FACTORS AFFECTING FOOT LOADING AND ULCER RISK IN DIABETES PATIENTS

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Factors Affecting Foot Loading and Ulcer Risk in Diabetes Patients

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Statement on Ethics

NHS REC reference: 239893. This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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2 STATEMENT OF DISRUPTION TO THE STUDY

This PhD thesis was originally planned with three experimental groups' viz. DPN-NU: people with diabetes and neuropathy but no history of foot ulcers, DFU: people with diabetes and neuropathy and a previous history of foot ulcers and CTRL: healthy controls without diabetes. The required number of participants were identified, and data collection was progressing on schedule, till sudden temporary university closure was announced due to Covid-19. At that stage, the data collection had completed in two of the target sample groups: DPN and CTRLs. While in DFU group, data collection had only been possible for n=2 participants. This low sample size in this group necessitated the exclusion of this group from the thesis, especially given a continued inability to recruit this at-risk population following the reopening of the university in fall 2020, due to a freeze in non-Covid related studies at the recruitment sites. This situation necessitated an informed revision of research strategy. To avoid altering the aims and objectives of the study, revisions in the approach were made to experimental chapters, the n=2 DFU participants were combined with the DPN-NU participants in order to create a single group (DPN) of people with DPN, irrespective of their ulcer history. This provides the limitation to the demonstration of plantar pressures as a proxy for ulcer risk without the ability to discuss differences in people with and without a history of ulceration. However, it is well established that DPN itself is the main indicator of ulcer risk, and that the differences that may have been observed between DPN-NU and DFU, it would have been difficult to establish the causal link: i.e. were differences due to previous ulcerations that have healed with different physiology, or were differences observed the root cause of the historical ulcer. Therefore, the route presented within this final thesis has been adjusted accordingly and discussions are presented with reference to the final populations.

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6 THESIS ABSTRACT

Diabetes is a number of diseases related to insulin imbalance, leading to hyperglycaemia and resulting in a variety of long-term pathophysiology. Neuropathy, one such long-term complication of diabetes, leads to a seven-fold increased risk for diabetic foot ulceration (DFU), having major socio-economic implications and affecting quality of life. A DFU accounts for more than 80% of total lower limb amputations, while 85% of diabetes-related lower limb amputations are avoidable(1). Early identification of the high-risk foot and ulcer prevention through timely clinical interventions, are key to averting a DFU.

This thesis aimed to investigate the effect of novel biomechanical and neuropathic factors underpinning DFU development. Specifically, the association between Achilles tendon mechanical properties, limited ankle-foot dorsiflexion and altered loading of the foot during gait were examined for their effects on plantar pressure development as a proxy for DFU risk. The thesis presents a series of cross-sectional studies conducted in people with diabetic peripheral neuropathy (DPN, n=13) and healthy controls (Ctrls, n=13).

Comparison of 4 pressure variables against common clinical risk factors identified peak plantar pressure and pressure-time integral the most appropriate proxy variables for DFU-risk and significantly correlated with established diabetic neuropathy indicators of DFU risk.

Investigation of Achilles tendon stiffness showed significantly higher stiffness in DPNs than Ctrls (DPN 80 Nmm^{-1} vs Ctrl 53Nmm^{-1}). Tendon stiffness was correlated with forefoot peak plantar pressure (rho=0.387, p<0.001). This study suggests that a stiffer ankle joint complex may alter the loading of the foot and therefore the pressures experienced under the foot.

Investigation of walking strategy revealed that when compared with controls, DPNs showed a 10% earlier heel-rise, 3.5 deg. reduced dorsiflexion (p<0.05), slower gait velocity and wider base (p<0.001). Tendon stiffness correlated with gait velocity (rho=-0.479, p<0.001), peak dynamic ankle dorsiflexion (rho=-0.427), vertical peak ground reaction force (rho=0.644, p<0.001) and peak plantar pressure at the 2nd toe, while walking. Thus, gait strategy and pressures changed in DPNs and significantly correlated with tendon stiffness.

This thesis concluded that increased stiffness of the ankle-foot joint complex is a key factor underpinning alterations to walking strategy and resulting in elevated forefoot plantar pressures and therefore increased DFU risk. The proposed early DFU risk assessments tools can impact pathways of delivering foot care to patients with diabetes.

7 GENERAL INTRODUCTION

Data from the International Diabetes Federation (IDF) (2) reveals that 537 million adults (20-79 years) are living with diabetes (almost 1 in 10 humans). This IDF diabetes atlas predicted the number to rise to 643 million by 2030 and 783 million by 2045, and states further that diabetes is responsible for 6.7 million deaths in 2021, which means 1 every 5 seconds corresponding to 12.2% of global deaths from all causes in this age group (20 and 79 years). Also, this report mentions that during the first wave of the Covid pandemic, people with diabetes had a 3.6-fold higher likelihood of being hospitalised due to COVID-19, compared to those without diabetes. In monetary terms, the IDF estimates that Diabetes caused at least USD 966 billion dollars in health expenditure – a 316% increase over the last 15 years. 541 million adults have Impaired Glucose Tolerance (IGT), which places them at high risk of type 2 diabetes.

It is estimated that almost 50% of diabetes and/or peripheral neuropathy population have the tendency to develop a foot ulcer during their lifetime (3–5) rendering diabetes as a major cause of lower limb amputation (6). The burden of diabetic foot pathology is ranked in the top 10 of all medical conditions (7). A systematic review by Zhang et. al (8), has stated that the prevalence of foot ulcers among diabetic patients ranges from 3% to 13% globally. The IDF estimates that every 30 seconds at least one limb is lost due to a diabetic foot ulcer (DFU) worldwide (9). It is estimated that a person with diabetes has a 25% lifetime risk of developing DFU (10). Patients with DFU have a greater than twofold increase in mortality compared with non-ulcerated diabetic patients (11). The risk for ulcer recurrence is high, with recurrence rates of 40% in the first year and 65% in the first 3 years after healing (12). Five-year mortality rates after ulceration were estimated to be ~40% (13). A DFU is preventable and evidence in the literature suggests that the early detection and treatment of diabetic foot complications could reduce the prevalence of ulceration by 44% to 85% (14).

In the UK, approximately 135 leg, foot and toe amputations take place on people with diabetes each week. Across the world this equates to an amputation caused by diabetes occurring every 20-30 seconds (1,15). People with diabetes are nine times more likely to experience a minor amputation and five times more likely to undergo a major amputation than counterparts without diabetes(16). Noticeably, 80-85% of all amputations caused by diabetes are largely preventable (1,17). Data from Diabetes UK suggest that four out of five

amputations in the UK are entirely preventable if more proactive care and a prompt referral to specialist teams had been in place(18).

The other issue directly related to diabetes, is diabetic peripheral neuropathy, which causes insensate distal regions of the lower limb causing foot dysfunctions. These foot issues also result in limb loss or mortality, as limb amputation risk is doubled in people with diabetes compared to their non-diabetic counterparts.

Diabetic Peripheral Neuropathy (DPN) has an association with autonomic as well as sensory neuropathy, which could also contribute to foot ulcerations. Risk factors leading to foot ulceration are multifactorial, however over 50% are thought to be due to a combination of peripheral neuropathy, foot deformity and trauma, with neuropathy often reported as predominant (19), which all contribute to the elevated pressures in the plantar regions of the foot(20)

Early recognition of diabetic neuropathy with interventions in time can leads to avoidance of deformities and foot ulceration issues. As such a single method for diagnosis and prediction of diabetic foot ulcers is not available. It is normally a combination of reviews in history, clinical interventions and imaging, which can help in assessment of risks of diabetic foot ulcers. Adherence to the evidence-based approach can help in reducing morbidity in people with diabetic foot ulcers as well as support the outcomes of clinical interventions (21).

The loss of sensory perception in the diabetic foot decreases the ability of an individual to detect high plantar pressure under the sole of their foot. In addition, cross-sectional studies have found patients with a history of plantar ulceration to have greater pressures than those without a history of ulceration (22). Previous studies have assessed diabetic plantar pressures using varying methods. Some are limited to barefoot analysis, which in itself may present a risk for diabetic patients (23), whom are advised never to walk barefoot to avoid injury. In-shoe analysis research has looked at walking trials only, without considering pressures throughout other daily activities.

Beyond DPN and the loss of protective sensation under the foot, the biomechanical strategy (24)and physiology (25) of people with diabetes is known to alter, and may contribute further to the development and risk of ulceration. Several common factors have been identified including joint range of motion deficits, foot deformities etc., but there still

remain a knowledge gap on how foot problems progress and what causes them. We know diabetic peripheral neuropathy causes changes in foot structure, affecting foot function and subsequently leading to increased plantar foot pressure, which is associated or linked with the risk of diabetic foot ulceration. Several other factors could potentially impact on the risk for diabetic foot ulceration including changes to the Achilles tendon properties, limited ankle-foot dorsiflexion range and altered foot loading through changes to gait biomechanics.

It has been shown from animal models of diabetes and in a small number of recent human studies, that diabetes increases the stiffness of tendon through non-enzymatic glycation. This stiffening of the Achilles tendon would limit the range of ankle dorsiflexion, thereby impacting upon the nature of foot loading (26) besides, other risk factors implicated in the development of DFU need to be studied.

Further focussed literatures reviews has been carried out within the individual chapters.

8 THESIS OBJECTIVE

This thesis will consider contributory factors to altered foot loading, foot deformities, Gait disturbances, soft tissue alterations that produce abnormally high forces of pressure as further risk factors for diabetic foot ulcer. Achilles tendon function in humans can influence foot function and is particularly relevant in people with diabetes for the development of diabetic foot ulcers. Linked to Achilles tendon stiffness, the proposed study will also examine the relationship between ankle dorsiflexion range of motion, and ankle joint passive tendon stiffness, with plantar foot pressure loading and ulceration risk during gait. The study will attempt to address some key questions for the at-risk patient population viz. peak dorsiflexion range of motion at foot-ankle complex, Achilles tendon stiffness alter plantar forefoot pressure loading during gait, thereby attempting to combine information and key theories suggested by past work regarding the effect of diabetes upon stiffening tissues and its role in development of ulceration.

Principal aim: Determine the effects of specific musculoskeletal, biomechanical and clinical factors underpinning diabetic foot ulcer development. Specifically**:**

1. To establish appropriate plantar pressure parameter(s) that can be used as a proxy for 'ulcer risk'.
2. Investigate differences in Achilles tendon stiffness and ankle joint function with diabetes and determine the relationship with forefoot plantar pressure.
3. Investigate the relationship between dynamic GAIT biomechanics, ankle stiffness and plantar pressures and their role in in determining foot ulcer risk.

Study Hypotheses: The study hypothesise that increased Achilles tendon stiffness would be associated with an increased ulcer risk, through the development of elevated forefoot pressures.

9 EXPERIMENTAL CHAPTER 1: PLANTAR PRESSURES AS A PROXY FOR DIABETIC FOOT ULCER RISK: ESTABLISHING APPROPRIATE PLANTAR PRESSURE PARAMETERS

9.1 ABSTRACT

Diabetic peripheral neuropathy (DPN) a major complication of diabetes is considered a major causative factor for the onset of diabetic foot ulcers. DPN impairs sensory and motor functions of the foot and ankle region causing musculoskeletal limitations that result in altered plantar pressures. This experimental chapter investigated the differences in plantar pressures between people with DPN and healthy controls and examined a range of different pressure variables for their utility in identifying ulcer risk. The aim was to establish the most relevant pressure parameter(s) to act as a proxy for diabetic foot ulcer risk. In-shoe pressure sensors were used to acquire data during walking in a gait laboratory at self-selected speed for n=13 healthy controls and n=15 people with DPN on a level walkway. Four key pressure variables were investigated: peak pressures, pressure-time integrals (force and pressure), force-time integral and a stance averaged peak pressure. Peak plantar pressure and pressure-time integrals significantly correlated with established markers of diabetic foot ulceration including duration of diabetes and severity of peripheral neuropathy (neuropathy disability score and vibration perception threshold). Peak plantar pressure and pressure-time integrals were identified as the most appropriate proxy measures for diabetic foot ulcer risk and taken forwards to be used in the subsequent chapters of the thesis.

9.2 INTRODUCTION

Distal symmetrical polyneuropathy or diabetic peripheral neuropathy (DPN) is a complication of diabetes (27) and is known as main reason of foot ulceration, that affects peripheral nervous system at sensory and motor component (28,29). Boulton et al. (30) defined DPN as "Diabetic neuropathy has been defined as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes". The complication of DPN leads to impaired pain sensation and loss of sensory feedback (31), further leading to Diabetic foot Ulcer (DFU) onset and progression (32). The general living quality is severely reduced due to DFU (33). recurrence rates are high, and the development of DFU are associated with high mortality due to infection (34) and mobility restrictions (35), leading to high health care costs (36). Lower extremity amputations possibility is fifteen times more in diabetics due to risk of DFU infection then people with no diabetes (37). A 15% increased risk of amputation is observed in people who have developed a DFU and average time of healing recovery is appx. 12 weeks, when not opting for surgery (38). Thus, the causes of DFU are multifactorial and make prevention a major challenge (28,39,40).

Demographically, it has been observed that males are at a 1.6 times more DFU risk in the western countries (41). DFU risk increased with age and diabetes period (42). Diabetes affects more than 4.8 million adults in the UK, 25% of have chances of developing diabetic foot ulcer (DFU) in their lifetime (43,44). In the United Kingdom Prospective diabetic study, a severity mapping found that at diagnosis itself 13% of study population was at risk of DFU (45) An annual cost in excess of £1 billion is spent by United Kingdom in management of DFU, appx. 1% of National Health Service budget (44), not including societal costs (33). Infection rates, hospitalisation and amputation rates are significantly higher in patients with non-healing DFUs (46) and over 125 such amputations are carried out in the UK every week (44).

