.68	131.08	<0.001**
	Own Shoes	78.77	48.45	11.8	230.43	
PT1/M10	Orthopaedic Shoes	205.24	65.81	94	371.05	<0.001**
	Own Shoes	169.21	77.39	29.9	429.7	
PT2/M11	Orthopaedic Shoes	145.71	62.01	38.4	382.3	<0.001**
	Own Shoes	120.97	50.45	43.55	308.05	
PT3/M12	Orthopaedic Shoes	127.62	46.62	60	279.7	<0.001**
	Own Shoes	102.71	47.85	19.9	315.75	
PMH1/M13	Orthopaedic Shoes	290.54	75.43	154.95	508.1	0.601
	Own Shoes	282.86	132.58	105.4	759.25	
PMH2/M14	Orthopaedic Shoes	301.52	79.00	171.5	587.05	0.005*
	Own Shoes	266.92	98.35	126.65	596.85	
PMH3/M15	Orthopaedic Shoes	267.12	74.99	134.5	509.9	<0.001**
	Own Shoes	231.35	84.94	118.25	514.35	
PMH4/M16	Orthopaedic Shoes	230.91	84.22	77.4	468.15	0.004*
	Own Shoes	199.80	74.00	97.4	432.65	
PMH5/M17	Orthopaedic Shoes	211.65	105.06	48.55	432.9	0.775
	Own Shoes	207.51	107.11	46.1	475.35	
PM midfoot/M18	Orthopaedic Shoes	112.17	49.88	43	362.25	0.107
	Own Shoes	100.15	58.87	11.7	228.7	
PL midfoot/M19	Orthopaedic Shoes	203.83	79.60	87.5	442.9	0.187
	Own Shoes	189.39	80.65	59.9	406.2	
PM heel/M20	Orthopaedic Shoes	363.90	92.11	190.25	640.7	0.002*
	Own Shoes	315.50	134.88	120.95	903.25	
PL heel/M21	Orthopaedic Shoes	394.16	131.60	212.55	1001.1	<0.001**
	Own Shoes	319.65	122.06	125.95	759.35	
Whole Dorsal surface PTI (KPa.s)	Orthopaedic Shoes	111.32	58.82	25.23	246.90	<0.001**
	Own Shoes	154.45	100.09	25.67	521.12	
Whole Plantar surface PTI (KPa.s)	Orthopaedic Shoes	408.69	126.84	231.00	1001.10	0.044*
	Own Shoes	371.86	139.76	129.95	903.25	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 7 Comparison between control group and diabetes group in PTI (KPa.s) in Own Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	29.75	28.12	3.75	132.15	0.026*
	Diabetes	22.40	21.32	2.07	120.77	
DT Lat/M2	Control	70.37	71.65	4.68	369.9	<0.001**
	Diabetes	45.96	28.54	14.68	130.07	
DMH1/M3	Control	78.10	73.32	10.95	303.02	0.492
	Diabetes	72.23	55.12	12.2	230.27	
DMH2/M4	Control	37.29	27.65	5.67	160.62	<0.001**
	Diabetes	26.92	19.66	1.32	92.53	
DMH3/M5	Control	34.20	34.31	3.95	199.38	<0.001**
	Diabetes	20.17	14.64	1	60.08	
DMH4/M6	Control	41.67	36.84	3.32	218.4	0.006*
	Diabetes	30.78	20.09	0	91.2	
DMH5/M7	Control	47.76	52.84	1.75	296.82	0.008*
	Diabetes	33.13	23.10	5.97	99.93	
DM midfoot/M8	Control	95.59	74.50	17.95	321.2	<0.001**
	Diabetes	145.62	101.63	25.67	521.12	
DL midfoot/M9	Control	54.23	43.18	10.92	194.72	<0.001**
	Diabetes	78.77	48.45	11.8	230.43	
PT1/M10	Control	204.12	99.87	57.35	629.05	0.003*
	Diabetes	169.21	77.39	29.9	429.7	
PT2/M11	Control	159.14	62.73	45.15	378.85	<0.001**
	Diabetes	120.97	50.45	43.55	308.05	
PT3/M12	Control	163.23	58.22	48.65	332.5	<0.001**
	Diabetes	102.71	47.85	19.9	315.75	
PMH1/M13	Control	248.59	114.75	97.1	661.05	0.033*
	Diabetes	282.86	132.58	105.4	759.25	
PMH2/M14	Control	262.57	90.09	114	536.8	0.722
	Diabetes	266.92	98.35	126.65	596.85	
PMH3/M15	Control	250.50	85.09	114.25	487.6	0.084
	Diabetes	231.35	84.94	118.25	514.35	
PMH4/M16	Control	232.58	83.41	121.85	460.5	0.002*
	Diabetes	199.80	74.00	97.4	432.65	
PMH5/M17	Control	227.73	99.77	64.3	592.4	0.132
	Diabetes	207.51	107.11	46.1	475.35	
PM midfoot/M18	Control	69.19	36.06	4.9	168.4	<0.001**
	Diabetes	100.15	58.87	11.7	228.7	
PL midfoot/M19	Control	156.37	73.62	23.3	410.4	<0.001**
	Diabetes	189.39	80.65	59.9	406.2	
PM heel/M20	Control	295.24	105.71	123.15	706.4	0.193
	Diabetes	315.50	134.88	120.95	903.25	
PL heel/M21	Control	332.62	133.80	144.7	754.55	0.438
	Diabetes	319.65	122.06	125.95	759.35	
Whole Dorsal surface PTI (KPa.s)	Control	144.27	83.78	33.37	369.90	0.392
	Diabetes	154.45	100.09	25.67	521.12	
Whole Plantar surface PTI (KPa.s)	Control	375.74	125.98	161.65	754.55	0.821
	Diabetes	371.86	139.76	129.95	903.25	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 8 Comparison between control group and diabetes group in PTI (KPa.s) in Orthopaedic Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	25.34	14.19	4.52	68.23	0.054
	Diabetes	30.84	28.68	1.27	185.73	
DT Lat/M2	Control	56.47	35.63	7.65	145.92	<0.001**
	Diabetes	39.41	20.49	12.08	120.87	
DMH1/M3	Control	31.54	15.44	1.38	80.05	<0.001**
	Diabetes	38.02	14.84	14.65	76.97	
DMH2/M4	Control	25.95	13.70	6.73	72.52	0.092
	Diabetes	29.48	18.65	4.47	88.82	
DMH3/M5	Control	21.33	20.29	0.38	109.83	0.682
	Diabetes	20.46	10.08	2.82	44.32	
DMH4/M6	Control	38.32	23.69	4.65	113.15	0.005*
	Diabetes	30.70	16.83	5.15	82.93	
DMH5/M7	Control	39.19	25.57	5.9	113.15	<0.001**
	Diabetes	24.96	13.19	5.05	74.98	
DM midfoot/M8	Control	79.39	43.87	14.38	207.78	<0.001**
	Diabetes	106.91	61.94	18.38	246.9	
DL midfoot/M9	Control	49.33	29.72	9.73	149.63	0.241
	Diabetes	53.75	27.90	8.68	131.08	
PT1/M10	Control	243.13	92.40	87.85	543.9	<0.001**
	Diabetes	205.24	65.81	94	371.05	
PT2/M11	Control	178.29	70.72	69	413.5	<0.001**
	Diabetes	145.71	62.01	38.4	382.3	
PT3/M12	Control	176.18	58.33	66.05	356.6	<0.001**
	Diabetes	127.62	46.62	60	279.7	
PMH1/M13	Control	265.03	78.56	117.35	595.4	0.011*
	Diabetes	290.54	75.43	154.95	508.1	
PMH2/M14	Control	280.88	76.78	113.05	449.25	0.042*
	Diabetes	301.52	79.00	171.5	587.05	
PMH3/M15	Control	260.99	72.47	118.95	430.15	0.521
	Diabetes	267.12	74.99	134.5	509.9	
PMH4/M16	Control	219.05	77.01	93.1	388.65	0.256
	Diabetes	230.91	84.22	77.4	468.15	
PMH5/M17	Control	217.53	81.37	57.65	425.25	0.626
	Diabetes	211.65	105.06	48.55	432.9	
PM midfoot/M18	Control	92.31	49.77	11.7	238.9	0.002*
	Diabetes	112.17	49.88	43	362.25	
PL midfoot/M19	Control	166.24	60.40	41.2	309.2	<0.001**
	Diabetes	203.83	79.60	87.5	442.9	
PM heel/M20	Control	323.13	79.97	180.25	517.55	<0.001**
	Diabetes	363.90	92.11	190.25	640.7	
PL heel/M21	Control	331.87	81.71	182.3	514.85	<0.001**
	Diabetes	394.16	131.60	212.55	1001.1	
Whole Dorsal surface PTI (KPa.s)	Control	100.29	34.64	27.28	207.78	0.072
	Diabetes	111.32	58.82	25.23	246.90	
Whole Plantar surface PTI (KPa.s)	Control	367.29	74.54	216.25	595.40	0.002*
	Diabetes	408.69	126.84	231.00	1001.10	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 9 Comparison between Orthopaedic Shoes and Own Shoes in CA (cm²) in control group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	7.58	0.97	5.09	8.50	0.892
	Own Shoes	7.59	0.95	5.58	8.50	
DT Lat/M2	Orthopaedic Shoes	7.69	0.78	6.82	8.62	0.244
	Own Shoes	7.80	0.77	6.82	8.62	
DMH1/M3	Orthopaedic Shoes	12.33	2.21	2.78	14.53	0.683
	Own Shoes	12.44	1.79	8.41	14.53	
DMH2/M4	Orthopaedic Shoes	11.62	2.24	5.27	14.00	0.612
	Own Shoes	11.49	1.86	7.09	14.00	
DMH3/M5	Orthopaedic Shoes	5.55	1.17	1.34	6.96	0.729
	Own Shoes	5.59	1.07	2.72	6.96	
DMH4/M6	Orthopaedic Shoes	5.83	1.23	1.47	7.65	0.223
	Own Shoes	5.63	1.36	0.21	7.65	
DMH5/M7	Orthopaedic Shoes	6.00	0.95	2.80	6.96	0.222
	Own Shoes	5.85	1.04	2.79	6.96	
DM midfoot/M8	Orthopaedic Shoes	18.03	3.54	8.37	21.39	0.002*
	Own Shoes	16.75	3.19	8.48	21.39	
DL midfoot/M9	Orthopaedic Shoes	21.99	3.39	14.28	28.17	<0.001**
	Own Shoes	19.08	5.29	7.06	27.96	
PT1/ M10	Orthopaedic Shoes	7.55	0.95	6.20	9.58	0.734
	Own Shoes	7.59	0.93	6.24	9.58	
PT2/M11	Orthopaedic Shoes	8.90	1.27	7.02	11.22	0.673
	Own Shoes	8.84	1.25	7.02	11.22	
PT3/M12	Orthopaedic Shoes	9.54	1.51	6.62	12.05	0.695
	Own Shoes	9.61	1.40	7.89	12.05	
PMH1/M13	Orthopaedic Shoes	12.38	1.62	9.49	15.49	0.650
	Own Shoes	12.47	1.62	9.95	15.49	
PMH2/M14	Orthopaedic Shoes	12.48	1.73	9.71	15.57	0.910
	Own Shoes	12.50	1.72	9.71	15.57	
PMH3/M15	Orthopaedic Shoes	6.22	0.71	5.13	7.53	1.000
	Own Shoes	6.22	0.71	5.13	7.53	
PMH4/M16	Orthopaedic Shoes	6.33	0.78	5.19	7.67	1.000
	Own Shoes	6.33	0.78	5.19	7.67	
PMH5/M17	Orthopaedic Shoes	6.43	0.96	5.12	8.52	1.000
	Own Shoes	6.43	0.96	5.12	8.52	
PM midfoot/M18	Orthopaedic Shoes	13.62	3.75	5.13	22.97	0.325
	Own Shoes	14.20	5.71	1.28	21.00	
PL midfoot/M19	Orthopaedic Shoes	25.08	3.62	19.04	30.96	0.403
	Own Shoes	24.67	4.34	12.69	30.96	
PM heel/M20	Orthopaedic Shoes	16.87	2.10	12.72	21.19	0.927
	Own Shoes	16.85	2.25	12.72	21.19	
PL heel/M21	Orthopaedic Shoes	23.28	3.02	19.05	28.81	0.910
	Own Shoes	23.32	3.14	17.79	28.81	
Whole Dorsal surface CA (cm ²)	Orthopaedic Shoes	96.61	12.35	61.56	114.94	0.005*
	Own Shoes	92.23	12.84	58.65	111.38	
Whole Plantar surface CA (cm ²)	Orthopaedic Shoes	148.68	19.84	114.47	189.49	0.890
	Own Shoes	149.03	21.31	104.26	185.60	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 10 Comparison between Orthopaedic Shoes and Own Shoes in CA (cm²) in diabetes group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	7.27	1.07	3.41	8.50	0.979
	Own Shoes	7.26	1.10	4.09	8.50	
DT Lat/M2	Orthopaedic Shoes	7.58	0.81	5.16	8.52	0.822
	Own Shoes	7.55	1.01	5.53	8.52	
DMH1/M3	Orthopaedic Shoes	12.39	1.55	9.25	14.53	0.242
	Own Shoes	12.64	1.61	9.78	14.53	
DMH2/M4	Orthopaedic Shoes	11.62	1.57	5.31	14.00	0.009*
	Own Shoes	10.92	2.24	6.95	14.00	
DMH3/M5	Orthopaedic Shoes	5.43	0.87	2.70	6.89	0.520
	Own Shoes	5.33	1.28	1.89	6.96	
DMH4/M6	Orthopaedic Shoes	5.36	1.13	3.47	6.82	0.723
	Own Shoes	5.42	1.57	0.00	6.96	
DMH5/M7	Orthopaedic Shoes	5.70	0.91	3.21	6.96	0.029*
	Own Shoes	5.40	1.09	3.21	6.96	
DM midfoot/M8	Orthopaedic Shoes	18.20	2.24	14.11	21.39	0.185
	Own Shoes	17.67	3.41	9.71	21.39	
DL midfoot/M9	Orthopaedic Shoes	21.68	3.05	11.30	27.96	<0.001**
	Own Shoes	17.88	5.13	7.15	27.96	
PT1/M10	Orthopaedic Shoes	7.63	1.04	6.20	9.58	0.975
	Own Shoes	7.62	0.99	6.24	9.58	
PT2/M11	Orthopaedic Shoes	8.88	1.33	6.90	11.22	0.084
	Own Shoes	8.60	1.03	7.13	10.26	
PT3/M12	Orthopaedic Shoes	9.78	1.61	6.67	12.05	0.781
	Own Shoes	9.72	1.65	7.34	12.05	
PMH1/M13	Orthopaedic Shoes	12.61	1.79	10.25	15.49	0.758
	Own Shoes	12.69	1.82	10.25	15.49	
PMH2/M14	Orthopaedic Shoes	12.82	1.78	10.27	15.57	0.746
	Own Shoes	12.90	1.80	10.27	15.57	
PMH3/M15	Orthopaedic Shoes	6.22	0.78	5.13	7.53	0.185
	Own Shoes	6.40	1.09	5.13	9.51	
PMH4/M16	Orthopaedic Shoes	6.34	0.89	5.19	7.67	0.725
	Own Shoes	6.38	0.90	5.19	7.67	
PMH5/M17	Orthopaedic Shoes	6.35	0.97	5.12	8.52	0.534
	Own Shoes	6.44	1.22	5.12	10.20	
PM midfoot/M18	Orthopaedic Shoes	14.21	2.98	5.18	19.09	<0.001**
	Own Shoes	16.22	4.13	5.18	22.97	
PL midfoot/M19	Orthopaedic Shoes	25.37	3.60	20.30	30.96	0.579
	Own Shoes	25.64	3.54	20.30	30.96	
PM heel/M20	Orthopaedic Shoes	17.16	2.34	13.98	21.19	0.666
	Own Shoes	17.30	2.38	13.98	21.19	
PL heel/M21	Orthopaedic Shoes	23.06	3.23	19.05	28.81	0.318
	Own Shoes	23.51	3.34	19.05	28.81	
Whole Dorsal surface CA (cm ²)	Orthopaedic Shoes	95.21	10.30	76.71	111.66	0.005*
	Own Shoes	90.08	15.49	59.88	114.55	
Whole Plantar surface CA (cm ²)	Orthopaedic Shoes	150.44	19.54	121.99	185.61	0.270
	Own Shoes	153.41	20.03	118.29	189.49	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 11 Comparison between control group and diabetes group in CA (cm²) in Own Shoes Using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	7.59	0.95	5.58	8.50	0.013*
	Diabetes	7.26	1.10	4.09	8.50	
DT Lat/M2	Control	7.80	0.77	6.82	8.62	0.029*
	Diabetes	7.55	1.01	5.53	8.52	
DMH1/M3	Control	12.44	1.79	8.41	14.53	0.358
	Diabetes	12.64	1.61	9.78	14.53	
DMH2/M4	Control	11.49	1.86	7.09	14.00	0.033*
	Diabetes	10.92	2.24	6.95	14.00	
DMH3/M5	Control	5.59	1.07	2.72	6.96	0.088
	Diabetes	5.33	1.28	1.89	6.96	
DMH4/M6	Control	5.63	1.36	0.21	7.65	0.266
	Diabetes	5.42	1.57	0.00	6.96	
DMH5/M7	Control	5.85	1.04	2.79	6.96	<0.001**
	Diabetes	5.40	1.09	3.21	6.96	
DM midfoot/M8	Control	16.75	3.19	8.48	21.39	0.033*
	Diabetes	17.67	3.41	9.71	21.39	
DL midfoot/M9	Control	19.08	5.29	7.06	27.96	0.078
	Diabetes	17.88	5.13	7.15	27.96	
PT1/M10	Control	7.59	0.93	6.24	9.58	0.793
	Diabetes	7.62	0.99	6.24	9.58	
PT2/M11	Control	8.84	1.25	7.02	11.22	0.112
	Diabetes	8.60	1.03	7.13	10.26	
PT3/M12	Control	9.61	1.40	7.89	12.05	0.584
	Diabetes	9.72	1.65	7.34	12.05	
PMH1/M13	Control	12.47	1.62	9.95	15.49	0.332
	Diabetes	12.69	1.82	10.25	15.49	
PMH2/M14	Control	12.50	1.72	9.71	15.57	0.080
	Diabetes	12.90	1.80	10.27	15.57	
PMH3/M15	Control	6.22	0.71	5.13	7.53	0.130
	Diabetes	6.40	1.09	5.13	9.51	
PMH4/M16	Control	6.33	0.78	5.19	7.67	0.601
	Diabetes	6.38	0.90	5.19	7.67	
PMH5/M17	Control	6.43	0.96	5.12	8.52	0.910
	Diabetes	6.44	1.22	5.12	10.20	
PM midfoot/M18	Control	14.20	5.71	1.28	21.00	0.002*
	Diabetes	16.22	4.13	5.18	22.97	
PL midfoot/M19	Control	24.67	4.34	12.69	30.96	0.063
	Diabetes	25.64	3.54	20.30	30.96	
PM heel/M20	Control	16.85	2.25	12.72	21.19	0.132
	Diabetes	17.30	2.38	13.98	21.19	
PL heel/M21	Control	23.32	3.14	17.79	28.81	0.665
	Diabetes	23.51	3.34	19.05	28.81	
Whole Dorsal surface CA (cm ²)	Control	92.23	12.84	58.65	111.38	0.241
	Diabetes	90.08	15.49	59.88	114.55	
Whole Plantar surface CA (cm ²)	Control	149.03	21.31	104.26	185.60	0.105
	Diabetes	153.41	20.03	118.29	189.49	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 12 Comparison between control group and diabetes group in CA (cm²) in Orthopaedic Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	7.58	0.97	5.09	8.50	0.020*
	Diabetes	7.27	1.07	3.41	8.50	
DT Lat/M2	Control	7.69	0.78	6.82	8.62	0.280
	Diabetes	7.58	0.81	5.16	8.52	
DMH1/M3	Control	12.33	2.21	2.78	14.53	0.831
	Diabetes	12.39	1.55	9.25	14.53	
DMH2/M4	Control	11.62	2.24	5.27	14.00	0.995
	Diabetes	11.62	1.57	5.31	14.00	
DMH3/M5	Control	5.55	1.17	1.34	6.96	0.392
	Diabetes	5.43	0.87	2.70	6.89	
DMH4/M6	Control	5.83	1.23	1.47	7.65	0.002*
	Diabetes	5.36	1.13	3.47	6.82	
DMH5/M7	Control	6.00	0.95	2.80	6.96	0.013*
	Diabetes	5.70	0.91	3.21	6.96	
DM midfoot/M8	Control	18.03	3.54	8.37	21.39	0.668
	Diabetes	18.20	2.24	14.11	21.39	
DL midfoot/M9	Control	21.99	3.39	14.28	28.17	0.464
	Diabetes	21.68	3.05	11.30	27.96	
PT1/M10	Control	7.55	0.95	6.20	9.58	0.553
	Diabetes	7.63	1.04	6.20	9.58	
PT2/M11	Control	8.90	1.27	7.02	11.22	0.884
	Diabetes	8.88	1.33	6.90	11.22	
PT3/M12	Control	9.54	1.51	6.62	12.05	0.235
	Diabetes	9.78	1.61	6.67	12.05	
PMH1/M13	Control	12.38	1.62	9.49	15.49	0.297
	Diabetes	12.61	1.79	10.25	15.49	
PMH2/M14	Control	12.48	1.73	9.71	15.57	0.130
	Diabetes	12.82	1.78	10.27	15.57	
PMH3/M15	Control	6.22	0.71	5.13	7.53	0.949
	Diabetes	6.22	0.78	5.13	7.53	
PMH4/M16	Control	6.33	0.78	5.19	7.67	0.897
	Diabetes	6.34	0.89	5.19	7.67	
PMH5/M17	Control	6.43	0.96	5.12	8.52	0.535
	Diabetes	6.35	0.97	5.12	8.52	
PM midfoot/M18	Control	13.62	3.75	5.13	22.97	0.180
	Diabetes	14.21	2.98	5.18	19.09	
PL midfoot/M19	Control	25.08	3.62	19.04	30.96	0.538
	Diabetes	25.37	3.60	20.30	30.96	
PM heel/M20	Control	16.87	2.10	12.72	21.19	0.314
	Diabetes	17.16	2.34	13.98	21.19	
PL heel/M21	Control	23.28	3.02	19.05	28.81	0.582
	Diabetes	23.06	3.23	19.05	28.81	
Whole Dorsal surface CA (cm ²)	Control	96.61	12.35	61.56	114.94	0.349
	Diabetes	95.21	10.30	76.71	111.66	
Whole Plantar surface CA (cm ²)	Control	148.68	19.84	114.47	189.49	0.492
	Diabetes	150.44	19.54	121.99	185.61	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 13 Comparison between Orthopaedic Shoes and Own Shoes in MF (N) in control group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	7.92	5.27	1.28	23.62	<0.001**
	Own Shoes	10.70	8.13	1.14	33.16	
DT Lat/M2	Orthopaedic Shoes	11.42	4.32	3.12	21.49	<0.001**
	Own Shoes	17.63	7.95	5.91	35.54	
DMH1/M3	Orthopaedic Shoes	16.81	9.21	2.55	36.21	<0.001**
	Own Shoes	27.83	16.93	3.81	74.03	
DMH2/M4	Orthopaedic Shoes	11.29	6.67	1.58	30.25	<0.001**
	Own Shoes	21.39	17.12	1.87	72.32	
DMH3/M5	Orthopaedic Shoes	5.58	3.22	0.99	14.22	<0.001**
	Own Shoes	9.57	6.69	1.3	30.53	
DMH4/M6	Orthopaedic Shoes	6.91	3.59	1.57	17.75	<0.001**
	Own Shoes	10.21	6.10	0.08	30.88	
DMH5/M7	Orthopaedic Shoes	6.09	2.62	2.22	14.62	<0.001**
	Own Shoes	9.10	5.73	0.69	21.92	
DM midfoot/M8	Orthopaedic Shoes	21.31	10.39	5.02	52.67	0.453
	Own Shoes	22.51	15.25	2.93	61.74	
DL midfoot/M9	Orthopaedic Shoes	15.51	10.04	5.59	48.55	0.615
	Own Shoes	16.20	11.94	1.66	56.38	
PT1/ M10	Orthopaedic Shoes	108.60	27.27	52.61	180.06	0.744
	Own Shoes	109.84	33.99	42.97	189.56	
PT2/M11	Orthopaedic Shoes	74.76	21.16	39.52	118.67	0.416
	Own Shoes	76.88	21.00	37.84	127.53	
PT3/M12	Orthopaedic Shoes	69.21	24.44	26.57	110.9	0.046*
	Own Shoes	74.99	22.45	32.08	118.98	
PMH1/M13	Orthopaedic Shoes	180.20	43.99	103.96	289.25	0.097
	Own Shoes	170.14	53.79	72.63	307.5	
PMH2/M14	Orthopaedic Shoes	166.03	39.97	94.94	257.67	0.218
	Own Shoes	172.31	42.63	85	259.19	
PMH3/M15	Orthopaedic Shoes	80.21	20.76	35.64	139.36	<0.001**
	Own Shoes	92.86	28.12	49.23	174.92	
PMH4/M16	Orthopaedic Shoes	69.18	19.95	27.69	119.79	<0.001**
	Own Shoes	80.97	23.25	45.31	153.02	
PMH5/M17	Orthopaedic Shoes	60.06	21.43	14.38	142.77	<0.001**
	Own Shoes	72.76	27.88	22.73	148.32	
PM midfoot/M18	Orthopaedic Shoes	23.70	19.47	2.14	100.31	<0.001**
	Own Shoes	36.04	28.31	1.28	123.45	
PL midfoot/M19	Orthopaedic Shoes	114.75	48.81	15.87	283.27	0.005*
	Own Shoes	134.94	66.38	7.85	285	
PM heel/M20	Orthopaedic Shoes	241.47	53.17	125.11	388.12	0.108
	Own Shoes	229.63	65.40	117.35	461.52	
PL heel/M21	Orthopaedic Shoes	350.80	63.08	192.61	497.81	0.002*
	Own Shoes	319.24	97.03	101.32	539.92	
Whole Dorsal surface MF (N)	Orthopaedic Shoes	25.41	9.29	8.85	52.67	<0.001**
	Own Shoes	34.74	16.51	7.56	74.03	
Whole Plantar surface MF (N)	Orthopaedic Shoes	350.85	63.10	192.61	497.81	0.026*
	Own Shoes	331.07	79.48	164.54	539.92	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 14 Comparison between Orthopaedic Shoes and Own Shoes in MF (N) in diabetes group using: Independent Sample t-test.

Foot Area /Mask no.	Tested Condition	Mean	SD	Min.	Max.	p
DT Med/M1	Orthopaedic Shoes	6.88	3.90	0.57	15.99	0.729
	Own Shoes	7.12	5.93	1.23	44.49	
DT Lat/M2	Orthopaedic Shoes	11.13	5.41	4.12	24.47	0.592
	Own Shoes	11.50	4.51	4.78	23.75	
DMH1/M3	Orthopaedic Shoes	20.59	9.66	4.02	42.32	<0.001**
	Own Shoes	32.32	20.74	8.63	100.18	
DMH2/M4	Orthopaedic Shoes	13.59	9.20	1.6	42.82	0.032*
	Own Shoes	16.24	8.83	2.78	34.56	
DMH3/M5	Orthopaedic Shoes	6.31	3.44	1.22	20.28	0.004*
	Own Shoes	8.08	5.23	0.58	24.51	
DMH4/M6	Orthopaedic Shoes	6.48	2.86	1.06	14.96	<0.001**
	Own Shoes	9.35	5.79	0	25.24	
DMH5/M7	Orthopaedic Shoes	4.19	1.74	1.34	10.11	<0.001**
	Own Shoes	7.35	3.92	1.39	15.66	
DM midfoot/M8	Orthopaedic Shoes	24.90	9.26	7.51	43.38	0.009*
	Own Shoes	29.36	15.08	7.19	58.41	
DL midfoot/M9	Orthopaedic Shoes	13.19	5.81	4.36	28.49	0.035*
	Own Shoes	15.42	9.20	2.16	39.23	
PT1/M10	Orthopaedic Shoes	94.51	31.23	37.12	186.84	0.604
	Own Shoes	92.08	37.33	26.64	195.56	
PT2/M11	Orthopaedic Shoes	58.00	23.42	23.11	110.64	0.660
	Own Shoes	59.46	25.07	22.75	132.23	
PT3/M12	Orthopaedic Shoes	48.03	20.13	20.81	120.07	0.678
	Own Shoes	49.18	20.68	16.38	112.98	
PMH1/M13	Orthopaedic Shoes	194.42	43.06	94.68	304.47	0.657
	Own Shoes	191.45	54.44	106.65	382.53	
PMH2/M14	Orthopaedic Shoes	186.22	29.88	135.57	262.59	0.044*
	Own Shoes	178.32	27.27	141.53	262.95	
PMH3/M15	Orthopaedic Shoes	85.65	21.34	38.11	136.68	0.974
	Own Shoes	85.55	21.14	43.93	135.31	
PMH4/M16	Orthopaedic Shoes	73.24	25.16	16.3	137.94	0.528
	Own Shoes	71.30	19.67	31.93	120.45	
PMH5/M17	Orthopaedic Shoes	58.02	27.66	13.2	131.54	0.457
	Own Shoes	60.72	25.55	13.43	113.71	
PM midfoot/M18	Orthopaedic Shoes	25.29	15.28	7.62	70.6	<0.001**
	Own Shoes	44.72	29.69	6.7	122.97	
PL midfoot/M19	Orthopaedic Shoes	133.08	52.67	55.9	291.12	0.004*
	Own Shoes	155.01	58.54	62.07	313.21	
PM heel/M20	Orthopaedic Shoes	257.44	62.26	163.98	432.28	0.433
	Own Shoes	251.03	57.67	145.93	384.78	
PL heel/M21	Orthopaedic Shoes	376.68	72.44	271.43	581.68	0.056
	Own Shoes	356.33	82.76	211.87	639.14	
Whole Dorsal surface MF (N)	Orthopaedic Shoes	27.41	9.44	7.51	43.38	<0.001**
	Own Shoes	38.24	19.43	13.75	100.18	
Whole Plantar surface MF (N)	Orthopaedic Shoes	376.86	72.30	271.43	581.68	0.060
	Own Shoes	356.89	82.63	211.87	639.14	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 15 Comparison between control group and diabetes group in MF (N) in Own Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	10.70	8.13	1.14	33.16	<0.001**
	Diabetes	7.12	5.93	1.23	44.49	
DT Lat/M2	Control	17.63	7.95	5.91	35.54	<0.001**
	Diabetes	11.50	4.51	4.78	23.75	
DMH1/M3	Control	27.83	16.93	3.81	74.03	0.066
	Diabetes	32.32	20.74	8.63	100.18	
DMH2/M4	Control	21.39	17.12	1.87	72.32	0.005*
	Diabetes	16.24	8.83	2.78	34.56	
DMH3/M5	Control	9.57	6.69	1.3	30.53	0.059
	Diabetes	8.08	5.23	0.58	24.51	
DMH4/M6	Control	10.21	6.10	0.08	30.88	0.271
	Diabetes	9.35	5.79	0	25.24	
DMH5/M7	Control	9.10	5.73	0.69	21.92	0.008*
	Diabetes	7.35	3.92	1.39	15.66	
DM midfoot/M8	Control	22.51	15.25	2.93	61.74	<0.001**
	Diabetes	29.36	15.08	7.19	58.41	
DL midfoot/M9	Control	16.20	11.94	1.66	56.38	0.578
	Diabetes	15.42	9.20	2.16	39.23	
PT1/M10	Control	109.84	33.99	42.97	189.56	<0.001**
	Diabetes	92.08	37.33	26.64	195.56	
PT2/M11	Control	76.88	21.00	37.84	127.53	<0.001**
	Diabetes	59.46	25.07	22.75	132.23	
PT3/M12	Control	74.99	22.45	32.08	118.98	<0.001**
	Diabetes	49.18	20.68	16.38	112.98	
PMH1/M13	Control	170.14	53.79	72.63	307.5	0.003*
	Diabetes	191.45	54.44	106.65	382.53	
PMH2/M14	Control	172.31	42.63	85	259.19	0.206
	Diabetes	178.32	27.27	141.53	262.95	
PMH3/M15	Control	92.86	28.12	49.23	174.92	0.027*
	Diabetes	85.55	21.14	43.93	135.31	
PMH4/M16	Control	80.97	23.25	45.31	153.02	<0.001**
	Diabetes	71.30	19.67	31.93	120.45	
PMH5/M17	Control	72.76	27.88	22.73	148.32	<0.001**
	Diabetes	60.72	25.55	13.43	113.71	
PM midfoot/M18	Control	36.04	28.31	1.28	123.45	0.022*
	Diabetes	44.72	29.69	6.7	122.97	
PL midfoot/M19	Control	134.94	66.38	7.85	285	0.015*
	Diabetes	155.01	58.54	62.07	313.21	
PM heel/M20	Control	229.63	65.40	117.35	461.52	0.008*
	Diabetes	251.03	57.67	145.93	384.78	
PL heel/M21	Control	319.24	97.03	101.32	539.92	0.002*
	Diabetes	356.33	82.76	211.87	639.14	
Whole Dorsal surface MF (N)	Control	34.74	16.51	7.56	74.03	0.134
	Diabetes	38.24	19.43	13.75	100.18	
Whole Plantar surface MF (N)	Control	331.07	79.48	164.54	539.92	0.015*
	Diabetes	356.89	82.63	211.87	639.14	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 16 Comparison between control group and diabetes group in MF (N) in Orthopaedic Shoes using: Independent Sample t-test.