The plantar side of foot, which bears the max. weight of the body goes under excruciatingly elevated and repeated pressures as DPN leads to Loss of protective sensation (LOPS). This high pressure leads to an elevated risk of DFUs which is evidently found in people having DFU history, when compared with people who have no history of diabetes or those who have not got ulceration despite diabetes (47).

Stokes et al (48), in their study had confirmed presence of highest loads at sites of ulcerations in DFUs. Researchers have indicated that plantar pressure can be viewed as a surrogate measure for DFU development (49,50). A healthy foot will exhibit fairly uniform pressure distribution (51–54), however, when foot deformities (55) and/or tissue breakdown (56) occur due to the interaction and combination of risk factors (42), it leads to unevenly distributed (57) and abnormally high plantar pressures (58). It is still not confirmed on the magnitude of plantar pressure with mild DPN (59), but evidences show that severe DPNs show elevated foot pressures (60) and those with obvious foot deformity (61)

Research studies have found various reasons for elevated plantar pressures (62–64) and few are mentioned as below:

- Changes to skeletal anatomy (foot deformity)

Small muscle atrophy affected by DPN may be causing reduced cushioning to relatively flatter areas of foot which would be in turn exposing those bony areas of foot to direct stresses (65). A comparison of DPNs and counterpart controls reveal that such changes mostly take place in mid-foot to forefoot region elevating pressures at those sites (66,67).

Toe extensors and flexors have a defined agonistic-antagonistic role in controlling their toe movements, which is disturbed by weakening of intrinsic plantar muscles due to motor neuropathy (68). Conditions such as prominent metatarsal head, toe region deformities may be routed through this reduced metatarsophalangeal plantar flexion (69). These Bony prominences concentrate force application on small areas where soft tissues are compressed between bony prominences e.g. the metatarsal heads in the foot, which may be qualifying them into definition of foot deformity (70). Association of foot deformities with elevation in plantar pressures has been reported by researchers (71).

Motor neuropathy has remained secondary topic of research when compared with well documented sensory and autonomic neuropathies (72–74) , although it well researched now that excessive and recurring pressures at sites of mechanical malformations have consequential tissue failures (69). Boulton et al (75) have theorised that such high pressure sites develop callosities, and LOPS in DPNs may cause thickening of these inflammations and with underneath haemorrhage resulting in traumatic ulcerations. Ulcers are directly

associated with peak plantar pressure sites (76). In majority of DFUs toe deformities and prominent metatarsal heads have been stated as main causative factors (77).

- Increased body mass

Intuitively, high body mass might be another factor that causes increased plantar pressure independent of diabetic neuropathy (59). Studies have assessed the influence of (78,79) different load carrying conditions (80), simulated changes in body (81,82) or body masses (83) and identified a correlation to peak plantar pressure (84). However, most studies have stated inconsistencies in the regional plantar pressure assessments of affected foot areas, which needs further research on dynamic Gait movements have on BMI and plantar pressures associations. (85).

- Changes to walking pattern

Patients with DPN use compensatory musculoskeletal mechanisms during Gait which include alteration in spatiotemporal, kinematic and kinetic aspects (86) to compensate for their sensory and motor system discrepancies, which can also effect high plantar pressures. An opinion that walking style may impact pressures is generally recognised and accordingly foot function (and the role lower limb region) during gait have been considered from different standpoints. Studies have also indicated that walking speeds may also be determining the distribution of plantar pressures (Stokes, 1975; Clark, 1980;). Stokes (88) indicated role of toe-out angle in increased load at first metatarsal head. Other gait aspects of stance duration and angular variation at MTPJ, foot landing and loading time or at duration at toe-off etc. can define the loading patterns but these intrinsic aspects of multidimensional kinematics requisite in-depth studies (90)

Whilst many studies analyse plantar pressures of the whole foot in people with (48,91–93) a smaller number of studies have reported pressure distributions of separate regions of the foot surface (94,95). Some researchers have reported higher rear foot pressure, while comparing plantar pressure in DPNs vs DMs vs Controls (96), while other meta-analysis (97) could not report any pressure differences in those plantar regions. Besides some studies could not establish correlation in forefoot pressures and sensory neuropathies but found associations with rear foot pressures (98). Gait events at or before heel strike in healthy populations, define rear foot pressure in non-diabetic healthy controls. (99). Forefoot pressures may have consequences from events at ankle joint during gait (100).

Quantification of Foot Pressures

Foot pressures can be quantified and expressed using different parameters with most studies having reported peak plantar pressure (PPP) (101–106) and/or pressure time integral (PTI)(107–109) as pertinent parameters for identifying risk of developing DFU. Peak plantar pressure represents the maximum amount of pressure in a given region of the foot during stance. Peak Plantar Pressure has been primarily used to investigate trauma to the soft tissue of diabetic foot to understand the onset of DFU impacted by altered plantar pressure distributions (76,110).

Researchers also believe that likelihood of skin breakdowns may not be the alone consequence of elevated plantar pressures and suggested that various other have advocated investigation of other procedures and approaches should be adopted in to forecast DFU risks (111). A multi-segmental geometry defines foot along with material properties of non-linear nature, which means a multifaceted behaviour can be expected with regard loading time and interaction of forces, pressures, and stresses acting on the plantar soft tissues (112,113). Another variable used to assess plantar pressures, that in contrast to PPP accounts for loading time, is the pressure–time integral (PTI), which is defined as the area under the pressure time curve, representing the cumulative pressure over the loading period (114). A number of retrospective studies have reported that plantar ulceration in DPNs may have associations with elevated PTIs (115). This variable (PTI) has been considered by some researchers as a more appropriate parameter to express DFU risk, compared to peak pressure because it incorporates time as well as pressure magnitude, which is suggested to be important (loading time) in ulcer formation (116) (117). This has been further reinforced by a observational studies meta-analysis, which discussed correlations of elevated PPP and PTI with DPN severity (118), though this meta-analysis indicated a limitation of those studies that there was no measure of whether the heightened pressures led to actual ulcer development. In general, there is paucity of studies that have conducted a longitudinal follow-up for patients developing a DFU. Researchers have shown a wide-ranging curiosity regarding the role of plantar pressures in DFU and PP threshold for forecasting foot ulceration onset (119–123).

Plantar Pressure Thresholds

Despite general agreement on the important role of high PPP in the onset/development of DFU, researchers are still inconclusive on a defined threshold of PPP over which DFU develop (124,125). One study has suggested a threshold for PPP of > 6 N/cm^2 for DFU

development (126), (67). A study by Owings et al. (127) suggested 200 kPa (20 N/cm^2) threshold for DFU development and this has perhaps been the most widely used and tested threshold to date, despite considerable uncertainty over its appropriateness. Another study revised PP threshold for people with DFU at >110 N/cm^2 (128), but other reports in view of detection of DFU at lower values have suggested reviews for the values (129). In certain studies even PPP >65 N/cm^2 in diabetics increased risk possibility by six fold (130). Therefore, whether due to differences in methodology or equipment, researchers have been unable to reach a consensus on a definitive PPP threshold to accept.

The overall PhD thesis aims to further understand the mechanisms underlying diabetic foot ulcer risk, through exploring the link between high foot pressure, tendon stiffness and limited ankle dorsiflexion during gait. This specific chapter enables the first step towards this overall aim by providing a comprehensive assessment of foot pressures across different regions of the foot and understanding which pressure parameter(s) most appropriately reflect diabetic foot ulcer risk. This chapter also tests the association between foot pressure and selected clinical variables relevant to DFU risk.

9.3 Aim:

Establish the most relevant pressure parameter(s) to act as a proxy for foot ulcer risk and identify associations between different foot pressure quantification methods with clinical parameters relevant to DFU risk in people with diabetic peripheral neuropathy.

9.3.1 Hypothesis:

DPN patients will have elevated foot pressure distribution compared to controls and this will be reflected more clearly in certain pressure variables than others. Certain variables quantifying plantar pressure underneath the foot will correlate with specific clinical variables.

9.4 METHODS

9.4.1 Ethical Consideration and Informed Consent

This research study involved NHS patients and thus had a requirement for NHS ethics approval for their participation. The researcher generated a new project study code (IRAS project ID: 239893, Ref. No. 18/NW/0274) at The Integrated Research Application System (IRAS, version 5.19, U.K.). Based on this process, GM East REC (Research Ethics Committee) granted favourable ethical opinion (Appendix 1). HRA (Health Regulatory Authority) and Health and Care Research Wales (HCRW) Approval (Appendix 2) was subsequently received, thus allowing research team to approach Manchester University NHS foundation trust (MFT) and Lancashire teaching Hospitals (LTHTR) hospitals for R&D approvals and issuance of research passport to access hospitals for recruitment of participants. Collaborating hospitals supported with recruitment activities, while testing and research activities were carried out at the Manchester Metropolitan University (MMU) research labs. Research Passport and R&D approvals from LTHTR was obtained (

Appendix 3) besides obtaining the R&D approval from MFT (PIN: B00125). Manchester Metropolitan University (Manchester U.K.) ethics clearance (via online ETHOS platform) was also obtained to commence the recruitment. In commensurations with NHS ethics on approaching the participants and information confidentiality, the identified eligible participants were provided with Participant Information Sheets (PIS) (Appendix 4) and were given a minimum 24 hours' time to study and respond through signed informed consents at the start of the first study visit.

9.4.2 Participants:

Two study participant groups were investigated:

DPN: People with Diabetic Peripheral Neuropathy. Diabetic patients with no previous history of ulceration, but mild to moderate diabetic neuropathy defined by a Vibration Perception Threshold (VPT) ≥ 15 Volts and/or modified Neuropathy Disability Score (mNDS) score ≥ 3;

Controls: Healthy Controls (CTRL) were people without Diabetes, sex matched to the DPN group.

9.4.2.1 Eligibility Criteria

Inclusion criteria for the DPN group

- Consenting people with Type 2 diabetes.
- Male, aged ≥18 years
- able to walk unaided for at least 30 steps
- Presence of diabetic peripheral neuropathy as defined above under 'Participants-Group DPN'.

Exclusion criteria for the DPN group

- Prior Achilles or gastrocnemius tendon lengthening surgery (or other major surgery involving these tendons)
- Patients with active/open foot ulcers (should be healed for >2 months)

- Presence of peripheral vascular disease, major vascular complications (palpable foot pulses required for inclusion)
- Unstable ischaemic heart and related major ischemic issues
- Body implants including metal or electronic implants in the body and cardiac pacemakers
- Neurological (other than diabetic aetiology), or rheumatic disease.
- Amputation of more than two small toes, the hallux, or any more proximal level
- Musculoskeletal injury/recent lower-limb surgeries affecting gait.
- Sequelae from poorly healed fractures.
- Patients on specific medications (e.g. Fluoroquinolones family) from a long duration which can cause Achilles tendon stiffness
- Charcot foot
- Unable to speak and comprehend English
- Unwilling or unable to comprehend informed consent

Inclusion criteria for Controls

- Consenting male, aged ≥18 years
- Should be able to walk unaided for at least 30 steps

Exclusion criteria for Controls

- Declared presence or history of diabetes
- Recent or previous Achilles or gastrocnemius tendon lengthening surgery (or other major surgery involving these tendons) or history of significant injury or morbidity of the Achilles tendon.
- Musculoskeletal injury/recent lower-limb surgeries affecting gait.
- Presence of peripheral vascular disease, major vascular complications (palpable foot pulses required for inclusion).

- Unstable ischaemic heart and related major ischemic issues
- Body implants including metal or electronic implants in the body and cardiac pacemakers
- Neurological or rheumatic disease.
- Amputation of more than two small toes, the hallux, or any more proximal level
- Sequelae from poorly healed fractures.
- Currently or recently taking Fluoroquinolones (due to impact on tendon properties)
- Charcot foot
- Marked foot deformity of any aetiology
- Unable to speak and comprehend English
- Unwilling or unable to comprehend informed consent

9.4.2.2 Sample Size

To estimate the required sample size, a power analysis was performed using values for one of the key variables of interest for the work of the PhD thesis: "Achilles Tendon Stiffness", and with the assumption of using F-test, ANOVA, fixed effect, omnibus one-way analysis of variance between the groups for statistical treatment of the data. Population standard deviations (0.45) and a difference between the groups considered to be clinically significant, have been derived from similar previous work by Coupe el al(131). For an effect size (f) of 0.56 (calculated from the data of Coupe et al., 2016), β-error 0.15 and an α-level of 0.05%, minimum 13 participants were required per group for this study. To account for any dropout or instances of participants not completing all study sessions a recruitment target was set at n=15 per group. Two participants in the DPN group were identified as having errors within their dataset post collection, so were recruited for again. One participant in the control data set also was identified as having errors within the dataset, however this was identified after the finish of recruitment, and based on the minimum of 13 per group, and it was decided not to re-open recruitment. Therefore, the final data set is presented for n=28 (CTRL: 13 & DPN: 15).

9.4.3 Study Design:

This study was cross-sectional, observational in design. Participants were selected based on the study inclusion and exclusion criteria defined above. The reason for selecting males for the study and to eradicate any confounding effects of gender on tendon mechanical characteristics (132,133). The main outcome variables included plantar pressure components, demographic and clinical variables.