Foot Area /Mask no	Group	Mean	SD	Min.	Max.	p
DT Med/M1	Control	7.92	5.27	1.28	23.62	0.090
	Diabetes	6.88	3.90	0.57	15.99	
DT Lat/M2	Control	11.42	4.32	3.12	21.49	0.650
	Diabetes	11.13	5.41	4.12	24.47	
DMH1/M3	Control	16.81	9.21	2.55	36.21	0.002*
	Diabetes	20.59	9.66	4.02	42.32	
DMH2/M4	Control	11.29	6.67	1.58	30.25	0.026*
	Diabetes	13.59	9.20	1.6	42.82	
DMH3/M5	Control	5.58	3.22	0.99	14.22	0.091
	Diabetes	6.31	3.44	1.22	20.28	
DMH4/M6	Control	6.91	3.59	1.57	17.75	0.314
	Diabetes	6.48	2.86	1.06	14.96	
DMH5/M7	Control	6.09	2.62	2.22	14.62	<0.001* *
	Diabetes	4.19	1.74	1.34	10.11	
DM midfoot/M8	Control	21.31	10.39	5.02	52.67	0.006*
	Diabetes	24.90	9.26	7.51	43.38	
DL midfoot/M9	Control	15.51	10.04	5.59	48.55	0.035*
	Diabetes	13.19	5.81	4.36	28.49	
PT1/M10	Control	108.60	27.27	52.61	180.06	<0.001* *
	Diabetes	94.51	31.23	37.12	186.84	
PT2/M11	Control	74.76	21.16	39.52	118.67	<0.001* *
	Diabetes	58.00	23.42	23.11	110.64	
PT3/M12	Control	69.21	24.44	26.57	110.9	<0.001* *
	Diabetes	48.03	20.13	20.81	120.07	
PMH1/M13	Control	180.20	43.99	103.96	289.25	0.013*
	Diabetes	194.42	43.06	94.68	304.47	
PMH2/M14	Control	166.03	39.97	94.94	257.67	<0.001* *
	Diabetes	186.22	29.88	135.57	262.59	
PMH3/M15	Control	80.21	20.76	35.64	139.36	0.047*
	Diabetes	85.65	21.34	38.11	136.68	
PMH4/M16	Control	69.18	19.95	27.69	119.79	0.164
	Diabetes	73.24	25.16	16.3	137.94	
PMH5/M17	Control	60.06	21.43	14.38	142.77	0.520
	Diabetes	58.02	27.66	13.2	131.54	
PM midfoot/M18	Control	23.70	19.47	2.14	100.31	0.489
	Diabetes	25.29	15.28	7.62	70.6	
PL midfoot/M19	Control	114.75	48.81	15.87	283.27	0.006*
	Diabetes	133.08	52.67	55.9	291.12	
PM heel/M20	Control	241.47	53.17	125.11	388.12	0.033*
	Diabetes	257.44	62.26	163.98	432.28	
PL heel/M21	Control	350.80	63.08	192.61	497.81	0.003*
	Diabetes	376.68	72.44	271.43	581.68	
Whole Dorsal surface MF (N)	Control	25.41	9.29	8.85	52.67	0.100
	Diabetes	27.41	9.44	7.51	43.38	
Whole Plantar surface MF (N)	Control	350.85	63.10	192.61	497.81	0.003*
	Diabetes	376.86	72.30	271.43	581.68	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 17 Comparison between Orthopaedic shoes and Own shoes according to time of Dorsal PP (% of Gait) in study groups using: Independent Sample t-test.

Group	Tested condition	Mean	SD	Min.	Max.	p
Control	Orthopaedic shoes	42.57	24.66	1.6	89.4	0.095
	Own shoes	45.20	19.07	1.7	82.6	
Diabetes	Orthopaedic shoes	45.05	25.71	0	98.7	0.266
	Own shoes	47.18	22.18	2	77.8	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 18 Comparison between control group and diabetes group according to time of Dorsal PP (% of Gait) in tested shoe conditions using: Independent Sample t-test.

Tested condition	Group	Mean	SD	Min.	Max.	p
Orthopaedic shoes	Control	42.57	24.66	1.6	89.4	0.189
	Diabetes	45.05	25.71	0	98.7	
Own shoes	Control	45.20	19.07	1.7	82.6	0.203
	Diabetes	47.18	22.18	2	77.8	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 19 Comparison between all Orthopaedic shoes and Own shoes time of Dorsal PP (% of Gait) using: Independent Sample t-test.