9.4.4 Assessments process

9.4.4.1 Anthropometric and clinical data collection

Standardized questionnaire and information formats were used to collect the data on social-demographic, clinical characteristics and lab testing.

9.4.4.2 Socio-Demographic data

Clinical data related to diabetes was also recorded for the DPN group which included duration of diabetes and list of medications. Anthropometrics included measurement of Height and Weight. BMI was calculated using NHS standard calculator for BMI. The NHS BMI calculator takes into account Height, Mass, Age, Gender, Ethnicity and Activity Level (Source: https://www.nhs.uk/live-well/healthy-weight/bmi-calculator/) to provide a numeric value of BMI level.

9.4.4.3 Well-being measurements

As part of socio-demographic reporting of participants, an assessment of wellbeing was done using the WHO-5 (World Health Organisation- Five) Well-Being Index (134), a short self-reported questionnaire which is a measure of current wellbeing.

9.4.4.4 Lifestyle Score

This study assessed the lifestyle of participants to understand lifestyle correlations with the risk of diabetic foot Ulcers (135) . A questionnaire was developed for lifestyle scoring, with broadly four categories of 1. Fitness awareness & consciousness, 2. General daily activity, 3. Daily exercise regime and 4. Eating habits & dietary regime. The queries were designed using WHO: Global recommendations on physical activity for health (136) and NHS eat well guide (137). A Likert scale (138) was used for scoring from 2 to 10 per category (2 points each for 5 ascending adherence levels). Using the Summative form of Likert scale, for four categories, a final participant score out of 40 (normalized to 100) was quantified.

9.4.5 Neuropathy tests

Clinical tests were performed with the patients to ascertain the neuropathy status of the participants. Modified Neuropathic disability score (NDS), (139)vibration perception threshold (VPT) and tactile sensation/loss of protective sensation (LOPS) tests (SWMT and Ipswitch test) were performed to assess the presence/extent of peripheral sensory neuropathy. The tests performed mainly refer to the assessment of large - fibre function, although the NDS assesses both large and small nerve fibres.

9.4.5.1 Neuropathy Disability Score

A modified neuropathy disability scoring (NDS) (140) system was used to assess participant's ability to detect very small applications of force, sharp/blunt, vibration, reflexes and temperature change in different areas of their feet. The recordings were taken in the prescribed format. To assess various components of NDS, the examiner used standardized traditional devices: 128 Hz tuning fork, reflex hammer, tiptherm and neurotip for the NDS testing. Site of testing was distal plantar surface of big toe (except for Achilles tendon Reflex test) of right and left feet. Only two individuals were tested on the edges of the tip of the hallux to avoid severe callus build up. Both feet were tested and scored independently, and the results were added together. The maximum score for the modified NDS is 10, indicating an identified loss of sensory modalities and absent reflexes. A score of six or more is considered to indicate an increased risk of foot ulceration (141).

The various tests for **NDS assessment** are described below. It is pertinent to mention that in all the test participants were not watching the procedures and were only required to give a pointed response based on the feeling whenever asked during/after completing a test. A dry run was done before every test, where the participant was informed on all aspects of the test and what was expected as a response from their end**:**

Ankle reflex (or Achilles reflex) test is done to measure the Ankle Jerk Reflex (S1, S2). It is performed on both legs, by gently bending the knee and holding the foot in dorsiflexion. The tendon hammer then drops onto the Achilles tendon. The response is plantar flexion of the foot with contraction of the gastrocnemius. With the participant sitting, the examiner dorsiflexed the foot and gently struck the Achilles tendon with the reflex hammer. In the absence of reflex, the test was repeated with reinforcement. In reinforcement for lower extremity reflexes, participants were asked to hook together the

fingers of each hand so each arm can forcefully pull against the other (Jendrassik manoeuvre), and the split second before the researcher was ready to tap the tendon, he would say "pull" to participant (142). It is key to compare the strength of reflexes elicited with each other. Reflexes were scored as zero (normal|) one (present with reinforcement) and two (absent with reinforcement).

To assess detection of **small temperature discrimination**, Tip-therm (TipTherm® Bailey Inst. Ltd. UK), was used, which assists in making an early diagnosis of distal symmetrical polyneuropathy. The participant's ability or failure to perceive variances was recorded as 0 and 1 respectively. Ambient room temperature of 23-25^{o}C was maintained while gently touching the dorsal (dorsum) surface of foot with tiptherm.

128-Hz standard tuning fork was used to test **vibratory sensation**. The tuning fork was put on the interphalangeal joint while it was still and secondly, when in vibration. An abnormal response was identified when the tested patients failed to perceive the vibration sensation while the examiner could. A score of "0" was recorded when participant was able to perceive the vibration and "1" was accorded in case of failure of perception.

To assess the ability to **detect sharp/blunt**, Pin prick testing was done. This was assessed using the Neurotip (Owen Munford^{TM}, Oxfordshire, UK), which consists of a disposable pin. Dorsal hallux (proximal to toenail) was chosen site for placing sharp side, with intent to avoid breaking and ensure only deforming of skin (143). Ability to identify sensation (sharp) was scored as "0" and inability to do the same was scored as "1".

9.4.5.2 Vibration Perception Threshold (VPT)

To test vibration perception, VPT test was performed using Neurothesiometer (Neurothesiometer- NV0592, Algeo UK,). This device has ability to measure lower VPTs than a tuning fork for measuring Vibration Perception Threshold. A non-recorded dry test was done first on end of great toe of either foot of participant, for extracting immediate response on feeling of any vibration sensation, while increasing from zero, the threshold of frequency to the point where the participant states they can feel the vibration. The cut-offs for severe neuropathy are neuropathy disability score ≥6 and/or vibration perception threshold ≥25 while mild to moderate diabetic neuropathy defined by a VPT ≥ 15 and/or NDS ≥ 3.

9.4.5.3 10-g Monofilament Test for Tactile sensation: LOPS test1

SWMT or Semmes-Weinstein monofilament test is used to assess sensory function and light touch perception. A large fibre nerve function impairment is indicated through inability of detection of sensation when pressed against chosen sites of foot (heel and 1st MT head).

9.4.5.4 Ipswich touch test (IpTT): LOPS Test2

Ipswich Touch Test was also performed for LOPS screening of study participants, besides the 10-g monofilament test. To check tactile sensation, Vas et al. (144) had developed IpTT which is a straightforward, low learning curve, non-instrumental tool to determine Loss Of Protective Sensation (LOPS). This has been developed for diabetic inpatients; with intent to decrease nosocomial foot ulceration (145). The identified toes in order of examining were tested, as recommended in IpTT protocol. The sites of test include the first, third, and fifth toes and the dorsum of the hallux for each foot (if available for testing). A dry run was done with participants while demonstrating the purpose and interaction during the actual test. Participants, while lying supine on examination bed with eyes closed, were asked to respond with a "Yes" on feeling a "touch" prompted by "light touch" of index finger of the examiner. A non-sensation in two or more locations of testing is considered as a failure in tactile senses and was recorded accordingly. IpTT simplifies touch sensation test from SWMT (Semmes-Weinstein Monofilaments perception Test). IpTT has shown analogous results when compared with 10 g monofilament test (146). The present study assessed the sensitivity and specificity of IpTT using Monofilament test as an outcome variable.

9.4.6 Plantar pressure assessment

The participants were asked to walk across a 10m walkway at their self-selected speed with insoles (F-Scan, Tekscan Inc., MA, USA) inserted into standardised shoes (post-surgical Darco sandal) directly underneath the participant's foot. The 10-metre walkway for level walking was a flat walking surface with appropriate slip-resistance and force platforms embedded into the floor midway along the walkway (use of the force platforms will be described in Chapter 2, where they are used for gait measurements). Start and finish lines were marked out to give an indication to participants on the length of walking, which ensured that they were not accelerating or decelerating within the motion capture volume. Participants stood at start of walkway and moved ahead looking towards an object kept in

straight line of their eyesight. Participants walked at their own routine walking natural speed (self-selected) and were signalled to stop where the walkway terminated at 10 metres.

There are other methods proposed for reproducibility of walking to elicit pattern of plantar pressure as mid-gait method (147). So, in our study for measurement purpose, a full gait cycle before a heel strike on first force plate and a complete gait cycle after toe-off from second force plate, were considered. This was repeated several times (5 times on an average) to generate sufficient data (10-12 nos. of stances). The other accepted in-shoe plantar pressure data measurement method “twelve steps” also conformed with our method (148) for sufficient no. of strides for collection of gait data.

F-Scan in-shoe sensors are flexible and embedded in the shoe such that measurements reflect the interface between the foot and the shoe. (149)(150). This sensor is designed to measure in-shoe plantar pressures and their distribution. The sensor insole consists of 955 individual pressure-sensing cells, evenly distributed at 5.05 mm intervals.

The basic F-Scan (Figure 1) system used in this study was tethered, meaning that it is a wired system, with a unit on ankle and a box on belt, so that wires are safely kept behind the participant and a person would carry the cables (in order to avoid interference with participant’s gait) while the participant walks.

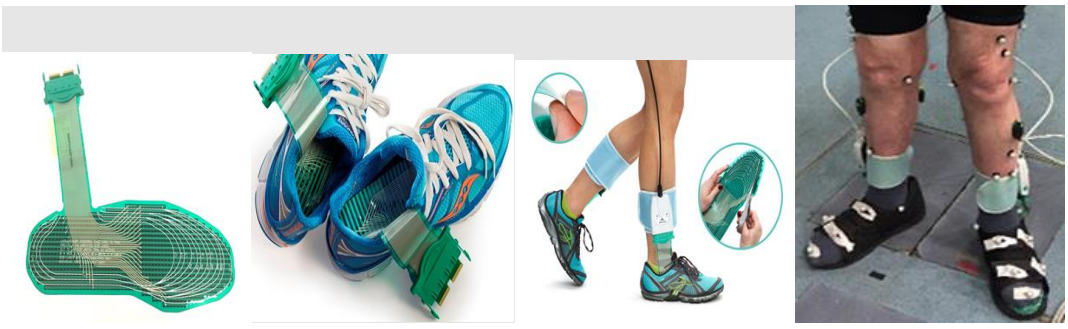

Figure 1 - In-Shoe pressure measurements using Tekscan Insoles.

Gait Analysis system. Source: Tekscan, https://www.tekscan.com/. Far right pane: A participant lower limb when prepared for testing.at MMU lab. T0.18 @2019.

The insoles were calibrated pre-data collection, using a Step/Walk calibration, where participant was asked to maintain balance for few seconds on alternate foots (one by one).

9.4.6.1 Foot Pressure Data Processing

Left foot analysis (standardised to this foot across all participants) was performed using Tekscan pressure measurement system (F-Scan Research Ver. 6.70-03, Tekscan Inc.). This

exercise was done to obtain measurements of various plantar pressure variables for the total foot and regional foot areas. The inbuilt facility in the software can divide up the foot into 12 segments:1st 2nd 3rd 4&5th Toe, 1st 2nd 3rd 4th, 5th Metatarsal, Midfoot, Medial Rearfoot and Lateral Hind foot. The Tekscan software automatically calculated the pressures across the different foot regions in addition to total foot pressure. The software also calculated the variables of: Peak Plantar Pressure (PPP), Pressure-Time Integral (PTI), Force-Time Integral (FTI) and Peak Pressure Stance Average (PPav).

Peak Plantar pressure (PPP) - (unit kPa) is defined as the highest pressure value recorded by each sensor over the entire period of the stance phase (151). This value represents the maximal load in an area under the foot during one step (111).

Pressure time integral (PTI) - (unit kPa·s/cm^2) defines the cumulative effect of pressure over time in a certain area of the foot and provides a value for the total load exposure of a foot sole area during one step. PTI represents the duration of mechanical stress on the foot. (152) and is the integral of Pressure/time curve for a stance.

Force Time Integral (FTI) - (unit N·s/cm^2) or "loading rate/impulse is the area under the curve of a force-time curve, which indicates the load of a certain area in relation to the time the area was loaded. This variable is a genuine integral of force over time in a particular foot sole area. However, unlike PTI, FTI does not account for the size of an area it is applied to. (109)

Peak Pressure Stance Average (PPAv) – (Unit kPa) is the peak value from an array of average of peak values of each "sensel" over the repeated stances. In this study, researcher used the F-Scan sensor (piezo-resistive technology), a thin multi-laminate construction of grid based sensor with 960 sensing elements called sensels (Figure 2), where each sensel is a square with side of 5.08 mm and area of 25.80 mm^2. Values of each sensel are first averaged over repeated stances, before a peak of all sensel values being taken. As people with DPN are known to make considerable walking adjustments resulting in ambulation at different over ground velocities; PPav normalizes the stance phase time to percent of gait cycle, thus accounting for this variation.

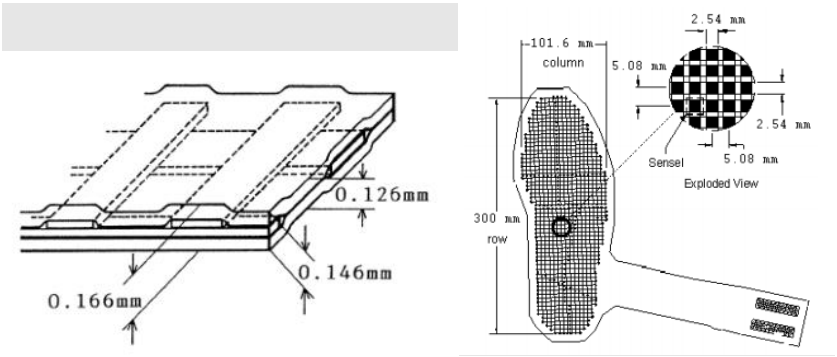

Figure 2: Sensor arrangement in Tekscan, F-Scan pressure mapping insoles.