Tested condition	Mean	SD	Min.	Max.	P
Orthopaedic shoes	43.66	25.14	0	98.7	0.047*
Own shoes	46.08	20.52	1.7	82.6	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 20 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of ACh on the dorsal surface in control group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	20.82	6.40a	6.25a	<0.001**
	SD	13.74	2.83	1.67	
100μA/60 seconds	Mean	28.50	6.72a	6.31a	<0.001**
	SD	19.25	2.76	1.67	
100μA/60 seconds	Mean	40.86	7.22a	6.50a	<0.001**
	SD	21.37	2.99	1.76	
100μA/60 seconds	Mean	52.47	7.57a	6.75a	<0.001**
	SD	21.78	3.45	1.86	
100μA/60 seconds	Mean	64.61	7.74a	6.73a	<0.001**
	SD	28.92	3.70	2.16	
100μA/60 seconds	Mean	75.30	8.29a	6.87a	<0.001**
	SD	34.93	4.61	2.30	
100μA/60 seconds	Mean	83.72	8.49a	7.02a	<0.001**
	SD	37.64	5.26	2.29	
100μA/60 seconds	Mean	97.32	8.85a	7.14a	<0.001**
	SD	43.88	5.50	2.28	
100μA/60 seconds	Mean	113.61	9.13a	7.45a	<0.001**
	SD	45.04	6.26	2.27	
100μA/60 seconds	Mean	124.31	9.32a	7.68a	<0.001**
	SD	50.19	6.17	2.48	
100μA/60 seconds	Mean	129.34	10.11a	7.53a	<0.001**
	SD	46.54	6.38	2.50	
100μA/60 seconds	Mean	129.06	10.65a	7.92a	<0.001**
	SD	47.85	6.89	2.71	
100μA/60 seconds	Mean	128.14	10.41a	7.82a	<0.001**
	SD	49.16	6.54	2.75	
100μA/60 seconds	Mean	130.24	10.20a	7.92a	<0.001**
	SD	51.97	6.45	2.71	
100μA/60 seconds	Mean	132.43	11.14a	8.40a	<0.001**
	SD	54.08	8.43	3.02	
Peak of Max response	Mean	142.99	18.27a	14.90a	<0.001**
	SD	39.56	8.19	3.64	
Change in response %	Mean	756.05	192.00a	149.65ab	<0.001**
	SD	467.04	80.08	66.86	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 21 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of SNP on the dorsal surface in control group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	24.64	7.37a	6.21a	<0.001**
	SD	12.18	4.41	2.39	
100μA/60 seconds	Mean	28.63	7.86a	6.43a	<0.001**
	SD	13.96	4.54	2.42	
100μA/60 seconds	Mean	44.27	11.67a	6.64a	<0.001**
	SD	26.18	18.53	2.54	
100μA/60 seconds	Mean	59.95	12.71a	6.57a	<0.001**
	SD	34.65	21.98	2.56	
100μA/60 seconds	Mean	73.97	12.10a	6.52a	<0.001**
	SD	43.86	16.70	2.42	
100μA/60 seconds	Mean	86.76	13.52a	6.65a	<0.001**
	SD	46.12	22.75	2.37	
100μA/60 seconds	Mean	96.52	15.41a	6.57a	<0.001**
	SD	49.37	29.93	2.41	
100μA/60 seconds	Mean	103.14	13.95a	6.87a	<0.001**
	SD	46.97	22.85	2.85	
100μA/60 seconds	Mean	111.09	12.49a	6.83a	<0.001**
	SD	47.18	16.40	2.73	
100μA/60 seconds	Mean	116.28	12.56a	7.30a	<0.001**
	SD	48.95	15.53	2.99	
100μA/60 seconds	Mean	119.73	12.06a	7.39a	<0.001**
	SD	47.11	13.06	3.20	
100μA/60 seconds	Mean	124.47	12.98a	7.45a	<0.001**
	SD	51.82	16.86	3.13	
100μA/60 seconds	Mean	119.07	13.47a	7.22a	<0.001**
	SD	43.26	18.23	2.93	
100μA/60 seconds	Mean	123.62	14.69a	7.54a	<0.001**
	SD	43.66	23.67	3.36	
100μA/60 seconds	Mean	123.51	15.48a	7.45a	<0.001**
	SD	45.73	26.16	3.53	
Peak of Max response	Mean	141.32	23.91a	14.66a	<0.001**
	SD	56.41	27.79	5.84	
Change in response %	Mean	517.86	199.67a	139.22ab	<0.001**
	SD	247.75	124.29	51.86	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 22 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of ACh on the plantar surface in control group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	45.22	3.66a	3.59a	<0.001**
	SD	32.22	1.54	1.01	
100μA/60 seconds	Mean	44.78	3.91a	3.69a	<0.001**
	SD	31.79	1.84	1.21	
100μA/60 seconds	Mean	59.97	4.04a	3.63a	<0.001**
	SD	39.06	1.98	1.23	
100μA/60 seconds	Mean	87.74	4.11a	3.67a	<0.001**
	SD	74.46	2.06	1.39	
100μA/60 seconds	Mean	105.96	4.05a	3.59a	<0.001**
	SD	104.81	2.00	1.23	
100μA/60 seconds	Mean	130.41	4.01a	3.67a	<0.001**
	SD	129.36	2.00	1.48	
100μA/60 seconds	Mean	143.90	4.06a	3.96a	<0.001**
	SD	134.62	1.96	2.12	
100μA/60 seconds	Mean	153.66	4.24a	3.68a	<0.001**
	SD	137.10	2.06	1.74	
100μA/60 seconds	Mean	164.49	4.42a	3.87a	<0.001**
	SD	138.38	2.10	2.12	
100μA/60 seconds	Mean	183.41	4.44a	3.84a	<0.001**
	SD	138.92	2.74	2.02	
100μA/60 seconds	Mean	193.87	4.52a	3.80a	<0.001**
	SD	144.86	2.88	2.15	
100μA/60 seconds	Mean	202.97	4.51a	3.94a	<0.001**
	SD	149.53	2.82	2.37	
100μA/60 seconds	Mean	212.48	4.63a	3.97a	<0.001**
	SD	150.96	2.83	2.33	
100μA/60 seconds	Mean	219.14	4.58a	3.99a	<0.001**
	SD	164.21	2.71	2.47	
100μA/60 seconds	Mean	218.60	4.40a	3.89a	<0.001**
	SD	168.44	2.29	2.29	
Peak of Max response	Mean	223.44	8.98a	8.13a	<0.001**
	SD	140.95	4.19	3.48	
Change in response %	Mean	491.46	146.20a	125.83a	<0.001**
	SD	355.82	63.87	70.15	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 23 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of SNP on the plantar surface in control group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	50.13	3.59a	3.50a	<0.001**
	SD	61.57	1.33	0.81	
100μA/60 seconds	Mean	48.54	3.62a	3.46a	<0.001**
	SD	60.26	1.32	0.95	
100μA/60 seconds	Mean	61.47	3.89a	3.63a	<0.001**
	SD	78.54	1.79	1.19	
100μA/60 seconds	Mean	84.66	4.05a	3.54a	<0.001**
	SD	83.00	1.90	1.01	
100μA/60 seconds	Mean	115.46	4.09a	3.56a	<0.001**
	SD	88.61	1.85	0.99	
100μA/60 seconds	Mean	143.24	4.01a	3.63a	<0.001**
	SD	121.10	1.74	1.05	
100μA/60 seconds	Mean	156.12	4.16a	3.59a	<0.001**
	SD	127.75	1.97	0.89	
100μA/60 seconds	Mean	163.81	4.43a	3.74a	<0.001**
	SD	134.38	2.34	1.14	
100μA/60 seconds	Mean	161.89	4.36a	3.68a	<0.001**
	SD	136.93	2.12	1.01	
100μA/60 seconds	Mean	163.39	4.15a	3.68a	<0.001**
	SD	135.21	1.89	0.90	
100μA/60 seconds	Mean	162.08	4.25a	3.62a	<0.001**
	SD	131.46	2.18	0.93	
100μA/60 seconds	Mean	162.21	4.20a	3.51a	<0.001**
	SD	129.28	1.84	0.78	
100μA/60 seconds	Mean	166.83	4.19a	3.88a	<0.001**
	SD	125.40	1.74	1.52	
100μA/60 seconds	Mean	155.81	4.22a	3.93a	<0.001**
	SD	119.80	1.72	1.66	
100μA/60 seconds	Mean	161.27	4.22a	4.10a	<0.001**
	SD	120.01	1.81	1.99	
Peak of Max response	Mean	203.12	8.00a	7.84a	<0.001**
	SD	138.22	3.59	3.97	
Change in response %	Mean	645.00	128.37a	118.30a	<0.001**
	SD	722.90	86.96	59.06	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 24 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of ACh on the dorsal surface in control group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	21.67	6.83a	6.56a	<0.001**
	SD	13.98	2.93	1.67	
100μA/60 seconds	Mean	29.31	7.19a	6.68a	<0.001**
	SD	18.89	2.96	1.71	
100μA/60 seconds	Mean	41.99	7.71a	6.97a	<0.001**
	SD	21.43	3.22	1.88	
100μA/60 seconds	Mean	53.93	8.06a	7.21a	<0.001**
	SD	21.97	3.65	1.99	
100μA/60 seconds	Mean	67.18	8.20a	7.17a	<0.001**
	SD	28.24	3.81	2.31	
100μA/60 seconds	Mean	77.18	8.75a	7.32a	<0.001**
	SD	34.39	4.79	2.39	
100μA/60 seconds	Mean	85.52	8.94a	7.43a	<0.001**
	SD	37.75	5.47	2.32	
100μA/60 seconds	Mean	100.07	9.30a	7.54a	<0.001**
	SD	43.25	5.65	2.37	
100μA/60 seconds	Mean	116.98	9.59a	7.86a	<0.001**
	SD	44.96	6.36	2.25	
100μA/60 seconds	Mean	125.59	9.75a	8.06a	<0.001**
	SD	50.01	6.28	2.55	
100μA/60 seconds	Mean	130.94	10.53a	7.97a	<0.001**
	SD	46.43	6.47	2.54	
100μA/60 seconds	Mean	130.67	11.03a	8.32a	<0.001**
	SD	47.55	6.95	2.71	
100μA/60 seconds	Mean	129.83	10.79a	8.20a	<0.001**
	SD	48.93	6.65	2.79	
100μA/60 seconds	Mean	131.99	10.62a	8.35a	<0.001**
	SD	51.59	6.62	2.82	
100μA/60 seconds	Mean	133.99	11.34a	8.73a	<0.001**
	SD	53.84	8.14	3.02	
Peak of Max response	Mean	142.99	18.27a	14.90a	<0.001**
	SD	39.56	8.19	3.64	
Change in response %	Mean	711.29	172.88a	136.28ab	<0.001**
	SD	431.85	75.75	61.02	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 25 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of SNP on the dorsal surface in control group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	25.30	7.96a	6.57a	<0.001**
	SD	12.54	4.49	2.50	
100μA/60 seconds	Mean	29.56	8.42a	6.85a	<0.001**
	SD	14.31	4.68	2.53	
100μA/60 seconds	Mean	45.66	12.01a	7.03a	<0.001**
	SD	27.78	17.55	2.65	
100μA/60 seconds	Mean	61.62	13.08a	6.93a	<0.001**
	SD	35.86	21.45	2.71	
100μA/60 seconds	Mean	75.58	12.54a	6.96a	<0.001**
	SD	44.35	16.60	2.57	
100μA/60 seconds	Mean	88.54	14.05a	7.07a	<0.001**
	SD	46.62	22.93	2.55	
100μA/60 seconds	Mean	98.11	15.71a	7.02a	<0.001**
	SD	49.84	29.33	2.63	
100μA/60 seconds	Mean	104.67	14.44a	7.30a	<0.001**
	SD	47.73	22.99	3.05	
100μA/60 seconds	Mean	112.80	13.02a	7.31a	<0.001**
	SD	48.05	16.52	2.93	
100μA/60 seconds	Mean	117.98	13.12a	7.72a	<0.001**
	SD	49.24	15.97	3.23	
100μA/60 seconds	Mean	121.38	12.45a	7.81a	<0.001**
	SD	47.80	13.02	3.37	
100μA/60 seconds	Mean	125.73	13.51a	7.85a	<0.001**
	SD	51.96	17.47	3.32	
100μA/60 seconds	Mean	121.68	13.93a	7.67a	<0.001**
	SD	44.66	18.48	3.17	
100μA/60 seconds	Mean	125.07	15.28a	7.93a	<0.001**
	SD	44.94	24.23	3.51	
100μA/60 seconds	Mean	125.04	15.95a	7.85a	<0.001**
	SD	46.76	26.15	3.67	
Peak of Max response	Mean	141.32	23.91a	14.66a	<0.001**
	SD	56.41	27.79	5.84	
Change in response %	Mean	502.76	174.99a	124.89ab	<0.001**
	SD	244.33	119.05	43.93	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 26 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of ACh on the plantar surface in control group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	47.27	3.91a	3.73a	<0.001**
	SD	33.03	1.78	1.06	
100μA/60 seconds	Mean	46.89	4.16a	3.81a	<0.001**
	SD	32.85	1.97	1.28	
100μA/60 seconds	Mean	61.91	4.29a	3.85a	<0.001**
	SD	39.58	2.17	1.35	
100μA/60 seconds	Mean	90.50	4.44a	3.92a	<0.001**
	SD	75.48	2.31	1.60	
100μA/60 seconds	Mean	107.88	4.40a	3.79a	<0.001**
	SD	102.87	2.36	1.33	
100μA/60 seconds	Mean	132.11	4.28a	3.95a	<0.001**
	SD	129.60	2.27	1.65	
100μA/60 seconds	Mean	145.65	4.38a	4.19a	<0.001**
	SD	135.60	2.23	2.21	
100μA/60 seconds	Mean	156.41	4.62a	3.91a	<0.001**
	SD	138.34	2.39	1.86	
100μA/60 seconds	Mean	170.08	4.70a	4.14a	<0.001**
	SD	137.31	2.31	2.18	
100μA/60 seconds	Mean	186.34	4.75a	4.05a	<0.001**
	SD	139.27	2.91	2.05	
100μA/60 seconds	Mean	196.50	4.78a	4.05a	<0.001**
	SD	145.32	3.02	2.19	
100μA/60 seconds	Mean	204.87	4.78a	4.17a	<0.001**
	SD	149.94	2.96	2.41	
100μA/60 seconds	Mean	213.75	4.90a	4.20a	<0.001**
	SD	151.28	2.93	2.35	
100μA/60 seconds	Mean	219.48	4.84a	4.19a	<0.001**
	SD	163.68	2.83	2.50	
100μA/60 seconds	Mean	218.87	4.69a	4.12a	<0.001**
	SD	167.36	2.50	2.32	
Peak of Max response	Mean	223.44	8.98a	8.13a	<0.001**
	SD	140.95	4.19	3.48	
Change in response %	Mean	460.66	130.92a	116.36a	<0.001**
	SD	344.35	53.81	64.56	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 27 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of SNP on the plantar surface in control group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	51.45	3.77a	3.76a	<0.001**
	SD	62.29	1.40	1.20	
100μA/60 seconds	Mean	49.90	3.83a	3.73a	<0.001**
	SD	60.77	1.41	1.36	
100μA/60 seconds	Mean	62.61	4.10a	3.91a	<0.001**
	SD	77.32	1.88	1.55	
100μA/60 seconds	Mean	85.68	4.24a	3.82a	<0.001**
	SD	80.51	1.95	1.42	
100μA/60 seconds	Mean	116.59	4.32a	3.82a	<0.001**
	SD	88.32	1.98	1.39	
100μA/60 seconds	Mean	144.77	4.26a	3.93a	<0.001**
	SD	120.54	1.85	1.41	
100μA/60 seconds	Mean	157.35	4.41a	3.90a	<0.001**
	SD	127.90	2.04	1.28	
100μA/60 seconds	Mean	163.84	4.63a	4.03a	<0.001**
	SD	135.86	2.35	1.49	
100μA/60 seconds	Mean	163.93	4.58a	3.96a	<0.001**
	SD	138.32	2.19	1.38	
100μA/60 seconds	Mean	165.48	4.41a	3.97a	<0.001**
	SD	135.62	2.00	1.34	
100μA/60 seconds	Mean	164.05	4.49a	3.92a	<0.001**
	SD	132.88	2.27	1.33	
100μA/60 seconds	Mean	163.78	4.44a	3.81a	<0.001**
	SD	130.22	1.98	1.24	
100μA/60 seconds	Mean	167.77	4.42a	4.14a	<0.001**
	SD	126.48	1.86	1.77	
100μA/60 seconds	Mean	157.93	4.44a	4.18a	<0.001**
	SD	120.67	1.86	1.86	
100μA/60 seconds	Mean	162.24	4.46a	4.39a	<0.001**
	SD	121.12	1.92	2.08	
Peak of Max response	Mean	203.12	8.00a	7.84a	<0.001**
	SD	138.22	3.59	3.97	
Change in response %	Mean	604.93	117.79a	103.58a	<0.001**
	SD	685.26	82.24	42.32	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 28 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	29.19	8.73a	6.19a	<0.001**
	SD	16.90	3.37	1.90	
100μA/60 seconds	Mean	38.33	8.76a	6.67a	<0.001**
	SD	23.74	3.25	2.12	
100μA/60 seconds	Mean	57.91	9.05a	6.35a	<0.001**
	SD	38.24	2.97	2.29	
100μA/60 seconds	Mean	66.30	9.32a	6.73a	<0.001**
	SD	41.27	3.04	2.33	
100μA/60 seconds	Mean	78.09	9.79a	6.94a	<0.001**
	SD	46.20	3.42	2.33	
100μA/60 seconds	Mean	87.43	9.77a	6.87a	<0.001**
	SD	49.41	3.56	2.30	
100μA/60 seconds	Mean	95.35	10.41a	7.07a	<0.001**
	SD	51.98	4.10	2.64	
100μA/60 seconds	Mean	100.72	10.81a	7.07a	<0.001**
	SD	55.12	4.52	2.60	
100μA/60 seconds	Mean	106.39	10.83a	7.39a	<0.001**
	SD	55.74	4.33	2.67	
100μA/60 seconds	Mean	102.57	11.05a	7.55a	<0.001**
	SD	49.67	4.35	3.02	
100μA/60 seconds	Mean	110.01	12.34a	7.53a	<0.001**
	SD	56.77	5.29	3.06	
100μA/60 seconds	Mean	110.50	11.76a	7.32a	<0.001**
	SD	66.23	5.43	2.81	
100μA/60 seconds	Mean	110.34	11.30a	7.57a	<0.001**
	SD	77.66	3.87	3.09	
100μA/60 seconds	Mean	107.41	11.89a	7.79a	<0.001**
	SD	78.45	4.88	2.95	
100μA/60 seconds	Mean	107.33	11.84a	7.83a	<0.001**
	SD	75.96	4.81	2.98	
Peak of Max response	Mean	141.10	20.40a	14.52a	<0.001**
	SD	85.93	8.03	5.34	
Change in response %	Mean	450.48	144.70a	136.01a	<0.001**
	SD	319.52	77.64	55.09	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 29 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	23.67	7.51a	7.29a	<0.001**
	SD	11.36	2.81	3.37	
100μA/60 seconds	Mean	22.95	8.29a	7.37a	<0.001**
	SD	9.62	4.25	3.32	
100μA/60 seconds	Mean	36.07	8.32a	7.37a	<0.001**
	SD	16.59	4.25	3.10	
100μA/60 seconds	Mean	63.04	8.83a	7.98a	<0.001**
	SD	39.50	4.34	3.51	
100μA/60 seconds	Mean	89.81	9.15a	8.35a	<0.001**
	SD	72.49	4.32	4.13	
100μA/60 seconds	Mean	113.62	9.31a	8.76a	<0.001**
	SD	101.29	4.20	4.70	
100μA/60 seconds	Mean	123.03	9.19a	9.03a	<0.001**
	SD	101.49	4.46	5.03	
100μA/60 seconds	Mean	129.67	9.21a	9.15a	<0.001**
	SD	100.12	4.77	5.24	
100μA/60 seconds	Mean	128.03	9.09a	8.99a	<0.001**
	SD	85.26	4.53	4.79	
100μA/60 seconds	Mean	134.79	8.99a	8.71a	<0.001**
	SD	84.67	5.04	4.34	
100μA/60 seconds	Mean	137.69	9.35a	8.82a	<0.001**
	SD	82.57	5.27	4.06	
100μA/60 seconds	Mean	138.11	9.61a	9.23a	<0.001**
	SD	83.54	5.53	4.43	
100μA/60 seconds	Mean	137.27	9.89a	9.55a	<0.001**
	SD	82.40	5.87	4.88	
100μA/60 seconds	Mean	135.26	10.29a	9.12a	<0.001**
	SD	76.21	6.23	4.32	
100μA/60 seconds	Mean	133.67	10.59a	9.00a	<0.001**
	SD	77.87	6.55	4.07	
Peak of Max response	Mean	164.80	18.56a	18.63a	<0.001**
	SD	97.46	9.48	7.96	
Change in response %	Mean	673.14	145.36a	171.43ab	<0.001**
	SD	469.64	55.33	84.82	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 30 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of ACh on the plantar surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	71.80	5.14a	5.19a	<0.001**
	SD	43.65	1.84	1.95	
100μA/60 seconds	Mean	84.01	5.49a	5.47a	<0.001**
	SD	47.37	2.13	2.07	
100μA/60 seconds	Mean	117.98	5.49a	5.63a	<0.001**
	SD	68.15	2.23	2.23	
100μA/60 seconds	Mean	165.60	5.52a	5.67a	<0.001**
	SD	99.90	2.04	2.32	
100μA/60 seconds	Mean	188.11	5.56a	5.72a	<0.001**
	SD	113.00	2.26	2.32	
100μA/60 seconds	Mean	203.95	5.67a	5.54a	<0.001**
	SD	128.43	2.48	2.07	
100μA/60 seconds	Mean	215.81	5.67a	5.77a	<0.001**
	SD	133.14	2.42	2.38	
100μA/60 seconds	Mean	222.55	6.01a	5.55a	<0.001**
	SD	133.47	2.40	2.12	
100μA/60 seconds	Mean	238.69	5.79a	5.78a	<0.001**
	SD	142.22	2.34	2.39	
100μA/60 seconds	Mean	246.98	6.03a	5.75a	<0.001**
	SD	152.56	2.86	2.37	
100μA/60 seconds	Mean	253.88	5.96a	5.98a	<0.001**
	SD	161.37	2.94	2.59	
100μA/60 seconds	Mean	248.19	5.85a	6.09a	<0.001**
	SD	161.78	3.03	2.72	
100μA/60 seconds	Mean	258.02	5.89a	6.01a	<0.001**
	SD	160.39	2.98	2.60	
100μA/60 seconds	Mean	260.47	5.91a	5.85a	<0.001**
	SD	151.49	2.87	2.51	
100μA/60 seconds	Mean	260.48	5.75a	5.73a	<0.001**
	SD	156.91	2.81	2.39	
Peak of Max response	Mean	314.91	11.48a	11.54a	<0.001**
	SD	167.14	4.50	4.20	
Change in response %	Mean	543.54	124.02a	130.29a	0.002*
	SD	585.51	53.23	60.42	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 31 Comparison between the three tested pressure conditions application on flux median (PU) during the iontophoresis of SNP on the plantar surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	67.52	5.45a	4.65a	<0.001**
	SD	45.70	4.00	1.93	
100μA/60 seconds	Mean	76.53	5.80a	4.74a	<0.001**
	SD	54.62	4.50	1.87	
100μA/60 seconds	Mean	90.69	5.95a	4.67a	<0.001**
	SD	66.88	5.67	1.85	
100μA/60 seconds	Mean	121.96	5.41a	4.80a	<0.001**
	SD	81.69	4.08	2.29	
100μA/60 seconds	Mean	157.38	5.99a	4.87a	<0.001**
	SD	98.94	5.52	2.07	
100μA/60 seconds	Mean	177.60	5.65a	4.69a	<0.001**
	SD	99.63	4.30	1.85	
100μA/60 seconds	Mean	193.88	5.97a	4.41a	<0.001**
	SD	109.66	5.60	1.57	
100μA/60 seconds	Mean	192.97	7.23a	4.41a	<0.001**
	SD	110.96	10.15	1.69	
100μA/60 seconds	Mean	196.31	7.39a	4.20a	<0.001**
	SD	113.92	11.03	1.70	
100μA/60 seconds	Mean	190.29	7.73a	4.50a	<0.001**
	SD	117.53	11.82	2.02	
100μA/60 seconds	Mean	189.45	6.81a	4.39a	<0.001**
	SD	117.53	9.22	1.90	
100μA/60 seconds	Mean	186.15	6.91a	4.37a	<0.001**
	SD	109.22	9.54	1.86	
100μA/60 seconds	Mean	184.92	6.58a	4.43a	<0.001**
	SD	101.43	9.17	2.00	
100μA/60 seconds	Mean	187.89	6.70a	4.63a	<0.001**
	SD	90.25	7.99	2.19	
100μA/60 seconds	Mean	181.20	7.17a	4.39a	<0.001**
	SD	81.34	10.18	1.77	
Peak of Max response	Mean	247.69	14.86a	9.55a	<0.001**
	SD	112.18	19.78	3.68	
Change in response %	Mean	332.47	141.91a	111.26ab	<0.001**
	SD	146.04	88.61	47.50	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 32 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	29.94	9.02a	6.45a	<0.001**
	SD	17.17	3.29	1.94	
100μA/60 seconds	Mean	39.73	9.14a	6.94a	<0.001**
	SD	24.75	3.30	2.21	
100μA/60 seconds	Mean	59.58	9.40a	6.74a	<0.001**
	SD	39.07	3.09	2.34	
100μA/60 seconds	Mean	68.04	9.68a	7.16a	<0.001**
	SD	42.22	3.21	2.55	
100μA/60 seconds	Mean	80.00	10.17a	7.35a	<0.001**
	SD	47.10	3.60	2.62	
100μA/60 seconds	Mean	89.33	10.15a	7.29a	<0.001**
	SD	50.18	3.71	2.61	
100μA/60 seconds	Mean	97.13	10.74a	7.53a	<0.001**
	SD	53.29	4.27	2.93	
100μA/60 seconds	Mean	102.41	11.25a	7.50a	<0.001**
	SD	56.19	4.78	2.92	
100μA/60 seconds	Mean	108.02	11.26a	7.85a	<0.001**
	SD	56.37	4.50	3.02	
100μA/60 seconds	Mean	105.26	11.43a	7.96a	<0.001**
	SD	51.32	4.47	3.32	
100μA/60 seconds	Mean	111.96	12.68a	7.98a	<0.001**
	SD	58.03	5.46	3.42	
100μA/60 seconds	Mean	112.27	12.12a	7.76a	<0.001**
	SD	67.68	5.51	3.08	
100μA/60 seconds	Mean	112.81	11.67a	7.99a	<0.001**
	SD	80.23	4.03	3.48	
100μA/60 seconds	Mean	109.59	12.23a	8.24a	<0.001**
	SD	80.03	5.03	3.26	
100μA/60 seconds	Mean	108.66	12.18a	8.25a	<0.001**
	SD	77.55	4.96	3.26	
Peak of Max response	Mean	141.10	20.40a	14.52a	<0.001**
	SD	85.93	8.03	5.34	
Change in response %	Mean	433.65	133.73a	125.65a	<0.001**
	SD	308.32	69.18	47.70	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 33 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Dorsum No pressure	Dorsum Ortho pressure	Dorsum Own pressure	p
0μA/60 seconds	Mean	24.44	8.05a	7.71a	<0.001**
	SD	11.41	3.09	3.60	
100μA/60 seconds	Mean	23.86	8.71a	7.81a	<0.001**
	SD	9.61	4.35	3.52	
100μA/60 seconds	Mean	37.58	8.76a	7.96a	<0.001**
	SD	17.74	4.41	3.34	
100μA/60 seconds	Mean	64.83	9.28a	8.43a	<0.001**
	SD	40.74	4.62	3.73	
100μA/60 seconds	Mean	92.24	9.73a	8.81a	<0.001**
	SD	73.75	4.66	4.25	
100μA/60 seconds	Mean	115.11	9.84a	9.19a	<0.001**
	SD	100.12	4.48	4.74	
100μA/60 seconds	Mean	125.05	9.67a	9.51a	<0.001**
	SD	102.17	4.79	5.03	
100μA/60 seconds	Mean	131.76	9.68a	9.63a	<0.001**
	SD	100.13	5.09	5.40	
100μA/60 seconds	Mean	131.05	9.61a	9.35a	<0.001**
	SD	87.20	4.90	4.84	
100μA/60 seconds	Mean	137.56	9.52a	9.23a	<0.001**
	SD	87.12	5.46	4.55	
100μA/60 seconds	Mean	140.41	9.84a	9.36a	<0.001**
	SD	84.96	5.59	4.20	
100μA/60 seconds	Mean	141.00	10.04a	9.73a	<0.001**
	SD	85.89	5.88	4.59	
100μA/60 seconds	Mean	139.51	10.34a	10.02a	<0.001**
	SD	84.40	6.23	5.00	
100μA/60 seconds	Mean	137.54	10.68a	9.76a	<0.001**
	SD	78.24	6.56	4.50	
100μA/60 seconds	Mean	136.17	10.95a	9.59a	<0.001**
	SD	79.59	6.83	4.33	
Peak of Max response	Mean	164.80	18.56a	18.63a	<0.001**
	SD	97.46	9.48	7.96	
Change in response %	Mean	643.82	127.73a	156.10ab	<0.001**
	SD	448.18	45.14	76.46	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 34 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of ACh on the plantar surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	74.37	5.39a	5.49a	<0.001**
	SD	44.50	1.90	2.05	
100μA/60 seconds	Mean	86.94	5.74a	5.73a	<0.001**
	SD	47.26	2.14	2.19	
100μA/60 seconds	Mean	119.68	5.75a	5.94a	<0.001**
	SD	67.90	2.22	2.31	
100μA/60 seconds	Mean	169.98	5.85a	5.98a	<0.001**
	SD	102.49	2.06	2.44	
100μA/60 seconds	Mean	192.55	5.86a	5.99a	<0.001**
	SD	115.84	2.29	2.38	
100μA/60 seconds	Mean	208.65	5.99a	5.85a	<0.001**
	SD	131.41	2.50	2.18	
100μA/60 seconds	Mean	220.53	5.97a	6.04a	<0.001**
	SD	136.63	2.42	2.49	
100μA/60 seconds	Mean	227.05	6.42a	5.93a	<0.001**
	SD	136.34	2.55	2.41	
100μA/60 seconds	Mean	242.06	6.14a	6.08a	<0.001**
	SD	144.28	2.46	2.52	
100μA/60 seconds	Mean	251.27	6.25a	6.07a	<0.001**
	SD	155.84	2.86	2.52	
100μA/60 seconds	Mean	257.97	6.25a	6.42a	<0.001**
	SD	163.25	2.97	2.72	
100μA/60 seconds	Mean	252.36	6.08a	6.43a	<0.001**
	SD	164.45	2.98	2.74	
100μA/60 seconds	Mean	262.03	6.17a	6.34a	<0.001**
	SD	162.67	2.94	2.65	
100μA/60 seconds	Mean	264.38	6.17a	6.24a	<0.001**
	SD	154.87	2.86	2.68	
100μA/60 seconds	Mean	264.65	6.07a	6.09a	<0.001**
	SD	159.12	2.84	2.63	
Peak of Max response	Mean	314.91	11.48a	11.54a	<0.001**
	SD	167.14	4.50	4.20	
Change in response %	Mean	516.13	112.01a	116.65a	0.002*
	SD	563.94	42.00	49.22	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 35 Comparison between the three tested pressure conditions flux mean (PU) during the iontophoresis of SNP on the plantar surface in diabetes group using: One Way analysis of variance.

Iontophoresis		Plantar No pressure	Plantar Ortho pressure	Plantar Own pressure	p
0μA/60 seconds	Mean	70.39	5.93a	4.92a	<0.001**
	SD	46.32	4.98	1.91	
100μA/60 seconds	Mean	78.38	6.45a	5.03a	<0.001**
	SD	55.31	5.73	1.98	
100μA/60 seconds	Mean	94.01	6.64a	5.01a	<0.001**
	SD	68.55	6.95	1.94	
100μA/60 seconds	Mean	124.09	6.09a	5.02a	<0.001**
	SD	81.68	5.36	2.25	
100μA/60 seconds	Mean	159.19	6.62a	5.22a	<0.001**
	SD	98.88	6.82	2.19	
100μA/60 seconds	Mean	180.58	6.26a	5.01a	<0.001**
	SD	101.00	5.60	1.97	
100μA/60 seconds	Mean	195.09	6.62a	4.81a	<0.001**
	SD	109.55	6.84	1.68	
100μA/60 seconds	Mean	195.65	7.63a	4.76a	<0.001**
	SD	111.51	10.55	1.86	
100μA/60 seconds	Mean	199.05	7.80a	4.51a	<0.001**
	SD	114.48	11.34	1.93	
100μA/60 seconds	Mean	193.60	8.03a	4.79a	<0.001**
	SD	118.05	11.92	2.13	
100μA/60 seconds	Mean	192.41	7.38a	4.69a	<0.001**
	SD	117.58	10.26	2.03	
100μA/60 seconds	Mean	189.99	7.48a	4.72a	<0.001**
	SD	110.35	10.20	1.97	
100μA/60 seconds	Mean	188.82	7.13a	4.72a	<0.001**
	SD	102.20	10.14	2.17	
100μA/60 seconds	Mean	190.06	7.30a	4.85a	<0.001**
	SD	91.38	9.12	2.19	
100μA/60 seconds	Mean	183.48	7.59a	4.67a	<0.001**
	SD	82.20	10.72	1.92	
Peak of Max response	Mean	247.69	14.86a	9.55a	<0.001**
	SD	112.18	19.78	3.68	
Change in response %	Mean	310.31	124.58a	96.93ab	<0.001**
	SD	139.64	67.91	38.99	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Post Hoc: a: significant difference with no pressure; b: significant difference with Ortho pressure