Source: F-Scan user manual, V. 6.51x. Tekscan Inc. research notes.@2016

9.5 STATISTICAL ANALYSIS

Statistical analyses included descriptive statistics, analysis of variance (ANOVA) and analysis of co-variance (ANCOVA). Differences between the groups (DPN and Controls) regarding the plantar pressure analysis were calculated using Independent Student's *t*-test. The correlations between socio-clinical and pressure variables were calculated with SPSS correlation bivariate analysis. The collected data was tabulated and analysed using software SPSS statistical software (Statistical Package for Social Sciences, IBM SPSS statistics Ver. 26, IBM corp.) for windows. All data and values have been expressed and presented as group mean ± SD.

9.5.1 Test of Normality

Overall normality was tested in SPSS using explore function. The data were explored for normality of distribution prior to inferential analysis. A test of normality was performed on the data. This test is used to determine whether sample data has been drawn from a normally distributed population (within some tolerance). In our case, independent t-test require a normally distributed sample population. As a statistical test to confirm hypothesis we used the Shapiro Wilk test. As majority of our data was not normal, we decided to use a nonparametric version of the test, which does not assume normality. Spearman correlation method computes the correlation between the rank of x and the rank of y variables. To perform test of Normality, in SPSS main menu "Analyse" function was selected followed by clicking on "Descriptive Statistics" and "Explore". In Explore window, selected variables from left pane to insert in "dependent variables" list. Then, clicked on

"Plots" and ticked the "normality plots with tests" to get information on normality. Test was run to obtain data. The output table presented the results from two tests of normality, namely the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test. The Shapiro-Wilk Test is more appropriate for small sample sizes (< 50 samples), so we used the Shapiro-Wilk test as our numerical means of assessing normality. As the test suggests, If the Sig. value of the Shapiro-Wilk Test is greater than 0.05, the data is normal, and If it is below 0.05, the data significantly deviate from a normal distribution. Spearman correlation method computes the correlation between the rank of x and the rank of y variables.

9.5.2 Independent samples Student's *t*-test

Differences between controls and DPN group were assessed using an Independent samples Student's *t*-test to determine if there is a significant difference between the means of two groups, which may be correlated in certain features. In SPSS software, where variables information and participant data were stored, "Analyse" function was clicked, where "compare means" option was selected followed by clicking on "Independent-Sample T Test" which opened the processing window. The dependent variables were selected from left pane (containing list of variables) and inserted in "Test Variable(s)" space. Grouping variable was "groups". As our grouping variable is numeric and continuous, we designated a cut point while defining group ("define group") to dichotomize the variable, which defines category indicators (groups) to use in the t-test. In "Options" a confidence level of 95% was selected for the confidence interval for the mean difference. To ensure how SPSS would handle missing values, the option to "exclude cases analysis by analysis" was selected. This when finished, click "OK" to run the Independent Samples t Test. All data and values have been expressed and presented as group mean ± SD and analysed with p value ≤ 0.05 was taken as statistically significant difference between groups. Despite non-normal distribution a T-test was used as it is less likely to generate type 1 errors.

9.5.3 ANCOVA (Assessing Age as a Covariate)

There were differences in Age between the groups (Table 1), therefore ANCOVA was used to assess for the effect of age as a covariate on all major variables.

9.5.4 Correlations

Correlation analysis was used to evaluate the strength of relationship between (state pressure variables) and the clinical measures. As the variables were non-parametric, a Spearman's rank-order correlation was used. Spearman's correlation coefficient measures the strength and direction of association between two ranked variables.

9.6 RESULTS

9.6.1 Demographics

Demographic data for controls and people with DPN are shown in Table 1. The DPN group being significantly older was adjusted for age in the analysis using an ANCOVA with age a covariate. The DPN group had a higher body mass compared to controls (Ctrls 84±6kg, DPN 92±15kg; means ± SD; p-value <.05, Sig ANCOVA p-value <.05) and BMI (p-value <.05, Sig ANCOVA p-value <.05) No significant differences were seen between groups in height. The DPN group had an average duration since diagnosis of 15 years (DPN 15±5 years, p<0.001) of known diagnosed diabetic condition. Two DPN patients had a previous history of foot ulcers.

Wellbeing score (Ctrls 86±15 vs DPN 78±25, score/100, p<0.001) as well as lifestyle score (Ctrls 75±22 vs DPN 50±17, score/100, p<0.001) were considerably lower in case of DPN group as compared to Controls.

Table 1 - Demographic and clinical characteristics of participants.

*Mean values ±SD (Std. Dev.) of all variables by group. CTRL: Healthy Controls, DPN. * Variable that showed significant (p>0.05) group effect when adjusted for Age as a covariate using an ANCOVA. ǂ Non-Parametric (Shapiro-Wilk).*

Variable	CTRL			DPN		SD	T-test p-value
Anthropometry							
Age (Years)	39	±	6	71	±	9	<0.001ǂ
Body mass (kg)	84	±	6	92	±	15	<.05*
Height (m)	1.77	±	0	1.8	±	0	0.36ǂ
BMI (Kg/m2) *	27	±	2	29	±	4	<.05*ǂ
Wellbeing score**	86	±	15	78	±	25	0.31*
Lifestyle Score***	75	±	22	50	±	17	<.05*
Clinical							
Neuropathy Disability Score	1	±	1	8	±	1.3	<.001*ǂ
VPT (Volts)	3	±	1.5	27	±	11	<.001*ǂ
Tactile Sense- LOPS	0	±	0	1	±	0.4	<.001*ǂ
Diabetes Duration (Years)	0	±	0	15	±	5	<.001*ǂ

9.6.2 Neuropathy

As expected, the DPN group had a higher NDS (Ctrls 1±1.04 vs, DPN 8±1.32, p<0.001) as compared to Ctrls. Similarly, VPT was considerably higher (Ctrls 3±1.5 vs, DPN 27±11.35 Volts, p<0.001) in DPN patients compared to controls (Table 1).

9.6.3 Plantar Pressures – total foot

Overall, total foot pressure data for controls and people with DPN is shown in Table 2. Pressure-Time Integral, Peak Plantar Pressure and Peak Pressure Stance Average were significantly higher in Diabetic group as compared to controls. In contrast FTI was significantly lower in the DPNs compared to Controls.

Table 2 - Total foot Pressure variables.
*Values presented as Means ±SD (Std. Dev.) for CTRL: Healthy Controls and DPN: Diabetic Peripheral Neuropathy. * Variable that shows significant (p>0.05) group effect when adjusted for Age as a covariate using an ANCOVA.*

Variable	CTRL		SD	DPN		SD	T-test p-value
Pressure Time Integral-PTI (kPa.s/Cm^2)	61	±	19	88	±	38	<.05*
Force Time Integral-FTI (N.s/cm^2)	62	±	5	53	±	7	<.001*
Peak Plantar Pressure-PPP (kPa)	518	±	202	896	±	489	<.05*
Peak Pressure Stance Average-PPav (kPa)	139	±	44	206	±	93	<.05*

9.6.3.1 Peak Plantar Pressure (PPP) across different foot regions

Peak Plantar Pressure across the foot regions are shown in Fig3. Peak pressure was higher in the forefoot regions of DPN patients as compared to controls. Hallux had higher PPP in DPN compared to Ctrls, and similarly 2nd, 3rd, 4 and 5th Toe, Metatarsal 1, 2, 3, 4 & 5 and Midfoot region had shown higher PPP in DPNs as compared to controls. The rear foot region showed no significant differences in peak pressures between Ctrls and DPN.

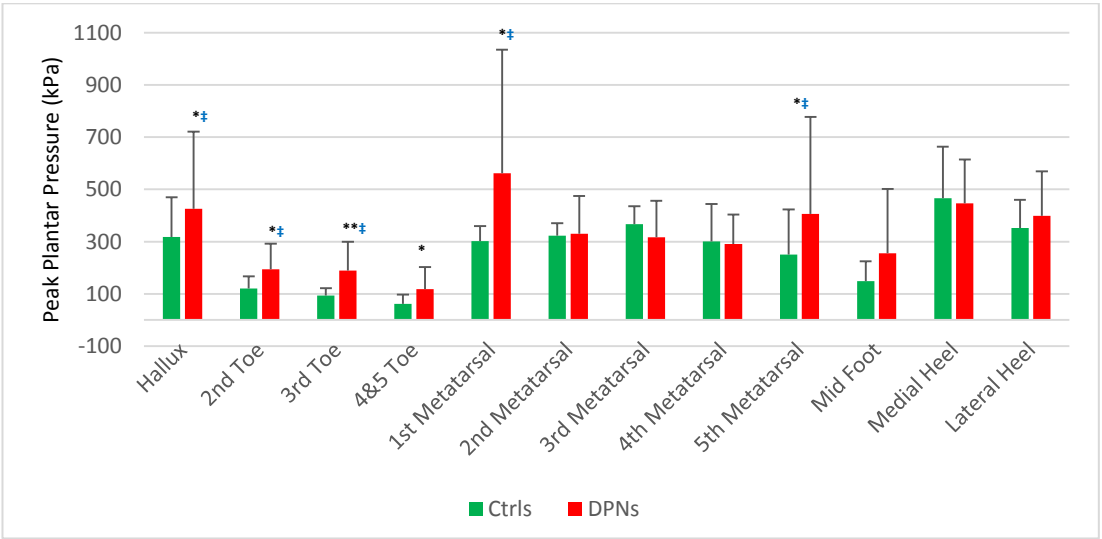

Figure 3 - Peak Plantar Pressure across the different regions of the foot.

*Shown for the DPN (Diabetic Peripheral Neuropathy) group (red bars) and control group (green bars). * (p<.05) and ** (p<.001) denotes significant difference at that specific foot region between DPN and Control groups. ǂ denotes a variable that shows significant (p<0.05) group effect when adjusted for Age as a covariate using an ANCOVA.*

9.6.3.2 Pressure-Time Integral (PTI) across different foot regions

The PTI across the different regions of the foot are shown in Figure 4. Few The forefoot region comprising toes and metatarsal showed significantly higher values in DPN patients. When analysing the foot in regions, higher PTIs were seen in the DPN group compared to the controls at selected toe regions. In the variable of PTI, in line with the total foot pressure, which was significantly higher (p<.05) in DPNs as compared to Controls, forefoot regions of Hallux (p<.05), 2nd Toe (p<.001), 3rd Toe (p<.001), 4&5 Toe (p<.05), 1st Metatarsal (<.05) and 5th Metatarsal showed elevated pressures as compared to controls. No significant differences were observed in PTI for the remaining regions of the foot.

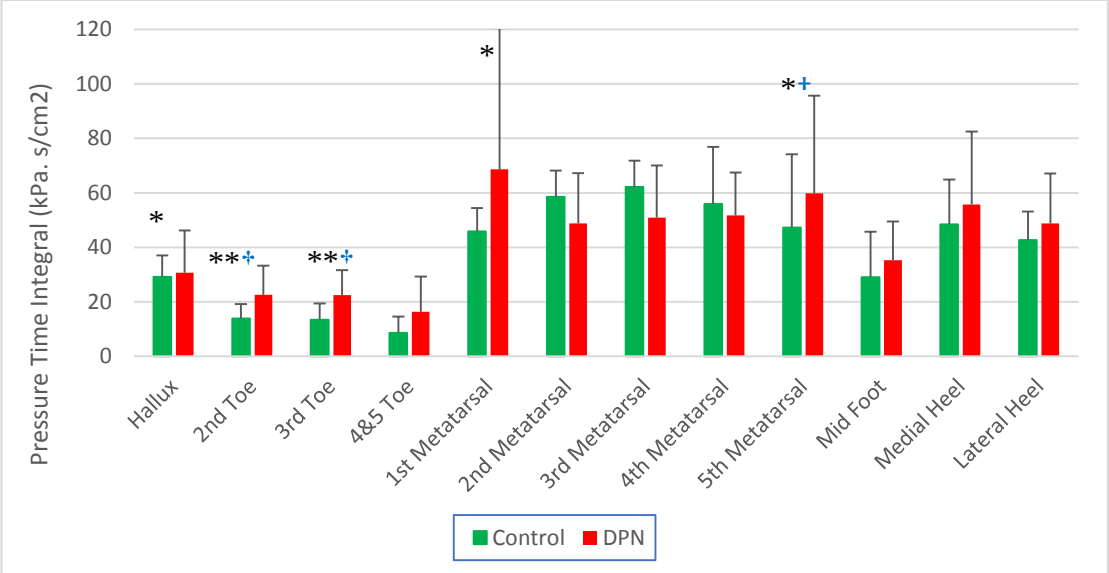

Figure 4- Pressure-Time Integral across the different regions of the foot.

*Shown for the DPN (Diabetic Peripheral Neuropathy) group (red bars) and control group (green bars). * (p<.05) and ** (p<.001) denotes significant difference at that specific foot region between DPN and Control groups. ǂ denotes a variable that shows significant (p<0.05) group effect when adjusted for Age as a covariate using an ANCOVA.*

9.6.3.3 Force-Time Integral (FTI) across different foot regions

The force-time integral across the different regions of the foot are shown in Figure 5. The FTI in general was lower for DPNs compared to controls, with the exception of the 2nd toe and Metatarsal 1.

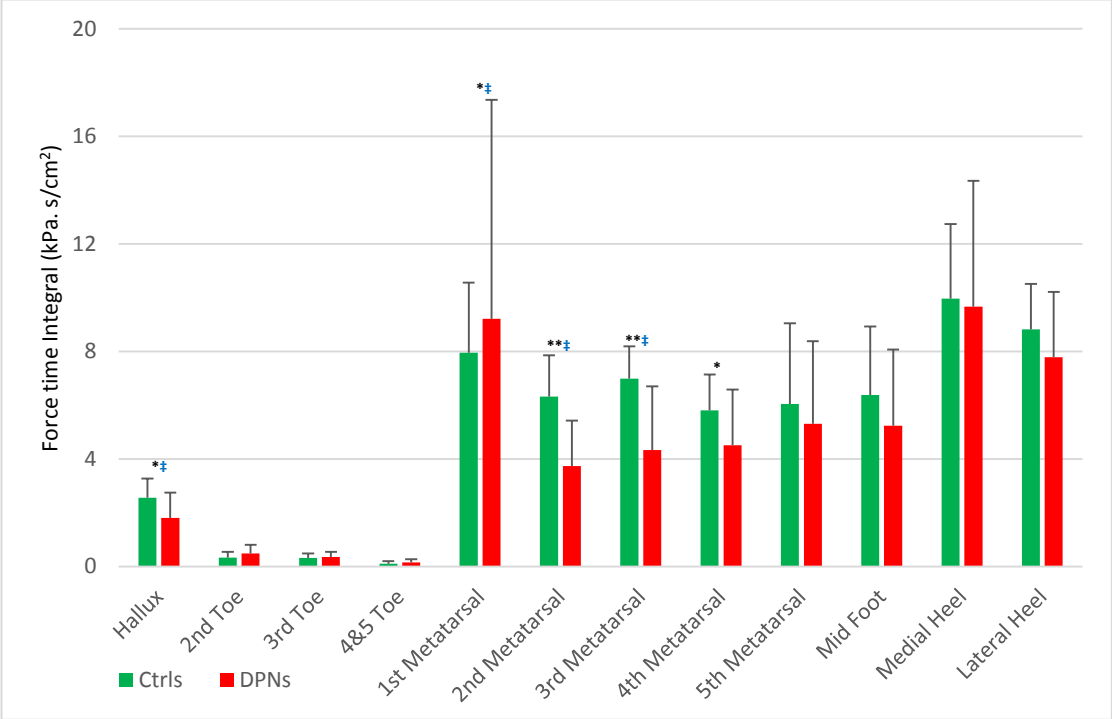

Figure 5 - Force-time integral across the different regions of the foot.

*Shown for the DPN (Diabetic Peripheral Neuropathy) group (red bars) and control group (green bars). * (p<.05) and ** (p<.001) denotes significant difference at that specific foot region between DPN and Control groups. ‡ denotes a variable that shows significant (p<0.05) group effect when adjusted for Age as a covariate using an ANCOVA.*

The forefoot FTI was lower in the DPN group at the 2nd Metatarsal (<.001), 3rd Metatarsal, (p<.001) and 4th Metatarsal (p<.05) and Hallux (p<.05), whereas 2nd, 3rd, 4&5 Toe and 5th Metatarsal though had elevated FTI in DPNs. showed no significant variations as compared to Controls. Midfoot and rear-foot regions showed no significant difference in FTI between two groups.

9.6.3.4 Plantar Pressure Stance Average (PP Av) across different foot regions

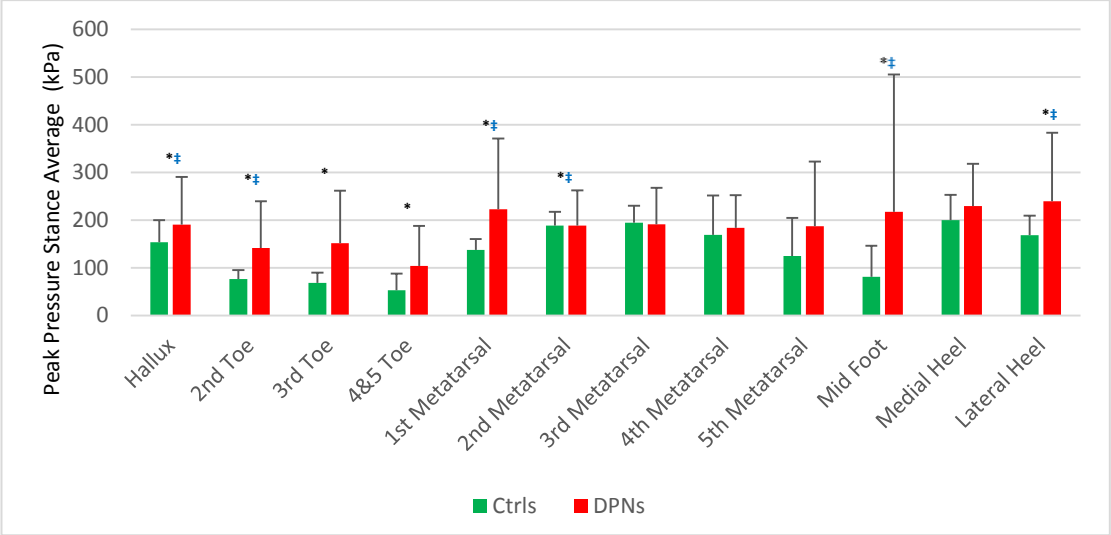

Figure 6 - Peak Plantar Pressure across the different regions of the foot.

*Shown for the DPN (Diabetic Peripheral Neuropathy) group (red bars) and control group (green bars). * (p<.05) and ** (p<.001) denotes significant difference at that specific foot region between DPN and Control groups. ‡ denotes a variable that shows significant (p<0.05) group effect when adjusted for Age as a covariate using an ANCOVA.*

The trend of total foot Peak Pressure stance average PPav being significantly higher (p<.05) in the DPN group as compared to controls continued for the specific forefoot regions, which was higher at the Hallux (<.05), 2nd Toe (p<.05), 3rd Toe (p<.05), 4&5 Toe (p<.05), 1st Metatarsal (p<.05), 2nd Metatarsal (<.05), 1st Metatarsal (p<.05), 2nd Metatarsal (<.001), 3rd Metatarsal, (p<.001) and 4th Metatarsal (p<.05) in the DPN group compared to controls. In contrast, PPav at the 3rd, 4th and 5th metatarsal heads showed no significant difference compared to controls. In the heel region PPav was higher in DPNs as compared to controls and reached significance for the lateral heel region.

9.6.4 Correlations

9.6.4.1 Peak plantar pressures and NDS

There was a positive correlation between peak plantar pressures and the NDS score (Figure 7).

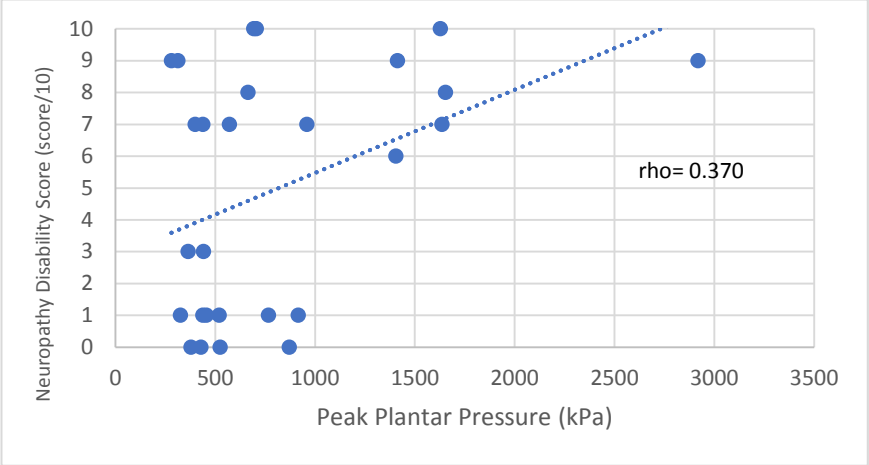

Figure 7 - Peak plantar pressure correlation with neuropathy disability score (NDS)

Shown for entire study group with linear trend line and correlation coefficient displayed.

9.6.4.2 Correlation between PTI and Age

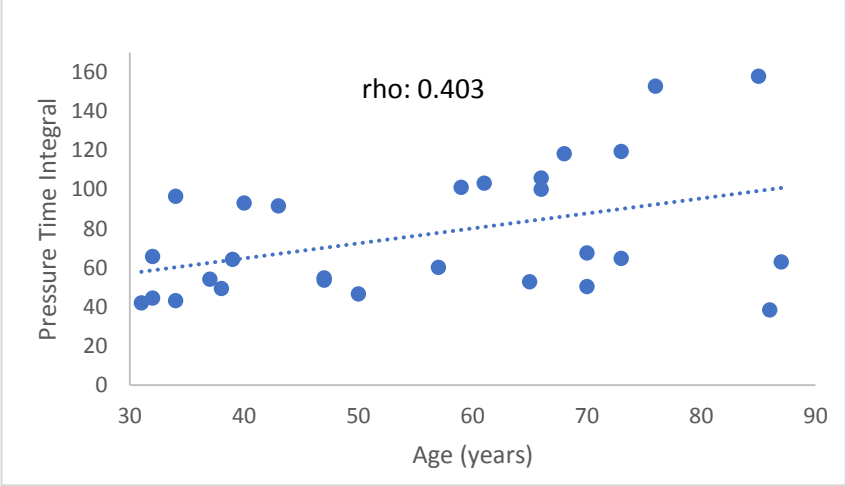

Figure - Pressure time integral (PTI) correlation with Age.

Shown for entire study group with linear trend line and correlation coefficient displayed.

There was a positive correlation between PTI and Age (Figure).

9.6.4.3 Correlation between diabetes duration and PPP

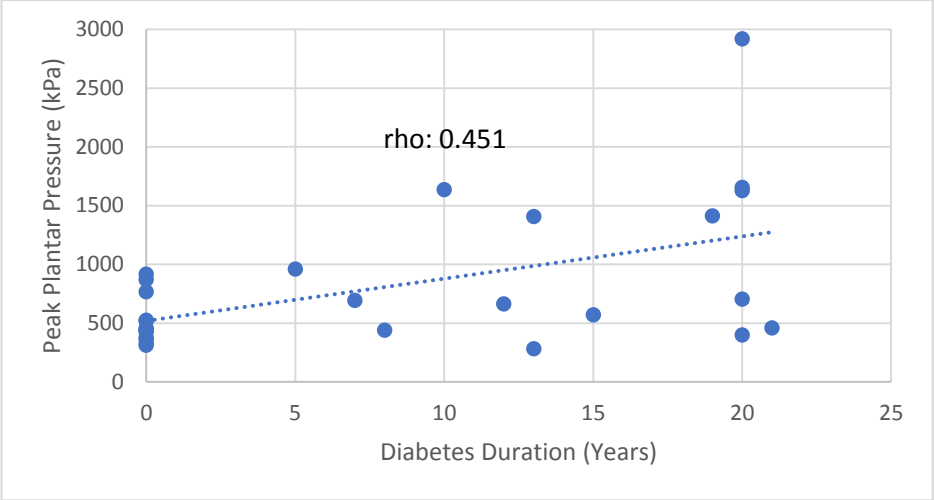

Figure 8 - Peak Plantar Pressure correlation with Diabetes Duration.

Shown for entire study group with linear trend line and correlation coefficient displayed.

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There was a positive correlation between PPP and diabetes duration (Figure 8).

9.6.4.4 Peak Pressure at 1stMetatarsal correlation with Vibration Perception Threshold

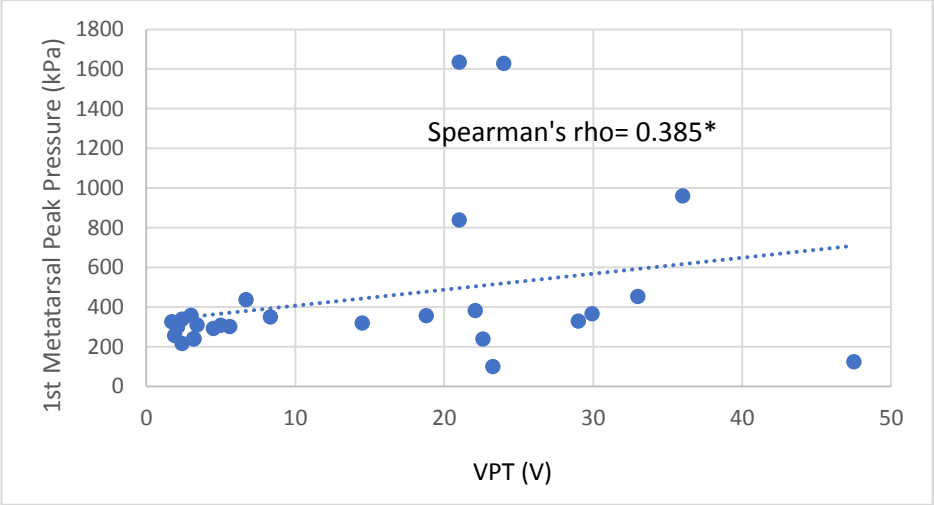

Figure 9 - Peak Pressure at 1st Metatarsal correlation with Vibration Perception

Shown for entire study group with linear trend line and correlation coefficient displayed.

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There was a positive correlation between Peak Pressure at 1stMetatarsal correlation with Vibration Perception Threshold (Figure 9).