Table 36 Comparison between control group and diabetes group median of flux (PU) on the dorsum of the foot with no pressure applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	20.82	13.74	8.8	69	0.115
	Diabetes	29.19	16.90	11.2	60.7	
100μA/60 seconds	Control	28.50	19.25	12.1	83.5	0.185
	Diabetes	38.33	23.74	11.4	78.6	
100μA/60 seconds	Control	40.86	21.37	14.8	96.7	0.103
	Diabetes	57.91	38.24	14.1	153.5	
100μA/60 seconds	Control	52.47	21.78	11.9	95.5	0.208
	Diabetes	66.30	41.27	13.3	162.5	
100μA/60 seconds	Control	64.61	28.92	22.9	142.8	0.297
	Diabetes	78.09	46.20	15.6	167.8	
100μA/60 seconds	Control	75.30	34.93	22.7	167.5	0.400
	Diabetes	87.43	49.41	19.4	169.4	
100μA/60 seconds	Control	83.72	37.64	31.3	188.1	0.448
	Diabetes	95.35	51.98	22.5	176.7	
100μA/60 seconds	Control	97.32	43.88	47.9	201	0.840
	Diabetes	100.72	55.12	22.1	182.2	
100μA/60 seconds	Control	113.61	45.04	53.6	225.8	0.675
	Diabetes	106.39	55.74	25.7	186.5	
100μA/60 seconds	Control	124.31	50.19	55.7	222.2	0.212
	Diabetes	102.57	49.67	27.1	178.1	
100μA/60 seconds	Control	129.34	46.54	55.4	226.3	0.276
	Diabetes	110.01	56.77	30.1	198.3	
100μA/60 seconds	Control	129.06	47.85	59.6	228.9	0.342
	Diabetes	110.50	66.23	38.8	281.9	
100μA/60 seconds	Control	128.14	49.16	62	237.1	0.413
	Diabetes	110.34	77.66	38.3	318.7	
100μA/60 seconds	Control	130.24	51.97	49.6	238.8	0.308
	Diabetes	107.41	78.45	27.5	312.4	
100μA/60 seconds	Control	132.43	54.08	35.9	242.1	0.261
	Diabetes	107.33	75.96	23.4	294.1	
Peak of Max response	Control	142.99	39.56	74.5	233.47	0.931
	Diabetes	141.10	85.93	40.1	369.01	
Change in response %	Control	756.05	467.04	238.36	1905.94	0.037*
	Diabetes	450.48	319.52	104.75	1113.00	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 37 Comparison between control group and diabetes group median of flux (PU) on the dorsum of the foot with no pressure applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	24.64	12.18	11	59	0.811
	Diabetes	23.67	11.36	11.6	49.2	
100μA/60 seconds	Control	28.63	13.96	12.6	68.2	0.186
	Diabetes	22.95	9.62	12	50.6	
100μA/60 seconds	Control	44.27	26.18	18.1	94.1	0.297
	Diabetes	36.07	16.59	12.7	69.4	
100μA/60 seconds	Control	59.95	34.65	15.8	121	0.807
	Diabetes	63.04	39.50	16.4	173.3	
100μA/60 seconds	Control	73.97	43.86	14.9	167.2	0.428
	Diabetes	89.81	72.49	14.9	252.9	
100μA/60 seconds	Control	86.76	46.12	18	170.4	0.300
	Diabetes	113.62	101.29	22.2	384.5	
100μA/60 seconds	Control	96.52	49.37	23	186	0.314
	Diabetes	123.03	101.49	39.4	372.2	
100μA/60 seconds	Control	103.14	46.97	34.7	194.6	0.304
	Diabetes	129.67	100.12	41.6	363.4	
100μA/60 seconds	Control	111.09	47.18	41.3	196.7	0.458
	Diabetes	128.03	85.26	43.2	364.6	
100μA/60 seconds	Control	116.28	48.95	44.3	212.5	0.421
	Diabetes	134.79	84.67	41.9	362	
100μA/60 seconds	Control	119.73	47.11	44.7	196.6	0.421
	Diabetes	137.69	82.57	47.9	366.5	
100μA/60 seconds	Control	124.47	51.82	39.2	210.9	0.556
	Diabetes	138.11	83.54	46.5	362.4	
100μA/60 seconds	Control	119.07	43.26	49.1	185.5	0.403
	Diabetes	137.27	82.40	47.1	366.2	
100μA/60 seconds	Control	123.62	43.66	49.8	181.4	0.572
	Diabetes	135.26	76.21	51.3	351.1	
100μA/60 seconds	Control	123.51	45.73	43.7	191.7	0.632
	Diabetes	133.67	77.87	52.2	363.9	
Peak of Max response	Control	141.32	56.41	56.26	242.25	0.376
	Diabetes	164.80	97.46	67.04	413.13	
Change in response %	Control	517.86	247.75	153.91	1087.50	0.214
	Diabetes	673.14	469.64	214.20	1607.15	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 38 Comparison between control group and diabetes group median of flux (PU) on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	6.40	2.83	4.1	17.2	0.034*
	Diabetes	8.73	3.37	3.3	16.7	
100μA/60 seconds	Control	6.72	2.76	4.3	17.1	0.035*
	Diabetes	8.76	3.25	3.4	15.7	
100μA/60 seconds	Control	7.22	2.99	4.4	18.2	0.081
	Diabetes	9.05	2.97	3.3	13.3	
100μA/60 seconds	Control	7.57	3.45	4.1	20.2	0.127
	Diabetes	9.32	3.04	3.5	14.4	
100μA/60 seconds	Control	7.74	3.70	4.3	20.2	0.103
	Diabetes	9.79	3.42	3.8	16.5	
100μA/60 seconds	Control	8.29	4.61	4.2	24.5	0.307
	Diabetes	9.77	3.56	3.9	16.5	
100μA/60 seconds	Control	8.49	5.26	4.3	27.8	0.251
	Diabetes	10.41	4.10	4.2	17.7	
100μA/60 seconds	Control	8.85	5.50	5.1	29.3	0.267
	Diabetes	10.81	4.52	4.4	19.2	
100μA/60 seconds	Control	9.13	6.26	4.6	33.5	0.374
	Diabetes	10.83	4.33	4.6	17.9	
100μA/60 seconds	Control	9.32	6.17	5.1	32.8	0.359
	Diabetes	11.05	4.35	4.7	19.9	
100μA/60 seconds	Control	10.11	6.38	5	32.2	0.278
	Diabetes	12.34	5.29	4.9	25.9	
100μA/60 seconds	Control	10.65	6.89	5.2	34.3	0.609
	Diabetes	11.76	5.43	4.7	26.7	
100μA/60 seconds	Control	10.41	6.54	4.9	31.5	0.643
	Diabetes	11.30	3.87	4.7	17.9	
100μA/60 seconds	Control	10.20	6.45	5.2	32.1	0.404
	Diabetes	11.89	4.88	4.6	22.1	
100μA/60 seconds	Control	11.14	8.43	4.8	42.8	0.774
	Diabetes	11.84	4.81	4.5	21.8	
Peak of Max response	Control	18.27	8.19	11.18	48.47	0.447
	Diabetes	20.40	8.03	8.39	37.53	
Change in response %	Control	192.00	80.08	113.73	458.38	0.089
	Diabetes	144.70	77.64	40.97	307.95	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 39 Comparison between control group and diabetes group median of flux (PU) on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	7.37	4.41	2	19.2	0.914
	Diabetes	7.51	2.81	2.7	12.9	
100μA/60 seconds	Control	7.86	4.54	2.2	19.8	0.779
	Diabetes	8.29	4.25	2.7	20.4	
100μA/60 seconds	Control	11.67	18.53	2.2	88.5	0.499
	Diabetes	8.32	4.25	2.7	21	
100μA/60 seconds	Control	12.71	21.98	2.4	104.5	0.507
	Diabetes	8.83	4.34	2.7	20.5	
100μA/60 seconds	Control	12.10	16.70	2.4	80.2	0.512
	Diabetes	9.15	4.32	2.9	19.7	
100μA/60 seconds	Control	13.52	22.75	2.8	108.3	0.485
	Diabetes	9.31	4.20	2.8	18.1	
100μA/60 seconds	Control	15.41	29.93	2.8	141	0.432
	Diabetes	9.19	4.46	2.8	19.5	
100μA/60 seconds	Control	13.95	22.85	3.3	109	0.437
	Diabetes	9.21	4.77	2.8	21.7	
100μA/60 seconds	Control	12.49	16.40	2.9	78.8	0.441
	Diabetes	9.09	4.53	2.9	21	
100μA/60 seconds	Control	12.56	15.53	3.1	75.7	0.400
	Diabetes	8.99	5.04	3.3	23.3	
100μA/60 seconds	Control	12.06	13.06	3.1	63.9	0.456
	Diabetes	9.35	5.27	2.9	24.9	
100μA/60 seconds	Control	12.98	16.86	3.7	81.7	0.464
	Diabetes	9.61	5.53	3.1	25.6	
100μA/60 seconds	Control	13.47	18.23	3.9	88.1	0.470
	Diabetes	9.89	5.87	2.9	26.8	
100μA/60 seconds	Control	14.69	23.67	3.6	113.6	0.489
	Diabetes	10.29	6.23	3	28.6	
100μA/60 seconds	Control	15.48	26.16	3.7	124.7	0.485
	Diabetes	10.59	6.55	3	30.3	
Peak of Max response	Control	23.91	27.79	6.64	137.13	0.481
	Diabetes	18.56	9.48	6.64	46.66	
Change in response %	Control	199.67	124.29	45.29	633.29	0.125
	Diabetes	145.36	55.33	74.17	261.72	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 40 Comparison between control group and diabetes group median of flux (PU) on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	6.25	1.67	3.2	8.6	0.932
	Diabetes	6.19	1.90	3.5	10.7	
100μA/60 seconds	Control	6.31	1.67	3.6	9	0.576
	Diabetes	6.67	2.12	4.1	12.2	
100μA/60 seconds	Control	6.50	1.76	3.3	9.7	0.831
	Diabetes	6.35	2.29	4.2	11.9	
100μA/60 seconds	Control	6.75	1.86	3.7	10	0.981
	Diabetes	6.73	2.33	4.2	11.6	
100μA/60 seconds	Control	6.73	2.16	3.8	11.1	0.780
	Diabetes	6.94	2.33	4.4	11.8	
100μA/60 seconds	Control	6.87	2.30	3.7	11.6	0.998
	Diabetes	6.87	2.30	4.3	12.3	
100μA/60 seconds	Control	7.02	2.29	4.1	12.7	0.949
	Diabetes	7.07	2.64	4.3	12.8	
100μA/60 seconds	Control	7.14	2.28	3.8	13.2	0.936
	Diabetes	7.07	2.60	4.3	12.7	
100μA/60 seconds	Control	7.45	2.27	4.2	13.8	0.940
	Diabetes	7.39	2.67	4.2	12.9	
100μA/60 seconds	Control	7.68	2.48	3.8	13.1	0.887
	Diabetes	7.55	3.02	4.2	13.6	
100μA/60 seconds	Control	7.53	2.50	4	13.5	0.997
	Diabetes	7.53	3.06	3.8	14.1	
100μA/60 seconds	Control	7.92	2.71	3.8	14.3	0.532
	Diabetes	7.32	2.81	4.3	13.2	
100μA/60 seconds	Control	7.82	2.75	3.8	14.5	0.809
	Diabetes	7.57	3.09	3.9	13.5	
100μA/60 seconds	Control	7.92	2.71	4.2	14.4	0.890
	Diabetes	7.79	2.95	4.2	13.9	
100μA/60 seconds	Control	8.40	3.02	3.9	15.8	0.584
	Diabetes	7.83	2.98	4.2	13.9	
Peak of Max response	Control	14.90	3.64	8.94	23.5	0.803
	Diabetes	14.52	5.34	8.07	27.51	
Change in response %	Control	149.65	66.86	29.87	274.80	0.525
	Diabetes	136.01	55.09	65.77	293.04	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 41 Comparison between control group and diabetes group median of flux (PU) on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	6.21	2.39	3	11.3	0.271
	Diabetes	7.29	3.37	3.3	13.9	
100μA/60 seconds	Control	6.43	2.42	3.5	12	0.341
	Diabetes	7.37	3.32	4	14.6	
100μA/60 seconds	Control	6.64	2.54	3.9	11.9	0.445
	Diabetes	7.37	3.10	4.2	13.8	
100μA/60 seconds	Control	6.57	2.56	3.7	11.7	0.177
	Diabetes	7.98	3.51	4.4	14.6	
100μA/60 seconds	Control	6.52	2.42	3.5	10.3	0.110
	Diabetes	8.35	4.13	4.2	17.1	
100μA/60 seconds	Control	6.65	2.37	3.5	11.8	0.090
	Diabetes	8.76	4.70	4.2	18.2	
100μA/60 seconds	Control	6.57	2.41	3.5	12.3	0.063
	Diabetes	9.03	5.03	3.9	19.4	
100μA/60 seconds	Control	6.87	2.85	3.3	14.2	0.108
	Diabetes	9.15	5.24	4.3	19.1	
100μA/60 seconds	Control	6.83	2.73	3.3	15.2	0.099
	Diabetes	8.99	4.79	4.5	17.4	
100μA/60 seconds	Control	7.30	2.99	3.4	16.1	0.260
	Diabetes	8.71	4.34	4.4	17.5	
100μA/60 seconds	Control	7.39	3.20	3.4	17.1	0.252
	Diabetes	8.82	4.06	4.4	17.8	
100μA/60 seconds	Control	7.45	3.13	3.7	16.9	0.172
	Diabetes	9.23	4.43	4.1	18.8	
100μA/60 seconds	Control	7.22	2.93	3.8	16.7	0.087
	Diabetes	9.55	4.88	4.2	21.8	
100μA/60 seconds	Control	7.54	3.36	3.6	18.5	0.230
	Diabetes	9.12	4.32	4.4	19.6	
100μA/60 seconds	Control	7.45	3.53	3.7	18.8	0.236
	Diabetes	9.00	4.07	3.9	18	
Peak of Max response	Control	14.66	5.84	6.64	24.99	0.098
	Diabetes	18.63	7.96	9.37	36.44	
Change in response %	Control	139.22	51.86	36.83	252.02	0.174
	Diabetes	171.43	84.82	84.80	340.80	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 42 Comparison between control group and diabetes group mean of flux (PU) on the dorsum of the foot with no pressure applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	21.67	13.98	9.93	71.03	0.126
	Diabetes	29.94	17.17	11.64	63.03	
100μA/60 seconds	Control	29.31	18.89	12.78	84.1	0.167
	Diabetes	39.73	24.75	11.53	86.85	
100μA/60 seconds	Control	41.99	21.43	15.57	96.34	0.098
	Diabetes	59.58	39.07	13.89	158.8	
100μA/60 seconds	Control	53.93	21.97	12.51	94.53	0.208
	Diabetes	68.04	42.22	13.53	167.35	
100μA/60 seconds	Control	67.18	28.24	25.34	143.13	0.323
	Diabetes	80.00	47.10	16.46	173.83	
100μA/60 seconds	Control	77.18	34.39	25.87	167.33	0.401
	Diabetes	89.33	50.18	20.62	173.75	
100μA/60 seconds	Control	85.52	37.75	31.93	187.96	0.455
	Diabetes	97.13	53.29	22.66	180.32	
100μA/60 seconds	Control	100.07	43.25	48.93	201.46	0.890
	Diabetes	102.41	56.19	22.07	186.86	
100μA/60 seconds	Control	116.98	44.96	54.94	225.13	0.604
	Diabetes	108.02	56.37	26.11	187.88	
100μA/60 seconds	Control	125.59	50.01	57.15	224.26	0.248
	Diabetes	105.26	51.32	27.82	184.31	
100μA/60 seconds	Control	130.94	46.43	56.81	226.84	0.290
	Diabetes	111.96	58.03	30.9	204.09	
100μA/60 seconds	Control	130.67	47.55	60.37	228.93	0.351
	Diabetes	112.27	67.68	38.25	287.61	
100μA/60 seconds	Control	129.83	48.93	63.49	236.78	0.443
	Diabetes	112.81	80.23	38.22	330.17	
100μA/60 seconds	Control	131.99	51.59	52.64	237.67	0.322
	Diabetes	109.59	80.03	28.22	321.12	
100μA/60 seconds	Control	133.99	53.84	36.69	242.13	0.262
	Diabetes	108.66	77.55	23.97	302.41	
Peak of Max response	Control	142.99	39.56	74.5	233.47	0.931
	Diabetes	141.10	85.93	40.1	369.01	
Change in response %	Control	711.29	431.85	228.69	1771.97	0.042*
	Diabetes	433.65	308.32	102.61	1065.32	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 43 Comparison between control group and diabetes group mean of flux (PU) on the dorsum of the foot with no pressure applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	25.30	12.54	11.56	61.54	0.835
	Diabetes	24.44	11.41	11.84	50.46	
100μA/60 seconds	Control	29.56	14.31	13.17	70.54	0.192
	Diabetes	23.86	9.61	12.4	50.77	
100μA/60 seconds	Control	45.66	27.78	18.13	97.05	0.332
	Diabetes	37.58	17.74	13.03	76.62	
100μA/60 seconds	Control	61.62	35.86	16.26	128.79	0.806
	Diabetes	64.83	40.74	16.52	178.42	
100μA/60 seconds	Control	75.58	44.35	15.61	169.21	0.412
	Diabetes	92.24	73.75	15.13	259.41	
100μA/60 seconds	Control	88.54	46.62	19.83	173.31	0.302
	Diabetes	115.11	100.12	22.91	372.21	
100μA/60 seconds	Control	98.11	49.84	24.36	185.23	0.310
	Diabetes	125.05	102.17	40.05	369.64	
100μA/60 seconds	Control	104.67	47.73	33.77	194.16	0.295
	Diabetes	131.76	100.13	42.89	373.05	
100μA/60 seconds	Control	112.80	48.05	41.6	196.75	0.434
	Diabetes	131.05	87.20	45.18	372.15	
100μA/60 seconds	Control	117.98	49.24	45.84	210.15	0.405
	Diabetes	137.56	87.12	42.26	372.67	
100μA/60 seconds	Control	121.38	47.80	46.88	199.2	0.406
	Diabetes	140.41	84.96	48.22	376.16	
100μA/60 seconds	Control	125.73	51.96	40.36	213.03	0.518
	Diabetes	141.00	85.89	47.82	371.93	
100μA/60 seconds	Control	121.68	44.66	48.69	184.07	0.425
	Diabetes	139.51	84.40	47.8	375.59	
100μA/60 seconds	Control	125.07	44.94	50.29	185.84	0.555
	Diabetes	137.54	78.24	52.35	359.86	
100μA/60 seconds	Control	125.04	46.76	43.28	192.66	0.607
	Diabetes	136.17	79.59	53.37	371.39	
Peak of Max response	Control	141.32	56.41	56.26	242.25	0.376
	Diabetes	164.80	97.46	67.04	413.13	
Change in response %	Control	502.76	244.33	157.04	1065.78	0.241
	Diabetes	643.82	448.18	212.22	1572.60	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 44 Comparison between control group and diabetes group mean of flux (PU) on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	6.83	2.93	4.19	17.96	0.045*
	Diabetes	9.02	3.29	3.41	16.65	
100μA/60 seconds	Control	7.19	2.96	4.51	18.33	0.076
	Diabetes	9.14	3.30	3.56	16.1	
100μA/60 seconds	Control	7.71	3.22	5	19.57	0.127
	Diabetes	9.40	3.09	3.45	14.45	
100μA/60 seconds	Control	8.06	3.65	4.67	21.36	0.181
	Diabetes	9.68	3.21	3.6	15.16	
100μA/60 seconds	Control	8.20	3.81	5	21.03	0.132
	Diabetes	10.17	3.60	4	16.74	
100μA/60 seconds	Control	8.75	4.79	4.41	25.61	0.354
	Diabetes	10.15	3.71	4.11	16.73	
100μA/60 seconds	Control	8.94	5.47	4.77	29.01	0.299
	Diabetes	10.74	4.27	4.26	18.72	
100μA/60 seconds	Control	9.30	5.65	5.71	30.38	0.289
	Diabetes	11.25	4.78	4.59	20.97	
100μA/60 seconds	Control	9.59	6.36	5.23	34.22	0.395
	Diabetes	11.26	4.50	4.82	18.7	
100μA/60 seconds	Control	9.75	6.28	5.45	33.64	0.386
	Diabetes	11.43	4.47	4.91	20.43	
100μA/60 seconds	Control	10.53	6.47	5.51	33.1	0.306
	Diabetes	12.68	5.46	5.13	26.74	
100μA/60 seconds	Control	11.03	6.95	5.66	34.76	0.620
	Diabetes	12.12	5.51	4.91	27.38	
100μA/60 seconds	Control	10.79	6.65	5.31	32.3	0.655
	Diabetes	11.67	4.03	4.93	19.06	
100μA/60 seconds	Control	10.62	6.62	5.45	33.39	0.439
	Diabetes	12.23	5.03	4.76	23.13	
100μA/60 seconds	Control	11.34	8.14	5.04	41.42	0.725
	Diabetes	12.18	4.96	4.59	22.99	
Peak of Max response	Control	18.27	8.19	11.18	48.47	0.447
	Diabetes	20.40	8.03	8.39	37.53	
Change in response %	Control	172.88	75.75	104.14	446.39	0.126
	Diabetes	133.73	69.18	40.83	295.90	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 45 Comparison between control group and diabetes group mean of flux (PU) on the dorsum of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	7.96	4.49	2.17	19.9	0.942
	Diabetes	8.05	3.09	2.86	15.16	
100μA/60 seconds	Control	8.42	4.68	2.45	20.54	0.856
	Diabetes	8.71	4.35	2.85	21.14	
100μA/60 seconds	Control	12.01	17.55	2.33	84.4	0.490
	Diabetes	8.76	4.41	2.76	22.04	
100μA/60 seconds	Control	13.08	21.45	2.45	102.37	0.506
	Diabetes	9.28	4.62	2.84	22.21	
100μA/60 seconds	Control	12.54	16.60	2.56	80.11	0.529
	Diabetes	9.73	4.66	2.96	21.86	
100μA/60 seconds	Control	14.05	22.93	2.98	109.5	0.489
	Diabetes	9.84	4.48	2.88	19.92	
100μA/60 seconds	Control	15.71	29.33	3.03	138.65	0.437
	Diabetes	9.67	4.79	2.94	21.64	
100μA/60 seconds	Control	14.44	22.99	3.51	109.99	0.438
	Diabetes	9.68	5.09	2.84	23.41	
100μA/60 seconds	Control	13.02	16.52	3.11	79.75	0.445
	Diabetes	9.61	4.90	3.03	23.02	
100μA/60 seconds	Control	13.12	15.97	3.22	78.02	0.411
	Diabetes	9.52	5.46	3.51	25.5	
100μA/60 seconds	Control	12.45	13.02	3.2	63.86	0.473
	Diabetes	9.84	5.59	2.98	26.72	
100μA/60 seconds	Control	13.51	17.47	3.77	84.78	0.466
	Diabetes	10.04	5.88	3.24	27.47	
100μA/60 seconds	Control	13.93	18.48	4.08	89.62	0.477
	Diabetes	10.34	6.23	3.01	28.93	
100μA/60 seconds	Control	15.28	24.23	3.88	116.5	0.481
	Diabetes	10.68	6.56	3.16	30.58	
100μA/60 seconds	Control	15.95	26.15	3.9	125.04	0.476
	Diabetes	10.95	6.83	3.18	32	
Peak of Max response	Control	23.91	27.79	6.64	137.13	0.481
	Diabetes	18.56	9.48	6.64	46.66	
Change in response %	Control	174.99	119.05	38.69	616.06	0.155
	Diabetes	127.73	45.14	74.17	216.61	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 46 Comparison between control group and diabetes group mean of flux (PU) on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	6.56	1.67	3.3	9.09	0.861
	Diabetes	6.45	1.94	3.49	11.15	
100μA/60 seconds	Control	6.68	1.71	3.89	9.51	0.696
	Diabetes	6.94	2.21	4.33	12.91	
100μA/60 seconds	Control	6.97	1.88	3.49	10.31	0.752
	Diabetes	6.74	2.34	4.59	12.52	
100μA/60 seconds	Control	7.21	1.99	3.91	10.86	0.950
	Diabetes	7.16	2.55	4.36	12.56	
100μA/60 seconds	Control	7.17	2.31	4	11.64	0.830
	Diabetes	7.35	2.62	4.54	13.51	
100μA/60 seconds	Control	7.32	2.39	4.05	12.06	0.967
	Diabetes	7.29	2.61	4.4	12.98	
100μA/60 seconds	Control	7.43	2.32	4.45	12.73	0.913
	Diabetes	7.53	2.93	4.42	14.25	
100μA/60 seconds	Control	7.54	2.37	4.12	13.55	0.965
	Diabetes	7.50	2.92	4.51	14.2	
100μA/60 seconds	Control	7.86	2.25	4.45	13.78	0.988
	Diabetes	7.85	3.02	4.29	14.78	
100μA/60 seconds	Control	8.06	2.55	3.91	13.82	0.925
	Diabetes	7.96	3.32	4.57	15.69	
100μA/60 seconds	Control	7.97	2.54	4.11	14.19	0.996
	Diabetes	7.98	3.42	4.06	16.35	
100μA/60 seconds	Control	8.32	2.71	3.93	14.46	0.573
	Diabetes	7.76	3.08	4.39	14.06	
100μA/60 seconds	Control	8.20	2.79	3.99	14.92	0.847
	Diabetes	7.99	3.48	4.05	15.97	
100μA/60 seconds	Control	8.35	2.82	4.31	15.27	0.916
	Diabetes	8.24	3.26	4.26	15.38	
100μA/60 seconds	Control	8.73	3.02	4.06	15.59	0.661
	Diabetes	8.25	3.26	4.32	14.85	
Peak of Max response	Control	14.90	3.64	8.94	23.5	0.803
	Diabetes	14.52	5.34	8.07	27.51	
Change in response %	Control	136.28	61.02	30.63	263.45	0.581
	Diabetes	125.65	47.70	64.24	255.00	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 47 Comparison between control group and diabetes group mean of flux (PU) on the dorsum of the foot with pressure in Own shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	6.57	2.50	3.14	11.98	0.279
	Diabetes	7.71	3.60	3.55	14.46	
100μA/60 seconds	Control	6.85	2.53	3.57	12.73	0.353
	Diabetes	7.81	3.52	4.5	15.24	
100μA/60 seconds	Control	7.03	2.65	4.15	12.71	0.365
	Diabetes	7.96	3.34	4.32	14.51	
100μA/60 seconds	Control	6.93	2.71	4	11.62	0.178
	Diabetes	8.43	3.73	4.64	15.15	
100μA/60 seconds	Control	6.96	2.57	3.66	11.25	0.119
	Diabetes	8.81	4.25	4.35	17.48	
100μA/60 seconds	Control	7.07	2.55	3.65	12.59	0.098
	Diabetes	9.19	4.74	4.71	18.38	
100μA/60 seconds	Control	7.02	2.63	3.64	13.19	0.066
	Diabetes	9.51	5.03	4.01	19.24	
100μA/60 seconds	Control	7.30	3.05	3.51	15.01	0.115
	Diabetes	9.63	5.40	4.6	19.4	
100μA/60 seconds	Control	7.31	2.93	3.51	16.16	0.132
	Diabetes	9.35	4.84	4.87	17.36	
100μA/60 seconds	Control	7.72	3.23	3.57	16.95	0.259
	Diabetes	9.23	4.55	5.02	18.5	
100μA/60 seconds	Control	7.81	3.37	3.58	17.83	0.236
	Diabetes	9.36	4.20	4.61	17.86	
100μA/60 seconds	Control	7.85	3.32	3.77	17.91	0.170
	Diabetes	9.73	4.59	4.28	19	
100μA/60 seconds	Control	7.67	3.17	3.91	17.69	0.097
	Diabetes	10.02	5.00	4.37	21.84	
100μA/60 seconds	Control	7.93	3.51	3.88	19.16	0.185
	Diabetes	9.76	4.50	4.76	19.99	
100μA/60 seconds	Control	7.85	3.67	3.9	19.33	0.209
	Diabetes	9.59	4.33	4.04	18.74	
Peak of Max response	Control	14.66	5.84	6.64	24.99	0.098
	Diabetes	18.63	7.96	9.37	36.44	
Change in response %	Control	124.89	43.93	37.44	215.18	0.137
	Diabetes	156.10	76.46	77.65	291.82	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 48 Comparison between control group and diabetes group median of flux (PU) on the Plantar of the foot with no pressure applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	45.22	32.22	11.8	122.3	0.046*
	Diabetes	71.80	43.65	10.9	150.3	
100μA/60 seconds	Control	44.78	31.79	8	120	0.006*
	Diabetes	84.01	47.37	12.3	166.8	
100μA/60 seconds	Control	59.97	39.06	11.8	130.4	0.003*
	Diabetes	117.98	68.15	11.6	218.6	
100μA/60 seconds	Control	87.74	74.46	14	280.4	0.012*
	Diabetes	165.60	99.90	16.5	324.2	
100μA/60 seconds	Control	105.96	104.81	14.9	421.3	0.033*
	Diabetes	188.11	113.00	23.5	369.7	
100μA/60 seconds	Control	130.41	129.36	9.2	515	0.104
	Diabetes	203.95	128.43	23.8	412.2	
100μA/60 seconds	Control	143.90	134.62	11.9	556	0.126
	Diabetes	215.81	133.14	29.7	463.5	
100μA/60 seconds	Control	153.66	137.10	18.9	571.6	0.146
	Diabetes	222.55	133.47	31.6	478.6	
100μA/60 seconds	Control	164.49	138.38	15.6	582.7	0.130
	Diabetes	238.69	142.22	37.5	541	
100μA/60 seconds	Control	183.41	138.92	11	583.5	0.208
	Diabetes	246.98	152.56	35.7	620.5	
100μA/60 seconds	Control	193.87	144.86	10.2	579.8	0.256
	Diabetes	253.88	161.37	38.8	663.9	
100μA/60 seconds	Control	202.97	149.53	9.5	577.8	0.399
	Diabetes	248.19	161.78	46	675.2	
100μA/60 seconds	Control	212.48	150.96	17.2	572.6	0.396
	Diabetes	258.02	160.39	51.7	681.2	
100μA/60 seconds	Control	219.14	164.21	10.6	576.8	0.452
	Diabetes	260.47	151.49	58.8	673.3	
100μA/60 seconds	Control	218.60	168.44	10.6	590.7	0.459
	Diabetes	260.48	156.91	46.2	692	
Peak of Max response	Control	223.44	140.95	33.39	588.58	0.089
	Diabetes	314.91	167.14	47.67	662.91	
Change in response %	Control	491.46	355.82	46.74	1272.23	0.746
	Diabetes	543.54	585.51	58.05	2298.87	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 49 Comparison between control group and diabetes group median of flux (PU) on the Plantar of the foot with no pressure applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	50.13	61.57	6.7	267.9	0.365
	Diabetes	67.52	45.70	22.8	184.5	
100μA/60 seconds	Control	48.54	60.26	8	270.6	0.167
	Diabetes	76.53	54.62	24.8	219	
100μA/60 seconds	Control	61.47	78.54	7.4	356.9	0.255
	Diabetes	90.69	66.88	23.6	230	
100μA/60 seconds	Control	84.66	83.00	7.9	376.3	0.194
	Diabetes	121.96	81.69	33	256.2	
100μA/60 seconds	Control	115.46	88.61	6.8	324.6	0.197
	Diabetes	157.38	98.94	34.5	366.1	
100μA/60 seconds	Control	143.24	121.10	11.1	363.5	0.378
	Diabetes	177.60	99.63	35	424.6	
100μA/60 seconds	Control	156.12	127.75	18.3	418.5	0.365
	Diabetes	193.88	109.66	40.5	464.4	
100μA/60 seconds	Control	163.81	134.38	12.6	496.7	0.499
	Diabetes	192.97	110.96	56.9	488.2	
100μA/60 seconds	Control	161.89	136.93	25.2	527.9	0.436
	Diabetes	196.31	113.92	53.3	495.7	
100μA/60 seconds	Control	163.39	135.21	13.4	551.5	0.543
	Diabetes	190.29	117.53	44.8	488	
100μA/60 seconds	Control	162.08	131.46	16.4	537.4	0.528
	Diabetes	189.45	117.53	52.9	471.4	
100μA/60 seconds	Control	162.21	129.28	12.3	518.6	0.567
	Diabetes	186.15	109.22	59.5	461	
100μA/60 seconds	Control	166.83	125.40	9.9	481.5	0.650
	Diabetes	184.92	101.43	79.3	452.8	
100μA/60 seconds	Control	155.81	119.80	11.3	469.4	0.392
	Diabetes	187.89	90.25	73	431.3	
100μA/60 seconds	Control	161.27	120.01	18.4	494.5	0.583
	Diabetes	181.20	81.34	68.7	384.4	
Peak of Max response	Control	203.12	138.22	32.07	502.48	0.315
	Diabetes	247.69	112.18	110.78	485.71	
Change in response %	Control	645.00	722.90	16.21	2484.31	0.110
	Diabetes	332.47	146.04	66.77	611.09	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 50 Comparison between control group and diabetes group median of flux (PU) on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.66	1.54	2.1	8.9	0.014*
	Diabetes	5.14	1.84	2.4	8.8	
100μA/60 seconds	Control	3.91	1.84	2.1	9.9	0.025*
	Diabetes	5.49	2.13	2.5	9	
100μA/60 seconds	Control	4.04	1.98	2.2	10.3	0.049*
	Diabetes	5.49	2.23	2.3	10	
100μA/60 seconds	Control	4.11	2.06	2.1	10.7	0.048*
	Diabetes	5.52	2.04	2.3	9.2	
100μA/60 seconds	Control	4.05	2.00	2.2	10	0.044*
	Diabetes	5.56	2.26	2.4	10.4	
100μA/60 seconds	Control	4.01	2.00	2.1	9.7	0.035*
	Diabetes	5.67	2.48	2.4	11.3	
100μA/60 seconds	Control	4.06	1.96	2	9.9	0.036*
	Diabetes	5.67	2.42	2.4	11.4	
100μA/60 seconds	Control	4.24	2.06	2	9.7	0.025*
	Diabetes	6.01	2.40	2.5	10.4	
100μA/60 seconds	Control	4.42	2.10	1.9	11.1	0.039*
	Diabetes	5.79	2.34	2.5	10.1	
100μA/60 seconds	Control	4.44	2.74	1.9	14.5	0.104
	Diabetes	6.03	2.86	2.5	12	
100μA/60 seconds	Control	4.52	2.88	1.9	15.2	0.155
	Diabetes	5.96	2.94	2.7	13.3	
100μA/60 seconds	Control	4.51	2.82	2	14.6	0.184
	Diabetes	5.85	3.03	2.6	13.9	
100μA/60 seconds	Control	4.63	2.83	2	13.6	0.211
	Diabetes	5.89	2.98	2.6	13.6	
100μA/60 seconds	Control	4.58	2.71	2.1	12	0.172
	Diabetes	5.91	2.87	2.8	12.6	
100μA/60 seconds	Control	4.40	2.29	2	11.2	0.126
	Diabetes	5.75	2.81	2.7	12.5	
Peak of Max response	Control	8.98	4.19	4.22	21.98	0.100
	Diabetes	11.48	4.50	4.38	20.16	
Change in response %	Control	146.20	63.87	78.91	322.58	0.284
	Diabetes	124.02	53.23	54.33	257.34	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 51 Comparison between control group and diabetes group median of flux (PU) on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.59	1.33	1.8	6.4	0.060
	Diabetes	5.45	4.00	2.4	18.5	
100μA/60 seconds	Control	3.62	1.32	1.9	6.1	0.047*
	Diabetes	5.80	4.50	2.5	20.9	
100μA/60 seconds	Control	3.89	1.79	2	9.2	0.134
	Diabetes	5.95	5.67	2.3	25.8	
100μA/60 seconds	Control	4.05	1.90	2	8.8	0.194
	Diabetes	5.41	4.08	2.3	19	
100μA/60 seconds	Control	4.09	1.85	2.2	8.3	0.157
	Diabetes	5.99	5.52	2.4	25	
100μA/60 seconds	Control	4.01	1.74	2.1	8	0.131
	Diabetes	5.65	4.30	2.4	19.7	
100μA/60 seconds	Control	4.16	1.97	2.1	8.6	0.187
	Diabetes	5.97	5.60	2.4	25.3	
100μA/60 seconds	Control	4.43	2.34	2	10.7	0.240
	Diabetes	7.23	10.15	2.5	43.4	
100μA/60 seconds	Control	4.36	2.12	2.2	9.5	0.237
	Diabetes	7.39	11.03	2.5	46.5	
100μA/60 seconds	Control	4.15	1.89	2.1	9.3	0.190
	Diabetes	7.73	11.82	2.5	49.7	
100μA/60 seconds	Control	4.25	2.18	2.3	10.6	0.237
	Diabetes	6.81	9.22	2.7	39.6	
100μA/60 seconds	Control	4.20	1.84	2	9.1	0.222
	Diabetes	6.91	9.54	2.6	40.9	
100μA/60 seconds	Control	4.19	1.74	2	8.4	0.261
	Diabetes	6.58	9.17	2.6	39.2	
100μA/60 seconds	Control	4.22	1.72	2	8.7	0.184
	Diabetes	6.70	7.99	2.8	35	
100μA/60 seconds	Control	4.22	1.81	2.1	9.3	0.210
	Diabetes	7.17	10.18	2.7	43.4	
Peak of Max response	Control	8.00	3.59	3.71	17.02	0.137
	Diabetes	14.86	19.78	4.38	84.87	
Change in response %	Control	128.37	86.96	65.78	401.81	0.654
	Diabetes	141.91	88.61	11.24	358.75	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 52 Comparison between control group and diabetes group median of flux (PU) on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.59	1.01	2.1	5.9	0.003*
	Diabetes	5.19	1.95	1.7	8.8	
100μA/60 seconds	Control	3.69	1.21	2.1	6.8	0.003*
	Diabetes	5.47	2.07	1.8	9	
100μA/60 seconds	Control	3.63	1.23	2.2	6.8	0.002*
	Diabetes	5.63	2.23	2.1	9.7	
100μA/60 seconds	Control	3.67	1.39	2.1	6.5	0.003*
	Diabetes	5.67	2.32	2.1	9.5	
100μA/60 seconds	Control	3.59	1.23	2.2	6.8	<0.001**
	Diabetes	5.72	2.32	2.2	10	
100μA/60 seconds	Control	3.67	1.48	2.1	7.6	0.004*
	Diabetes	5.54	2.07	2.3	8.6	
100μA/60 seconds	Control	3.96	2.12	2	9.6	0.024*
	Diabetes	5.77	2.38	2.2	11.1	
100μA/60 seconds	Control	3.68	1.74	2	8.3	0.007*
	Diabetes	5.55	2.12	2.1	8.1	
100μA/60 seconds	Control	3.87	2.12	1.9	11	0.018*
	Diabetes	5.78	2.39	2.3	9.3	
100μA/60 seconds	Control	3.84	2.02	1.9	9.7	0.015*
	Diabetes	5.75	2.37	2.2	10.2	
100μA/60 seconds	Control	3.80	2.15	1.9	9.7	0.010*
	Diabetes	5.98	2.59	2.3	11.3	
100μA/60 seconds	Control	3.94	2.37	1.9	10.9	0.018*
	Diabetes	6.09	2.72	2.4	11.8	
100μA/60 seconds	Control	3.97	2.33	2	10.4	0.020*
	Diabetes	6.01	2.60	2.4	12.2	
100μA/60 seconds	Control	3.99	2.47	1.9	11.1	0.035*
	Diabetes	5.85	2.51	2.3	10.6	
100μA/60 seconds	Control	3.89	2.29	1.9	10.2	0.027*
	Diabetes	5.73	2.39	2.3	9.1	
Peak of Max response	Control	8.13	3.48	3.8	16.92	0.013*
	Diabetes	11.54	4.20	4.34	16.9	
Change in response %	Control	125.83	70.15	13.14	284.52	0.845
	Diabetes	130.29	60.42	50.67	257.34	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 53 Comparison between control group and diabetes group median of flux (PU) on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.50	0.81	2.4	5.7	0.022*
	Diabetes	4.65	1.93	2.1	9.4	
100μA/60 seconds	Control	3.46	0.95	2.2	6.5	0.012*
	Diabetes	4.74	1.87	1.7	8.5	
100μA/60 seconds	Control	3.63	1.19	2.2	7.7	0.037*
	Diabetes	4.67	1.85	1.7	7.9	
100μA/60 seconds	Control	3.54	1.01	2.1	6.8	0.035*
	Diabetes	4.80	2.29	1.8	10.1	
100μA/60 seconds	Control	3.56	0.99	2.4	6.9	0.018*
	Diabetes	4.87	2.07	2.1	8.5	
100μA/60 seconds	Control	3.63	1.05	2.5	7	0.039*
	Diabetes	4.69	1.85	1.6	7.3	
100μA/60 seconds	Control	3.59	0.89	2.7	6.8	0.044*
	Diabetes	4.41	1.57	2	7.6	
100μA/60 seconds	Control	3.74	1.14	2.6	6.6	0.164
	Diabetes	4.41	1.69	1.5	7.8	
100μA/60 seconds	Control	3.68	1.01	2.3	6.7	0.261
	Diabetes	4.20	1.70	1.5	7.8	
100μA/60 seconds	Control	3.68	0.90	2.3	6.6	0.113
	Diabetes	4.50	2.02	1.4	8.2	
100μA/60 seconds	Control	3.62	0.93	2.6	6.9	0.124
	Diabetes	4.39	1.90	1.5	8.9	
100μA/60 seconds	Control	3.51	0.78	2.5	5.9	0.071
	Diabetes	4.37	1.86	1.4	8.4	
100μA/60 seconds	Control	3.88	1.52	2.5	8.5	0.360
	Diabetes	4.43	2.00	1.4	8.2	
100μA/60 seconds	Control	3.93	1.66	2.1	9.6	0.288
	Diabetes	4.63	2.19	1.3	9.8	
100μA/60 seconds	Control	4.10	1.99	2.2	11.6	0.662
	Diabetes	4.39	1.77	1.4	7.7	
Peak of Max response	Control	7.84	3.97	4.78	22.03	0.202
	Diabetes	9.55	3.68	4.45	17.42	
Change in response %	Control	118.30	59.06	61.21	286.51	0.708
	Diabetes	111.26	47.50	11.24	190.31	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 54 Comparison between control group and diabetes group mean of flux (PU) on the Plantar of the foot with no pressure applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	47.27	33.03	12.41	120.7	0.046*
	Diabetes	74.37	44.50	11.33	150	
100μA/60 seconds	Control	46.89	32.85	10.09	120.82	0.006*
	Diabetes	86.94	47.26	12.53	167.84	
100μA/60 seconds	Control	61.91	39.58	12.5	136.06	0.003*
	Diabetes	119.68	67.90	12.29	210.06	
100μA/60 seconds	Control	90.50	75.48	14.53	285.09	0.012*
	Diabetes	169.98	102.49	17.1	334.55	
100μA/60 seconds	Control	107.88	102.87	14.66	408.37	0.029*
	Diabetes	192.55	115.84	23.98	382.12	
100μA/60 seconds	Control	132.11	129.60	9.78	518.31	0.049*
	Diabetes	208.65	131.41	24.1	423.73	
100μA/60 seconds	Control	145.65	135.60	13.9	559.91	0.117
	Diabetes	220.53	136.63	29.59	475.79	
100μA/60 seconds	Control	156.41	138.34	21.17	576.73	0.142
	Diabetes	227.05	136.34	32.19	485.69	
100μA/60 seconds	Control	170.08	137.31	16.02	584.81	0.143
	Diabetes	242.06	144.28	37.8	552.29	
100μA/60 seconds	Control	186.34	139.27	13.32	585.41	0.204
	Diabetes	251.27	155.84	35.82	632.6	
100μA/60 seconds	Control	196.50	145.32	10.6	581.59	0.248
	Diabetes	257.97	163.25	39.11	669.41	
100μA/60 seconds	Control	204.87	149.94	11.03	579.29	0.380
	Diabetes	252.36	164.45	45.86	681.33	
100μA/60 seconds	Control	213.75	151.28	17.49	576.58	0.372
	Diabetes	262.03	162.67	51.48	687.87	
100μA/60 seconds	Control	219.48	163.68	11.21	573.45	0.417
	Diabetes	264.38	154.87	58.46	680.92	
100μA/60 seconds	Control	218.87	167.36	11.01	587.19	0.419
	Diabetes	264.65	159.12	46.77	696.15	
Peak of Max response	Control	223.44	140.95	33.39	588.58	0.089
	Diabetes	314.91	167.14	47.67	662.91	
Change in response %	Control	460.66	344.35	51.80	1240.94	0.721
	Diabetes	516.13	563.94	58.37	2213.42	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 55 Comparison between control group and diabetes group mean of flux (PU) on the Plantar of the foot with no pressure applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	51.45	62.29	6.87	272.72	0.330
	Diabetes	70.39	46.32	23.63	188.99	
100μA/60 seconds	Control	49.90	60.77	8.3	273.25	0.164
	Diabetes	78.38	55.31	25.6	220.3	
100μA/60 seconds	Control	62.61	77.32	8.06	351.63	0.221
	Diabetes	94.01	68.55	24.61	231.43	
100μA/60 seconds	Control	85.68	80.51	8.62	366.07	0.174
	Diabetes	124.09	81.68	34.26	263.13	
100μA/60 seconds	Control	116.59	88.32	7.62	322.51	0.189
	Diabetes	159.19	98.88	36.2	368.02	
100μA/60 seconds	Control	144.77	120.54	11.85	366.85	0.359
	Diabetes	180.58	101.00	36.07	430.63	
100μA/60 seconds	Control	157.35	127.90	18.35	418.3	0.366
	Diabetes	195.09	109.55	41.84	465.78	
100μA/60 seconds	Control	163.84	135.86	14.37	501.32	0.465
	Diabetes	195.65	111.51	58.7	492.63	
100μA/60 seconds	Control	163.93	138.32	25.19	533.74	0.430
	Diabetes	199.05	114.48	55.05	500.66	
100μA/60 seconds	Control	165.48	135.62	14.71	552.75	0.526
	Diabetes	193.60	118.05	46.26	491.93	
100μA/60 seconds	Control	164.05	132.88	17.02	543.06	0.517
	Diabetes	192.41	117.58	54.92	475.89	
100μA/60 seconds	Control	163.78	130.22	12.94	523.15	0.534
	Diabetes	189.99	110.35	61.35	466.51	
100μA/60 seconds	Control	167.77	126.48	10.58	486.85	0.601
	Diabetes	188.82	102.20	80.65	456.65	
100μA/60 seconds	Control	157.93	120.67	12.22	474.81	0.395
	Diabetes	190.06	91.38	73.63	436.15	
100μA/60 seconds	Control	162.24	121.12	18.99	499.26	0.563
	Diabetes	183.48	82.20	69.13	387.65	
Peak of Max response	Control	203.12	138.22	32.07	502.48	0.315
	Diabetes	247.69	112.18	110.78	485.71	
Change in response %	Control	604.93	685.26	17.48	2348.29	0.112
	Diabetes	310.31	139.64	62.81	585.86	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 56 Comparison between control group and diabetes group mean of flux (PU) on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.91	1.78	2.24	10.26	0.023*
	Diabetes	5.39	1.90	2.48	9.17	
100μA/60 seconds	Control	4.16	1.97	2.15	10.69	0.029*
	Diabetes	5.74	2.14	2.54	9.39	
100μA/60 seconds	Control	4.29	2.17	2.23	11.51	0.060
	Diabetes	5.75	2.22	2.39	9.57	
100μA/60 seconds	Control	4.44	2.31	2.19	12.1	0.069
	Diabetes	5.85	2.06	2.36	9.05	
100μA/60 seconds	Control	4.40	2.36	2.3	11.94	0.076
	Diabetes	5.86	2.29	2.44	10.51	
100μA/60 seconds	Control	4.28	2.27	2.13	11.5	0.042*
	Diabetes	5.99	2.50	2.4	11.6	
100μA/60 seconds	Control	4.38	2.23	2.09	11.54	0.046*
	Diabetes	5.97	2.42	2.42	11.6	
100μA/60 seconds	Control	4.62	2.39	2.09	11.3	0.039*
	Diabetes	6.42	2.55	2.54	10.64	
100μA/60 seconds	Control	4.70	2.31	1.96	12	0.086
	Diabetes	6.14	2.46	2.54	9.94	
100μA/60 seconds	Control	4.75	2.91	2	15.27	0.136
	Diabetes	6.25	2.86	2.61	12.35	
100μA/60 seconds	Control	4.78	3.02	1.94	16.03	0.162
	Diabetes	6.25	2.97	2.75	13.39	
100μA/60 seconds	Control	4.78	2.96	2.04	15.49	0.208
	Diabetes	6.08	2.98	2.63	13.92	
100μA/60 seconds	Control	4.90	2.93	2.07	14.55	0.213
	Diabetes	6.17	2.94	2.64	13.72	
100μA/60 seconds	Control	4.84	2.83	2.25	13.42	0.179
	Diabetes	6.17	2.86	2.84	12.76	
100μA/60 seconds	Control	4.69	2.50	2.01	12.7	0.135
	Diabetes	6.07	2.84	2.76	12.6	
Peak of Max response	Control	8.98	4.19	4.22	21.98	0.100
	Diabetes	11.48	4.50	4.38	20.16	
Change in response %	Control	130.92	53.81	77.08	255.98	0.268
	Diabetes	112.01	42.00	48.11	198.32	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 57 Comparison between control group and diabetes group mean of flux (PU) on the Plantar of the foot with pressure in Orthopaedic shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.77	1.40	1.86	6.63	0.037*
	Diabetes	5.93	4.98	2.48	22.88	
100μA/60 seconds	Control	3.83	1.41	2.06	6.42	0.043*
	Diabetes	6.45	5.73	2.54	26.1	
100μA/60 seconds	Control	4.10	1.88	2.16	9.67	0.126
	Diabetes	6.64	6.95	2.39	31.15	
100μA/60 seconds	Control	4.24	1.95	2.19	9.07	0.163
	Diabetes	6.09	5.36	2.36	24.38	
100μA/60 seconds	Control	4.32	1.98	2.19	8.57	0.160
	Diabetes	6.62	6.82	2.44	30.31	
100μA/60 seconds	Control	4.26	1.85	2.08	8.37	0.145
	Diabetes	6.26	5.60	2.4	25.16	
100μA/60 seconds	Control	4.41	2.04	2.19	8.98	0.179
	Diabetes	6.62	6.84	2.42	30.47	
100μA/60 seconds	Control	4.63	2.35	2.06	10.73	0.225
	Diabetes	7.63	10.55	2.54	45.15	
100μA/60 seconds	Control	4.58	2.19	2.25	9.86	0.222
	Diabetes	7.80	11.34	2.54	47.8	
100μA/60 seconds	Control	4.41	2.00	2.14	9.51	0.189
	Diabetes	8.03	11.92	2.61	50.18	
100μA/60 seconds	Control	4.49	2.27	2.32	10.43	0.230
	Diabetes	7.38	10.26	2.75	43.87	
100μA/60 seconds	Control	4.44	1.98	2.02	9.31	0.200
	Diabetes	7.48	10.20	2.63	43.73	
100μA/60 seconds	Control	4.42	1.86	2.05	8.71	0.248
	Diabetes	7.13	10.14	2.64	43.19	
100μA/60 seconds	Control	4.44	1.86	2.07	9.01	0.180
	Diabetes	7.30	9.12	2.84	39.63	
100μA/60 seconds	Control	4.46	1.92	2.18	9.54	0.207
	Diabetes	7.59	10.72	2.76	45.66	
Peak of Max response	Control	8.00	3.59	3.71	17.02	0.137
	Diabetes	14.86	19.78	4.38	84.87	
Change in response %	Control	117.79	82.24	59.46	372.83	0.797
	Diabetes	124.58	67.91	12.92	270.93	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 58 Comparison between control group and diabetes group mean of flux (PU) on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of ACh using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0 μ A/60 seconds	Control	3.73	1.06	2.24	6.06	0.002*
	Diabetes	5.49	2.05	1.76	9.17	
100 μ A/60 seconds	Control	3.81	1.28	2.15	7.13	0.003*
	Diabetes	5.73	2.19	1.84	9.39	
100 μ A/60 seconds	Control	3.85	1.35	2.23	7.01	0.002*
	Diabetes	5.94	2.31	2.26	9.76	
100 μ A/60 seconds	Control	3.92	1.60	2.19	7.81	0.005*
	Diabetes	5.98	2.44	2.16	9.67	
100 μ A/60 seconds	Control	3.79	1.33	2.2	6.94	<0.001**
	Diabetes	5.99	2.38	2.28	9.89	
100 μ A/60 seconds	Control	3.95	1.65	2.13	8.3	0.006*
	Diabetes	5.85	2.18	2.39	8.81	
100 μ A/60 seconds	Control	4.19	2.21	2.09	10.15	0.026*
	Diabetes	6.04	2.49	2.24	10.97	
100 μ A/60 seconds	Control	3.91	1.86	2.09	8.98	0.008*
	Diabetes	5.93	2.41	2.14	9.15	
100 μ A/60 seconds	Control	4.14	2.18	1.96	11.29	0.020*
	Diabetes	6.08	2.52	2.38	9.41	
100 μ A/60 seconds	Control	4.05	2.05	2	10.03	0.014*
	Diabetes	6.07	2.52	2.29	10.35	
100 μ A/60 seconds	Control	4.05	2.19	1.94	10.04	0.007*
	Diabetes	6.42	2.72	2.39	11.38	
100 μ A/60 seconds	Control	4.17	2.41	1.95	11.11	0.014*
	Diabetes	6.43	2.74	2.48	11.57	
100 μ A/60 seconds	Control	4.20	2.35	2.05	10.65	0.017*
	Diabetes	6.34	2.65	2.46	12	
100 μ A/60 seconds	Control	4.19	2.50	1.92	11.35	0.026*
	Diabetes	6.24	2.68	2.37	10.77	
100 μ A/60 seconds	Control	4.12	2.32	1.94	10.55	0.025*
	Diabetes	6.09	2.63	2.35	9.73	
Peak of Max response	Control	8.13	3.48	3.8	16.92	0.013*
	Diabetes	11.54	4.20	4.34	16.9	
Change in response %	Control	116.36	64.56	14.61	267.80	0.988
	Diabetes	116.65	49.22	48.11	198.32	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 59 Comparison between control group and diabetes group mean of flux (PU) on the Plantar of the foot with pressure in Own shoes applied during the iontophoresis of SNP using: Independent Sample t-test.