9.6.4.5 Correlation between PPP at 2nd Toe and VPT

There was a positive correlation between PPP at 2nd Toe and VPT (Figure 10).

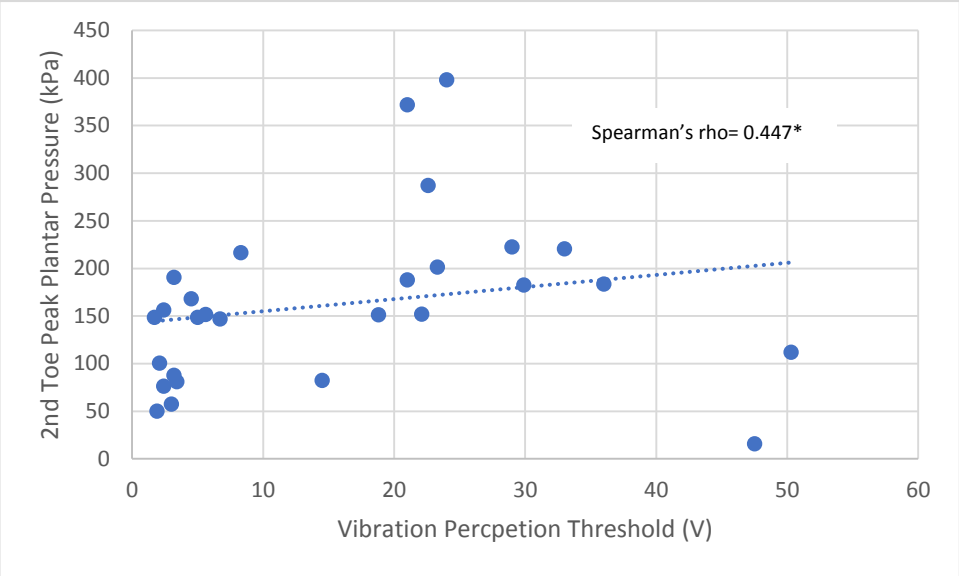


Figure 10- Peak Pressure at 2nd Toe correlation with Vibration Perception Threshold.

Shown for entire study group with linear trend line and correlation coefficient displayed.

9.6.5 Associations of plantar pressure, Demographics & Clinical variable

Table 3-Associations of plantar pressure, Demographics & Clinical variable

Variable	Region	Body Mass (kg)	Diabetes Duration	VPT	NDS
Pressure-time integral (PTI)	Total Foot	-0.03	.461*	0.317	0.252
	1st Metatarsal	-0.033	0.168	0.18	0.269
	2nd Metatarsal	-.405*	-0.193	-0.318	-0.296
	First Toe	-0.235	0.375*	0.175	0.201
Force-time integral (FTI)	Total Foot	-.680**	-.529**	-.636**	-0.665**
	1st Metatarsal	-0.241	-0.229	-0.134	-0.185
	2nd Metatarsal	-0.310	-0.532**	-0.565**	-0.559**
	First Toe	-0.192	-0.381	-0.354	-0.268
Peak Plantar Pressure (PPP)	Total Foot	0.008	0.451*	0.395*	0.370*
	1st Metatarsal	0.067	0.306	0.385*	.378*
	First Toe	-0.201	0.212	0.163	0.262
	2nd Toe	0.015	0.443*	0.447*	0.371*
Plantar Pressure Stance Average (PPav)	Total Foot	0.026	0.472*	0.370*	0.308
	1st Metatarsal	-0.064	0.253	0.287	0.324
	2nd Metatarsal	-0.315	0.026	-0.18	-0.115
	First Toe	-0.228	0.165	0.105	0.218
PTI	Forefoot	-0.371*	0.162	-0.011	0.063
	Rear Foot	0.004	0.256	0.136	0.072
FTI	Forefoot	-0.558**	-0.369*	-0.411*	-0.434*
	Rear Foot	-0.201	-0.164	-0.236	-0.296
PPP	Forefoot	-0.108	0.382*	0.390*	0.367*
	Rear Foot	-0.08	-0.003	-0.087	-0.110
PPav	Forefoot	-0.139	0.320	0.214	0.217
	Rear Foot	0.06	0.271	0.128	0.093

9.7 DISCUSSION

9.7.1 Pressure parameters

The present study shows clear foot pressure differences between DPN patients and controls without diabetes, simultaneously assessing a variety of pressure parameters and pressure regions, which have not been previously investigated together. This study has quantified four variables of plantar pressure: peak plantar pressure (PPP), Pressure-time Integral (PTI), Force-Time Integral (FTI) and Peak Pressure Stance Average (PPav). High pressure alone does not give rise to DFU, but coupled with other risk factors including an insensitive foot and foot deformities aberrant plantar pressures substantially increase the risk of DFU (62). Total foot PPP, PTI and PPav were found to be higher in patients with DPN as compared to controls, while total foot FTI was lower in DPNs in comparison with controls.

In the current study an elevated total foot peak plantar pressure was observed in DPN as compared to Controls (DPN 896 kPa vs Ctrls 518 kPa; Table 1), similar findings to those of Armstrong et al. (153) who also found significantly higher total foot mean PPP for patients with healed ulcers compared to controls who were diabetics with no history of ulceration (Ctrls 627 kPa vs DFU 831 KPa). Heightened peak pressures in people with DPN is one of the most historically researched measures of pressure, and has commonly been shown as an eminent surrogate for diabetic foot (64,154,155). In the current study the midfoot PPP was higher in DPNs as compared to controls (Ctrls 149 kPa vs DPN 255 kPa) in line with Bacarin et al. (156) findings that DPN/DFU groups had higher peak plantar pressure than control subjects with midfoot peak pressure being significantly higher for people with DPN than without Ctrl (Figure 3).

The current study showed that total foot pressure-time integral was higher in DPNs in comparison with controls (DPN 88 kPa.s vs Ctrls 61 kPa), commensurate with other studies showing significantly higher PTI in DPNs when compared to controls (157). In the current study, although plantar pressure in the midfoot and rear-foot region of DPNs was higher when compared to controls, although this did not reach significance. A study by Bacarin et. al. (156) found that DPN/DFU groups showed that the pressure-time integral was significantly higher in people with DPN at both midfoot (DPN: 69kPa.s; Ctrls: 37 kPa.s) and rearfoot (DPN: 103kPa.s; Ctrls: 83 kPa.s) regions.

The presents study found lower total foot FTI in DPN patients compared to controls (DPN 53 N.s vs Ctrls 62 N.s). Force-time integral, has also been assessed as a parallel outcome measure besides PTI and PPP in plantar pressure threshold studies (63,158–161) . Stess et al. (63) found significantly higher values in peak plantar pressure and pressure-time integral levels in people with DPN and a history of foot ulceration when compared to controls, with the highest pressure present under the 4th and 5th metatarsal heads. Despite these differences in peak pressures, Stress et al. (63) showed no differences existed between groups when comparing FTI levels. However, Melai et al. (157) found some relevance of testing the clinical relationship of the specific quotient of FTI and contact area with plantar tissue damage. As FTI, unlike PTI does not account for the area of force application, a higher FTI in a region does not automatically mean that the skin tissue of this area is at risk of overloading. This indicates that FTI might not be an appropriate proxy measure for DFU risk.

The variable of Peak Plantar Stance Average (PPav) has been used for plantar pressure and vertical force mapping (162,163) in previous studies with individuals with pathologies. As mentioned earlier, Peak Pressure Stance Average is a peak taken from an average of each pixel within the pressure sensor across repeated steps, rather than a peak of the peak values from each stance period. In the current study, PPav was higher in DPNs as compared to controls (DPN 206 kPa vs Ctrls 139 kPa kPa). This was an expected outcome as this variable is a derivative of PPPA. In a retrospective study on diabetic foot patients in comparison with people with DFU amputations was conducted by Borg et al. (172), where they considered maximum amount of pressure during stance (each pressure measurement trial produced five stance phases, whose mean peak pressure value was used for evaluation) and concluded that pressures have considerable impact on the progression of DFUs, when expressed by using the PPav variable.

The key difference between PPP and PPAv is if the peak occurs on adjacent “sensels” each stance, then the overall averaged peak for each of those sensors is considerably lower than the actual peak for the entire foot (measured by PPP) per stance, which is amply justified by overall lower values found in PPav as compared to PPP, and also that PPav values for total foot may not be the most appropriate measure when the interest in a study is to highlight differences in peak pressures. It may be interesting to observe further when discussing the region wise effect of PPav, as each region is covered by multiple sensels. Diabetic participants with progressively severe DPN have greater in-shoe vertical pressures

than controls. An earlier study had indicated a threshold of 200 kPa for vertical plantar pressure within in-shoe pressure research (165) highlighting those at risk of diabetic foot. Furthermore, another study reported 51% of patients who ulcerated to have pressures above the threshold (166), while in our study the majority of DPN patients (including two who had previously ulcerated) showed much higher vertical in-shoe pressure then the mentioned threshold of 200 KPa.

A study was conducted on people with healed plantar ulcers in bare-foot and in-shoe alternatives for pressure mapping by Owings et al (127), which showed 556kPa as barefoot mean pressure on site of previous ulceration, which was lower than the mean DPN plantar pressure reported in researcher's current study (896 kPa) and PPP values in other published series (167–169) indicating a clear methodological difference between the magnitude of Owings results and those of other studies including this one. However, Owings still reported lower peak pressures in controls than persons with DPN, despite the differences in magnitudes to other studies. Additionally, the range peak pressures during walking trials reported by Owings et al. (127) was large, viz. 107–1192 kPa (both shod and barefoot) indicating that whilst increased pressures occur at healed ulcer sites, this is not always the site of highest pressure. Whilst mean in-shoe peak pressure at prior ulcer location averaged 207 kPa, under half that of barefoot pressures, Owings et al (127)found barefoot peak pressure only predicted 35% of the variance of in-shoe peak pressure. Given the additional factors impacting in-shoe pressure, the key advantage of in-shoe pressure assessment is that of real-world applicability, in a population that is recommended to always wear shoes to protect their feet, is that it provides a more accurate representation of pressures applied out-side of the laboratory environment.

9.7.2 Regional pressure assessment

In addition to the total foot assessment, this study investigated further where the differences resulting in the group variations for the total foot variables came from, by assessing pressure across 12 distinct regions of the foot.

This study identified that whilst there is an increased PPP for the total foot as mentioned above, when assessing the foot regionally, differences between DPN and control groups are seen as higher values in the DPN group at the Hallux, 2nd toe, 3rd toe and 4 and 5th toes, as well as the 1st and 2nd metatarsal head regions. The greatest group differences in PPP

were observed in the regions of Hallux (Ctrls 318 kPa vs 425kPa,) and 1st Metatarsal (Ctrls 301 DPN 562 kPa). However, no significant group differences were observed for the mid or rear foot peak plantar pressures. These observations are in line with other studies (170) that showed that the highest plantar pressure occurred at the first metatarsal head in diabetics. Perry et al. (170) suggested that the diabetes-related stiffening of the plantar soft tissues at the pad of the first toe and first metatarsal head may cause this elevated PPP. Another study (171) showed a 160% increase at 1st MTH for Young's modulus. The current study results are in line with the study by A. Gefan (172) which theorised that during gait highest loading occurs at distal medial region of forefoot subjecting people with DPN with elevated DFU risk. In the current study, the total foot differences observed for PPP were underpinned primarily by changes at the forefoot, but not the rear-foot region.

In the current study only a few regions in the forefoot showed a significant elevation in PTI in the DPN group compared to controls. Midfoot and rear-foot regions showed no significant variations when measuring PTI between these two groups. The total foot differences in PTI between groups were therefore also underpinned by higher pressure in the forefoot region for the DPN group, but not the rear-foot. Stess et al. (63) found significant increases in peak plantar pressure ($P < 0.004$) and pressure time integral ($P < 0.0004$) levels in the DFU group when compared with controls and the highest pressure was present under the 4th and 5th metatarsal heads.

Similarly to PPP and PTI, PPAv showed between group differences in the forefoot region in the current study. PPAv is also the only variable to show differences in the mid-foot and heel regions. Fernando et al. (96) found a significant increase in peak plantar pressure at the hindfoot in DPN patients as compared to non-neuropathic diabetes patients and healthy controls, but the more recent meta-analysis by Hazari et al. (97) found no significant differences at the hind foot.

The FTI was the only variable which showed lower values in the DPN group compared to controls for both the whole foot as well as forefoot region. However, as discussed earlier, this variable does not take into account the contact area. Assuming force remains constant, the smaller an area a force is applied to, the greater the pressure. Therefore, FTI unlike PTI is not normalised to the area of force application and as such it might be difficult to relate this variable to plantar tissue damage without also accounting for the area the force is

applied to, as demonstrated by Melai et al. (157). As PPP and PTI account for contact area, these variables demonstrated more relevance as DFU proxy measures in the current study.

Region wise analysis of PPP, PTI, PPav indicated that the variations (elevations) in DPN patients in total foot pressures were significantly contributed by forefoot variations (elevations) and not through mid and rear-foot regions. The current study found most between group differences at the forefoot region, and this is where the majority of foot deformities (173,174) are present. Lower limb tissue properties and their morphological changes in people with DPN (175–178) may also be causing stiffness in the Achilles tendon during walking (179) (180), which could be resulting in higher pressures in forefoot region of DPNs. Therefore, to investigate reasons for elevations in forefoot pressures in DPN, the current thesis will assess the aspects of lower limb tissue stiffness likely resulting from non-enzymatic glycation of collagen in diabetes (181) and movement parameters e.g. range of motion, gait adjustments in DPNs.