Iontophoresis	Group	Mean	SD	Min.	Max.	p
0μA/60 seconds	Control	3.76	1.20	2.51	8.04	0.035*
	Diabetes	4.92	1.91	2.35	9.26	
100μA/60 seconds	Control	3.73	1.36	2.22	8.72	0.028*
	Diabetes	5.03	1.98	1.78	8.66	
100μA/60 seconds	Control	3.91	1.55	2.29	9.68	0.072
	Diabetes	5.01	1.94	1.84	8.08	
100μA/60 seconds	Control	3.82	1.42	2.18	9.14	0.060
	Diabetes	5.02	2.25	1.88	9.54	
100μA/60 seconds	Control	3.82	1.39	2.57	9.13	0.027*
	Diabetes	5.22	2.19	2.28	8.99	
100μA/60 seconds	Control	3.93	1.41	2.57	9.05	0.066
	Diabetes	5.01	1.97	1.69	7.89	
100μA/60 seconds	Control	3.90	1.28	2.86	8.94	0.079
	Diabetes	4.81	1.68	2.12	8.18	
100μA/60 seconds	Control	4.03	1.49	2.66	8.77	0.205
	Diabetes	4.76	1.86	1.56	8.32	
100μA/60 seconds	Control	3.96	1.38	2.4	8.83	0.325
	Diabetes	4.51	1.93	1.54	8.49	
100μA/60 seconds	Control	3.97	1.34	2.4	8.95	0.173
	Diabetes	4.79	2.13	1.43	8.68	
100μA/60 seconds	Control	3.92	1.33	2.74	9.02	0.185
	Diabetes	4.69	2.03	1.59	9.34	
100μA/60 seconds	Control	3.81	1.24	2.73	8.5	0.102
	Diabetes	4.72	1.97	1.45	8.81	
100μA/60 seconds	Control	4.14	1.77	2.75	9.38	0.395
	Diabetes	4.72	2.17	1.43	8.66	
100μA/60 seconds	Control	4.18	1.86	2.21	9.62	0.336
	Diabetes	4.85	2.19	1.42	8.97	
100μA/60 seconds	Control	4.39	2.08	2.23	11.41	0.687
	Diabetes	4.67	1.92	1.6	8.2	
Peak of Max response	Control	7.84	3.97	4.78	22.03	0.202
	Diabetes	9.55	3.68	4.45	17.42	
Change in response %	Control	103.58	42.32	57.96	215.52	0.637
	Diabetes	96.93	38.99	12.92	161.54	