9.7.3 At risk of ulceration demonstration

Socio-Demographic parameters showed significant variations between controls and DPN groups. Anthropometric parameters e.g. body mass and BMI were higher in DPNs, while lifestyle habits and wellbeing scores were lower in the DPN population compared to controls.

Clinical variables including neuropathy status, were assessed during the current study as factors other than plantar pressure associated with the likelihood of ulceration (182). In order to establish a clear comparison between individuals likely to ulcerate, the controls and DPN groups were compared for these factors. Age and diabetes duration was higher in DPNs and risk of and it has been established that age and diabetes tenure escalates risk for DFU and limb loss (183,184).

A high mean (modified) Neuropathic Disability Score (8/10) in the DPN population under study was also a significant risk factor for diabetic foot ulceration (185). As previously reported, both high foot pressures (≥6 kg/cm2) (126) and diabetic neuropathy are independently associated with onset of diabetic foot risks, with the latter having the greater magnitude of effect. (186). Another neuropathy test, the VPT also revealed a very high average threshold of 27 Volts for the DPN group, indicating severe neuropathy. A high

VPT has been reported to be an independent predictor of foot ulceration (187,188).It has been observed that internal and external traumas including foot deformities, ingrowth of nail, hyperkeratosis, foreign bodies, acute foot trauma; are not detected as foot is insensate and consistent loading at same sites can heighten DFU risk (189). A study of two groups of diabetic individuals having normal and elevated plantar pressures was conducted by Kästenbauer et. al (83), which revealed that the high plantar pressure group (at the forefoot) showed a significant positive correlation with body mass and VPT.

The LOPS variables of monofilament testing and IpTT showed marked differences ($p<.001$) between controls and DPNs. In the current study researcher performed a statistical analysis, in which the IpTT test results showed a sensitivity of 100% and specificity of 93.3%, when monofilament test results were used as outcome variable. This amply justifies usage of IpTT test as a simpler tool, low-learning curve test for screening of sensory losses, which is highly predictive of risk of consequential ulceration (190).

Wellbeing and lifestyle were assessed in both study groups and a lower score for both parameters were found in the DPN group as compared to controls. People with low daily activity have alteration in foot morphology and material properties making them susceptible to plantar soft tissue disruptions (191); in line with tissue stress theory (192) to the patho-mechanics of formation of DFU. As per this theory a sudden change in activity level of individuals with DPN can render their foot unable to tolerate the increased stress (pressure) and a DFU forms. This is also supported by a recent 8-year prospective study showing that sedentary time was the strongest predictor of DFU in patients with DPN(193). A prospective study by Armstrong et al. (194) provided further perception into the link between physical activity and DFU formation in individuals with DPN.

Therefore, all major clinical variables including Duration of diabetes, Neuropathy disability score, LOPS and VPT which have been considered in the current study demonstrate that the DPN population represent a higher risk of foot ulceration compared to the control group, providing a suitable comparison for identifying differences in pressures relevant to ulcer risk.

9.7.4 Correlations and comparison of pressure parameters

9.7.4.1 Pressure variables correlation with demographic and clinical factors

Diabetes duration and pressures

Within total foot and regional PPP parameters the study found some correlations with assessed demographic and clinical variables. Both total foot and forefoot PPP correlated with diabetes duration (Rho=0.451 and rho=0.382 respectively, $p<0.05$). This is in line with other similar studies (195,196) that have found as duration of diabetes increased, peak plantar pressure increased significantly under the forefoot area (specially 2nd - 4th MPJ). Similarly to PPP, total foot PTI and first toe PTI correlated with diabetes duration (Rho=0.461 & 0.375 respectively, $p<0.05$), which is in line with similar studies (197). In comparison, FTI showed an inverse correlation with diabetes duration, which was in opposition to the results of PTI and PPP, with duration. The FTI at total foot and 2nd metatarsal, showed a significant (<.001) and first toe and forefoot showed significant ($p<.05$) inverse correlations with diabetes duration (Rho= -.529, -.532, -0.381 and -0.369) respectively.

As already discussed, the current study found that FTI was lower in people with DPN than controls; given no mechanical link between lower force values and increased likelihood of ulceration it is likely that the reduction in FTI is related to an adaptation in foot placement strategy. However, these correlations do demonstrate that PPP and PTI may be better indicators of ulceration risk compared to FTI. Total foot PPav showed a significant correlation (Rho=.472) with diabetes duration, but no correlation of diabetes duration was observed with forefoot or rear-foot.

Neuropathy and Pressures correlation

Similar to the associations with duration of diabetes, the current study showed that the PPP across the whole foot, forefoot and 1st metatarsal was moderately correlated with VPT (Rho= 0.395, 0.385, 0.390) and modified NDS (Rho=0.370, 0.378, 0.367). Similar studies (198–201) have previously demonstrated similar associations; which suggest that individuals with DPN and DFUs have elevated PPP, and their risk of ulceration was highly associated with the severity of neuropathy (assessed via VPT and mNDS), and high PPP variables especially the forefoot peak plantar pressure. Most previous studies have studied PPP as a general parameter for associations with small fibre neuropathy, and this study has attempted to visualize specific plantar regions also to define regional PPP relationships with

neuropathy severity. Current study has not found correlation of rear-foot PPP with studied demographic and clinical parameters, supporting the hypothesis that it is predominantly forefoot pressures that are elevated in people with DPN.

In contrast to PPP, there were no correlations between PTI parameters with neuropathy severity indicators. Although, no specific reports are found in the literature significantly correlating VPT/NDS with PTI, but indications are available in studies showing some positive correlations between PTI and VPT (202). Peak Pressure of Stance Average (PPav) showed a significant correlation of Total foot only with VPT (Rho=0.370), with no correlations between the regional PPAv variables, again in contrast to PPP. The FTI at total foot, 2nd metatarsal and forefoot depicted a highly significant inverse correlation of Rho= -0.636, -0.565 & -0.411 respectively, with VPT. The inverse correlation signifies that lower FTI at forefoot is associated with increased severity of neuropathy. Similarly, on the same plantar regions as for VPT, modified NDS also showed highly significant inverse correlations. As with diabetes duration, this inverse correlation between FTI and peripheral neuropathy, given no mechanical link between lower force values and increased likelihood of ulceration, it is plausible that those with DPN may be walking more cautiously (203) and have altered foot loading time and the consequent reduction in force time integral is related to such adaptation in gait strategy.

The NDS and VPT (reflecting diabetic neuropathy severity) are key risk factors of the diabetic foot (204), which can directly lead to ulceration (141,205). PPP at total foot, forefoot regions showed good correlations with these important predictors of foot ulceration, therefore demonstrating a reason to believe that PPP may be a good predictor, although rear-foot PPP did not correlate, therefore it still needs to be examined further, but it is likely mechanical loading strategy is affecting the forefoot predominantly(200). In comparison to PPP, whilst PTI correlated with the duration of diabetes, the lack of correlation with sensory loss may render it is a less useful predictor of DFU. Similarly, the inverse associations seen with FTI again, whilst of potential further interest regarding loading strategy alterations, it is unlikely to show a route to DFU, limiting the potential of FTI as an indicator of DFU risk.

Body mass and pressures correlation

The PPP and body mass did not show any correlation in this study in agreement with previous findings from Cavanagh et a. (206) who reported plantar pressure and body mass were not correlated. Though studies have found body mass/BMI as relevant in prediction

of diabetic foot ulcers (207,208) but conflicting views have been found on correlation between increased foot pressures in diabetic at risk population and Body mass in relevant studies, where few studies found some correlations with plantar pressures (209) and some calling body mass as poor predictor of plantar pressures (210). . It also depends upon the structure and deformity of the foot. The PTI at 2nd metatarsal and forefoot had significant ($p<0.05$) inverse correlation of rho=-0.405 and rho=-0.370 with body mass. FTI at total foot and forefoot showed a significant inverse correlation of -0.680 and -0.558 with body mass. We would theorise that this could be possibly due to changes in fatty tissue prevalence, with higher body mass resulting in greater soft tissue to distribute the forces over, therefore decreasing pressure.

In the current study lack of associations between foot pressures and body mass has been observed, although body mass has been found to be relevant to ulceration by some earlier studies, but given the poor correlation with pressure variables, and studies that report there is no association with ulceration, it provides a weaker influence on development of ulcers than duration of disease and loss of sensation.

9.7.5 Suitable foot pressure variable

The current study showed a similar pattern of results for PPP, PTI and PPav, which showed higher values in DPN as compared to Controls and also changes were mainly observed in forefoot region and they showed good correlations with key known DFU-risk variables. The FTI showed inverse correlations with some clinical variables as mentioned above, but its relevance in foot loading strategy do not lead in the direction of increased DFU risk, as it is unclear how lower force application would increase DFU risk, thus making FTI a less likely a candidate to surrogate for DFU risk indications. PPP and PTI are suitable, but PTI accounts for time and also the current study's findings found better correlations with PPP.

A large number of studies have found suitability of PPP measurement in the prediction of diabetic foot ulceration (76,211–215), whereas there are other studies which have emphasised on PTI as a better predictor of diabetic foot (212,216–218) as it accounts for both pressure magnitudes, but also loading time. Our findings indicate that whilst PPP and PTI show similar patterns of correlations with relevant clinical variables, the study outcomes indicate that PPP might be considered the stronger predictor due to the higher number of associations with clinical risk variables.

Studies have also suggested that it may be counterproductive to report PTI in addition to PPP (219–221). Past work has shown PPP to be the clinically more relevant parameter (224,225)

Longer diabetes and neuropathy duration may be leading to crosslinking of collagen (226) tissues via the Maillard reaction (227), thus pulling on the metatarsal heads and stiffening/thickening of the plantar fascia and Achilles tendon together with neuropathy may further increase pressure in the forefoot (228).

Although the PPav variable provided some additional information on rear-foot variations, the current study is concentrating on forefoot variations, and thus PPav can be subservient to PPP for our decision making in selecting suitable variable representing the objectives of this study. The FTI variable can only be considered useful when accounting for contact area, otherwise its inability in identifying differences between study groups and the inverse correlations with some clinical parameters, which may be due to gait adaptations in DPNs, do not lead our investigation in support of this variable as an important proxy for DFU risk.

9.8 LIMITATIONS

The high age variation amongst the compared groups was a limiting factor and wherever applicable, this study has verified significance of variance between the DPNs and Controls by using ANCOVA for studying effect of age as a covariate. This indicated changes in significance levels in certain variables but overall, the p-value (even after taking age as a covariate) remained consistent in key parameters in the study.

This study had selected men as study participants, since studies have shown that foot ulceration is more common in men with diabetes than in women (229,230) and the mechanisms are likely similar across both genders.

There is also a debate between the efficacies of assessing shod or in-shoe pressures. Caselli et al. (231) found that barefoot peak pressure ratios are associated with a high risk of foot ulceration, compounding this, it is self-evident that barefoot walking is unaffected by the mechanics of the shoe, whereas shoe mechanics and the fit of footwear will vary from shoe-to-shoe, thereby impacting the pressures measured (232). However, in-shoe techniques monitor interface between the foot and shoe, which are genuine representation of how

forces are experienced during the foot during most of the day. Besides, it is a fact that Patients with DPN are recommended to always walk shod. In-shoe assessments provide detailed and vigorous assessments for statistical comparisons (233). The higher pressure values shown in the DPN group in the current study agree with similar in-shoe pressure studies (234–236).

The final limitation is that these indicators or proxies of foot pressure cannot guarantee the development of DFU. However, without a large longitudinal follow-up consisting of pre- and post-pathology evaluation, the approach as adopted in this current cross-sectional study would provide the suitable 'current' indicators of ulceration. Most studies investigate at risk groups, as was the case in our study, rather than investigating a large cohort and following them over long time to see, if they ulcerate. This is a limitation of current study, as the real assessment of predicted progression and temporal comparisons in a longitudinal study would be an optimum method to determine the outcomes from baseline and test the validity of hypothesis.

9.9 CONCLUSION

This study, aimed at comparing plantar pressure measure, recruited diabetic cohort (DPN, n=15) based on moderate to severe neuropathy (besides other eligibility criteria) and gender matched healthy controls (Ctrls n=13). The investigations assessed pressure variables included Peak plantar pressure (PPP), pressure time integral (PTI), force-time integral (FTI) and peak pressure stance average (PPAv) for within study group comparisons and correlations with markers of diabetes comorbidities. The diabetic participants were at different stages of the natural history of pathology effected by duration of diabetes, neuropathy severity levels, behavioural and lifestyle parameters. These socio-clinical parameters have established links to severity of diabetic comorbidity and thus increased risks of diabetic foot.

The Diabetic cohort with peripheral neuropathy when compared with controls, showed diminished sensorimotor functions in foot & ankle region with neuropathy values of NDS and VPT appx. 90% higher in diabetes, besides 35% reduction in their lifestyle activities; in conformity with previous studies on socio-clinical differences in DPN vs Control. Potentiality of socio-clinical pathological impairments resulting in musculoskeletal limitations causing altered plantar pressures, were also explored, whose outcomes in

agreement with previous researches, confirmed that diabetic population have altered plantar pressure distribution.