p >0.05 NS; *p <0.05 S; **p <0.001 HS

Table 60 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.465*	0.039*
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.495*	0.026*
Peak of Max response	-0.390	0.089
Change in response % (Using Median)	0.240	0.309
Change in response % (Using Mean)	0.306	0.189

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 61 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.242	0.304
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.216	0.361
Peak of Max response	-0.370	0.108
Change in response % (Using Median)	-0.157	0.508
Change in response % (Using Mean)	-0.221	0.349

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 62 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.227	0.337
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.254	0.280
Peak of Max response	-0.332	0.152
Change in response % (Using Median)	-0.207	0.381
Change in response % (Using Mean)	-0.189	0.425

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 63 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.221	0.348
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.209	0.377
Peak of Max response	-0.073	0.759
Change in response % (Using Median)	0.126	0.597
Change in response % (Using Mean)	0.122	0.608

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 64 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.257	0.274
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.270	0.250
Peak of Max response	-0.392	0.087
Change in response % (Using Median)	-0.124	0.602
Change in response % (Using Mean)	-0.144	0.544

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 65 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.211	0.372
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.242	0.305
Peak of Max response	-0.199	0.401
Change in response % (Using Median)	-0.103	0.664
Change in response % (Using Mean)	-0.040	0.868

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 66 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.179	0.450
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.195	0.411
Peak of Max response	-0.032	0.895
Change in response % (Using Median)	0.061	0.799
Change in response % (Using Mean)	0.075	0.752

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 67 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in control group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	0.175	0.461
Baseline Label 1/0 μ A/60 seconds (Mean)	0.195	0.410
Peak of Max response	0.313	0.178
Change in response % (Using Median)	0.337	0.146
Change in response % (Using Mean)	0.352	0.128

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 68 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.004	0.990
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.031	0.913
Peak of Max response	-0.191	0.495
Change in response % (Using Median)	-0.212	0.447
Change in response % (Using Mean)	-0.186	0.507

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 69 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.339	0.216
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.400	0.139
Peak of Max response	-0.545*	0.036*
Change in response % (Using Median)	-0.396	0.144
Change in response % (Using Mean)	-0.359	0.189

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 70 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.323	0.240
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.357	0.191
Peak of Max response	-0.220	0.431
Change in response % (Using Median)	-0.041	0.885
Change in response % (Using Mean)	0.056	0.844

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 71 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.419	0.120
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.390	0.151
Peak of Max response	-0.323	0.240
Change in response % (Using Median)	-0.210	0.453
Change in response % (Using Mean)	-0.183	0.513

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 72 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	0.040	0.887
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.009	0.974
Peak of Max response	-0.228	0.414
Change in response % (Using Median)	-0.290	0.294
Change in response % (Using Mean)	-0.232	0.405

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 73 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.191	0.495
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.227	0.416
Peak of Max response	-0.227	0.415
Change in response % (Using Median)	-0.221	0.429
Change in response % (Using Mean)	-0.161	0.568

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 74 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.074	0.794
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.110	0.696
Peak of Max response	-0.316	0.251
Change in response % (Using Median)	-0.309	0.263
Change in response % (Using Mean)	-0.276	0.319

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 75 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in diabetes group using Pearson correlation coefficient.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.116	0.680
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.134	0.634
Peak of Max response	-0.177	0.527
Change in response % (Using Median)	-0.047	0.867
Change in response % (Using Mean)	-0.060	0.833

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 76 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.290	0.215
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.373	0.105
Peak of Max response	-0.244	0.301
Change in response % (Using Median)	-0.002	0.995
Change in response % (Using Mean)	-0.096	0.686

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 77 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.267	0.255
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.205	0.387
Peak of Max response	-0.200	0.398
Change in response % (Using Median)	0.075	0.753
Change in response % (Using Mean)	0.027	0.910

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 78 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	0.041	0.865
Baseline Label 1/0 μ A/60 seconds (Mean)	0.030	0.900
Peak of Max response	-0.155	0.514
Change in response % (Using Median)	-0.280	0.232
Change in response % (Using Mean)	-0.221	0.349

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 79 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.102	0.668
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.089	0.710
Peak of Max response	0.066	0.782
Change in response % (Using Median)	0.050	0.835
Change in response % (Using Mean)	0.011	0.965

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 80 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.230	0.329
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.298	0.202
Peak of Max response	-0.343	0.139
Change in response % (Using Median)	-0.132	0.578
Change in response % (Using Mean)	-0.143	0.548

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 81 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.178	0.452
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.235	0.319
Peak of Max response	-0.251	0.286
Change in response % (Using Median)	-0.135	0.569
Change in response % (Using Mean)	-0.023	0.925

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 82 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.179	0.450
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.197	0.405
Peak of Max response	-0.117	0.622
Change in response % (Using Median)	0.006	0.980
Change in response % (Using Mean)	0.003	0.990

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 83 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in control group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	0.102	0.667
Baseline Label 1/0 μ A/60 seconds (Mean)	0.104	0.663
Peak of Max response	0.164	0.490
Change in response % (Using Median)	0.265	0.259
Change in response % (Using Mean)	0.292	0.212

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 84 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.041	0.884
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.018	0.950
Peak of Max response	-0.282	0.308
Change in response % (Using Median)	-0.204	0.467
Change in response % (Using Mean)	-0.257	0.355

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 85 Correlation between Orthopaedic shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.239	0.390
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.275	0.321
Peak of Max response	-0.321	0.243
Change in response % (Using Median)	-0.318	0.248
Change in response % (Using Mean)	-0.257	0.355

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 86 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.312	0.257
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.400	0.140
Peak of Max response	-0.271	0.328
Change in response % (Using Median)	0.036	0.899
Change in response % (Using Mean)	0.086	0.761

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 87 Correlation between Orthopaedic shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.508	0.053
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.579	0.024*
Peak of Max response	-0.489	0.064
Change in response % (Using Median)	-0.075	0.791
Change in response % (Using Mean)	-0.046	0.869

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 88 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of ACh on the dorsal surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	0.020	0.945
Baseline Label 1/0 μ A/60 seconds (Mean)	0.043	0.879
Peak of Max response	-0.175	0.533
Change in response % (Using Median)	-0.275	0.321
Change in response % (Using Mean)	-0.264	0.341

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 89 Correlation between Own shoes Dorsal PP (KPa) and Flux (PU) during the iontophoresis of SNP on the dorsal surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.093	0.742
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.121	0.666
Peak of Max response	-0.311	0.260
Change in response % (Using Median)	-0.254	0.362
Change in response % (Using Mean)	-0.189	0.499

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 90 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of ACh on the plantar surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	-0.005	0.985
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.054	0.850
Peak of Max response	-0.325	0.237
Change in response % (Using Median)	-0.293	0.289
Change in response % (Using Mean)	-0.261	0.348

Correlation is highly significant at the 0.001 level (2-tailed). **

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

Table 91 Correlation between Own shoes Plantar PP (KPa) and Flux (PU) during the iontophoresis of SNP on the plantar surface in diabetes group using Spearman correlation test.

Flux (PU)	PP (KPa)	
	r	p
Baseline Label 1/0 μ A/60 seconds (Median)	0.007	0.980
Baseline Label 1/0 μ A/60 seconds (Mean)	-0.018	0.950
Peak of Max response	-0.104	0.713
Change in response % (Using Median)	-0.161	0.567
Change in response % (Using Mean)	-0.232	0.405

Correlation is highly significant at the 0.001 level (2-tailed). **

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