In between group comparisons of overall foot pressure showed an elevated PPP (896 vs 518 kPa, p<.05), PTI (88 v s61 kPa.s/Cm^2, p<.05) and PPAv (206 vs 139 kPa, p<.05) and a lower value of FTI (53 vs 62 N.s/cm2, p<.001) in DPNs when compared to Ctrls. Significantly higher PPP and PTI in DPNs at Hallux, 1^{st} & 2^{nd} Toe (p<.05), 1^{st} & 2^{nd} Metatarsal (p<.001) and overall forefoot region (p<.05) were observed, while Significantly lower FTI value (p<.05) was observed in metatarsal regions of DPNs. PPAv in DPNs showed significantly higher values in midfoot (p<.05) and Heel region (p<.05). Significant correlation of PPP at total foot, 2^{nd} Toe, 1^{st} metatarsal and forefoot were observed with diabetes duration and neuropathy indicators (Vibration perception and neuropathy disability score). PTI at total and 1^{st} Toe showed moderate correlation with diabetes duration. FTI, showed inverse correlations with diabetes pathology indicators. PPav, except at total foot, showed fewer and insignificant correlations with diabetes indicators. Though the PPav variable provided some additional information on mid and rear-foot variations, the current study is concentrating on forefoot variations. The inverse correlations of FTI with some clinical variables may have some different consequences, but it relevance in foot loading strategy do not lead in the direction of increased diabetes foot risk, as it is unclear how lower force application would increase DFU risk, thus making FTI a less likely a candidate.

Thus, diabetic cohort demonstrated that Peak plantar pressure and pressure-time integrals significantly correlated with established markers of diabetic foot ulceration including duration of diabetes and severity of peripheral neuropathy. Thus, Considering the differences from controls and associations with demographic and clinical factors associated with at risk feet, PPP (representing magnitudes of plantar pressure) and PTI (representing the duration and magnitude of plantar pressure) are best correlated with physiological indicators of diabetic foot ulcer risk. In this cohort PPP and PTI have been identified as the best pressure indicators of ulceration risk within this cohort.

10 EXPERIMENTAL CHAPTER 2: THE ROLE OF ACHILLES TENDON STIFFNESS IN FOREFOOT PRESSURE DEVELOPMENT

10.1 ABSTRACT

People with diabetes are known to have high collagen cross-linking and Advanced Glycated Endproducts (AGEs) accumulation leading to stiffer tissues. This includes increased Achilles tendon stiffness. This change in tissue mechanical properties is likely to exert an impact upon biomechanical behaviour during movements. One key suggestion is that stiffer ankle joint complexes may alter the loading of the foot and therefore the pressures experienced under the foot. The current study aims to investigate changes in Achilles tendon stiffness in patients with DPN and their link to elevated plantar pressure loading during gait.

Ankle tendon stiffness properties were assessed for n=15 people with diabetic peripheral neuropathy (DPN) and n=13 healthy controls (Ctrl). Achilles tendon forces were calculated from dynamometry of the ankle for a plantarflexion contraction with simultaneous measurement of Achilles tendon tissue elongation using ultrasound imaging. Tendon length, Moment arm and Cross-sectional area of the tendon were measured using Magnetic Resonance Imaging (MRI). Slopes of individual force-elongation curves provided calculations of Stiffness and accordingly young's modulus was also calculated. Voluntary and Assisted Range of Motion were also measured at the ankle joint. Achilles tendon stiffness in people with peripheral neuropathy was significantly higher than controls (DPN 80 Nmm^{-1} vs Ctrl 53Nmm^{-1}). Achilles tendon stiffness was moderately correlated with forefoot peak plantar pressure (rho=0.387). Patients with DPN were seen to have higher tendon stiffness compared to controls and thus reduced ankle-foot dorsiflexion; additionally this was linked to higher forefoot plantar pressures, thereby indicating the increased stiffness of tissues in people with diabetes as a potential risk factor for foot ulceration.

10.2 INTRODUCTION

Achilles tendon or "tendo magnus" exhibits mechanical properties that are subject to change and can influence the functional performance of the surrounding joints. Limitations in dorsiflexion range of motion (237) and increased ankle (238) and tendon stiffness (239) have been reported in individuals with diabetes . Achilles tendon function in humans can influence foot function and is particularly relevant in people with diabetes for the development of diabetic foot ulcers.

Collagen provides tensile strength to tendon, constituting <70% weight of tendon. Adaptations to the Achilles tendon's dimensions material properties and glycation of collagen can result in tendon stiffness changes, altering joint movements and gait patterns in case of people with diabetes, thus a potential contributory factor to diabetic foot ulceration.

It has been shown from animal models of diabetes (240–243) and in a small number of recent human studies (244–249) that diabetes increases the stiffness of tendon likely through non-enzymatic glycation. Soft tissue biomechanical veracity is modified through glycation, which results in stiffness elevation, as evidenced by animal models (250–252).

Role of Advanced Glycated Endproducts

Tendon Stiffening results from alterations to the properties of elastin and collagen fibres as a result of non-enzymatic glycosylation and excessive advanced glycation end-products (AGEs) deposition. Ahmed (253) in his extensive research on pathophysiological mechanism of AGEs, has implicated these proteins/lipids, which gets glycated when exposed to sugars and can stimulate development of diabetic complications. Basically, collagen cross-links are generated through two different pathways. One which is beneficial, is the enzymatically driven hydroxylysine-derived aldehyde pathway, while the other is the non-enzymatic glycation or oxidation-induced AGE cross-link (254), which is assumed to be causing decline of the functionalities (biological, mechanical) in tendons and associated modalities (255). AGEs abate only when its linking protein is degraded, thus AGEs accumulation is substantial in lower turnover tissues e.g. bones and tendon (256).

In prolonged diabetes collagen (type I) impairs in flexibility and increased acid insolubility, this in-turn correlates with the accumulation of non-enzymatic AGEs crosslinks (257). Elderly people with diabetes may have higher impact of AGE crosslinking (258) (259) as they have slow but from a longer duration build-up of AGEs crosslinks through long-lived

proteins; this in conjunction with high levels of glucose due to diabetes, causes protein glycation.

A critical review on the merits and limitations of major techniques used for the measurement of AGEs has been undertaken by Ashraf, Ahmed et al. (260). The Immunohistochemistry technique has the advantage that tissue localization of AGEs can be determined, and its colocalization with RAGEs can also be determined (261) but it lacks sensitivity and reproducibility (262). The ELISA (Enzyme-linked Immunoassay) technique is currently most frequently used and is rapid (263), but specificity of antibodies is often difficult to characterize and due to steric constraints, all epitopes are not accessible to the antibodies (264). The high-performance liquid chromatography (HPLC) technique provides precise quantification of AGEs (265) but has cumbersome chromatographic systems and long retention time (266). LC/MS (liquid chromatography-tandem mass spectrometry) is considered by far the most accurate technique (267) but carries the disadvantage of being a highly expensive method (260). Ultra-Violet visible spectroscopy is a quick preliminary tool for initial monitoring of glycation reaction (268) but at the same time it is not appropriate for quantitative estimation of glycation products(269). Boronate affinity chromatography is a simple and efficient technique for AGEs measurements (270) but it has the disadvantage of nonspecific interactions between boronate and non-glycosylated proteins (271). Fluorescent phenylboronate gel electrophoresis is yet again a simple, cost-effective detection and analysis tool for glycated proteins and provides direct visualization of glycated proteins (272), but it is only suitable for the analysis of samples with limited complexity (273). Fluorescence spectroscopy, as discussed further below, is most commonly used methods for the measurement of AGEs, but it has the limitation of being unable to determine the Nonfluorescent AGEs (274).

Effectiveness of skin autofluorescence reader in measurement of AGEs

As discussed, Advanced glycation end products (AGEs) are a group of heterogeneous molecules formed by non-enzymatic reactions and are reactive metabolites under physiological and pathological conditions(275), so AGEs can be divided into fluorescent and non-fluorescent forms, as well as cross and non-cross-linked types, where the underlying mechanisms of AGE formation include the Maillard reaction, the polyol and glycolysis pathways (276). With this as background, fluorescence spectroscopy seems straightforward and a regularly adopted method for the measurement of AGEs.

Fluorescent AGEs show a distinctive fluorescent spectrum at 440 nm subsequent to excitation at 370 nm (277). This property of AGEs has been used commonly to investigate their accumulation at a tissue level (278). Fluorescent AGEs can be detected from blood /urine samples or from tissues, but researchers believe that measurement of AGEs from blood and urine samples may not reflect the actual tissue level, as the accumulation of AGEs in the body is dependent on the half-life of the glycated protein, whilst AGEs are known to accumulate for almost a lifetime in long-lived proteins (e.g. skin collagen and cartilage proteins), which coincides with sites of pathological manifestations of diabetes, which makes assays of tissues as medium of choice for detecting and measuring AGEs rather than plasma samples (279).

Miniaturization in instrumentation has facilitated development of handheld autofluorescence readers or fluorimeters e.g. AGE reader (DiagnOptics, Groningen, The Netherlands), TruAge™ Scanner (Morinda, Long Island City, NY) and other devices like Optical AGE Sensor (Sharp, Tokyo, Japan), which measures glycation of blood vessels. These fluorimeters illuminate 1 cm^2 area of skin with a wavelength band of about 300–420 nm and the emitted light from the illuminated skin is observed over a wavelength range from 300 to 600 nm. Autofluorescence is measured by dividing the average emitted light intensity per nm over 420–600 nm range by the average emitted light intensity per nm over the 300–420 nm excitation range. Over specific time intervals skin autofluorescence is measured at identified body sites. Research studies have found higher skin autofluorescence in people with diabetes in comparison to age matched controls (280,281). Although these skin autofluorescence readers offer authenticity, ease of handling and a bedside options of usage, but the analytical limitations should be considered alongside which includes that these AGE readers can't detect non-fluorescent AGEs, besides interferences from fluorophore and oxidation adducts like N-formylkynurenine (NFK) alters specificity of detected fluorescent AGEs. Another issue observed with the AGE reader is that as several fluorophores contribute to the global detection of fluorescence, thus an accurate quantitative calibration is a challenge to achieve in AGE readers (260).

Tendon mechanical properties, adaptations and influence on functional performance

Foot is a complicated structure with internal geometries for accommodation of soft/hard tissues, mechanical onslaughts and varied reaction by tissues as response to loading (282). Researchers have been intrigued with intra-foot and inter foot-ankle complex mechanisms leading to altered plantar loading; whilst Achilles tendon stiffness as a potential factor

increasing the mechanical loading underneath the plantar surface. A large population (30%) among elderly diabetics have complains related to foot region. These if not timely addressed could result in imbalances and posture instability (283) gait performance (284)foot pathology/deformity and ulceration (285) in the diabetes population.

The previous experimental chapter discussed the role of elevated foot pressure as a proxy for foot ulcer risk. Now the current study investigated further changes in soft tissue mechanical properties, as a potential indicator of diabetic foot ulcer risk (286). The pathophysiology pathway to DFU describes glycation as a major contributor (287). The clinically well know pathway to collagen cross-links is through sustained hyperglycaemia ensuing glycation of proteins, damages mechanical strength of collagens (288).

Gait processes leading to high pressures with increased Achilles tendon stiffness

Limitations in ankle range of motion and increases to Achilles tendon stiffness may have important implications for elevated plantar loading and ulcer formation (289). The excessive loads on plantar surface during walking, could lead to a sizeable population (20%) among diabetics at DFU risk (290). Research has indicated that joint stiffness in conjunction with impaired range of motions in foot ankle region, could a factor in elevated loading at plantar sites. (291,292).

In DPNs loss of motor nerve function is usually associated with toe deformity, bringing changes in foot structure and gait strategies (293), which elevates mechanical stress in form of compression and shear forces. Examining ankle and foot joint range of motion will enable a clearer impression of the effects of DPN on forefoot pressures and diabetic foot ulcer risk. A regular gait has foot rollover, whereas reduced ankle and MTPJ dorsiflexion constraints this mechanism (294), which may lead to higher plantar pressures (295) and a higher risk of ulceration (296). People with diabetic neuropathy and ulceration were found to have a higher incidence of limited ankle dorsiflexion compared to non-diabetic people and diabetic patients without neuropathy.

Studies have found substantial effects of diabetes-induced alterations including: increased Achilles tendon stiffness (131), stiffer plantar fascia properties and more prominent first metatarsophalangeal joints (anatomical & functional); with implications upon foot loading and gait strategy (297). The metatarsophalangeal joint is imperative for standard ambulation and any restrictions in the range of motion at this joint has an impact upon the toe-off phase of gait (298). For normal functioning, the first metatarsophalangeal joint should have a minimum of 35 to 40 degrees of dorsiflexion, although the expected range

would usually be 70-90 degrees and normal plantarflexion range is around 45 degrees (299). Quantitative characterization studies of gait kinematics in people with DPN and other studies on people with gait impairments have reported a range of MTPJ dorsiflexion varying from 14 to 40 degree with an average of 23 to 30 degrees (300–302).

The research aims to understand the relation between tendon stiffness, foot and ankle joint range of motion and plantar pressure loading during ambulation in patients with diabetic neuropathy.

Increased tendon stiffness causes increased forefoot DFU risk

During the late stance phase of the gait cycle patients with DPN are hypothesised to dwell on the forefoot area, unless they chose to flex at the MTP joint, in which case higher pressure will remain across the MTP joints and toes. All these deliberated factors lead us to believe that increased Achilles tendon stiffness may cause reduced ankle dorsiflexion and possibly earlier heel lift which increases pressure and time on forefoot regions. The current research aims to investigate the role of increased/altered Achilles tendon stiffness and limited ankle-foot joint dorsiflexion, which in turn may be associated with elevated plantar pressures and when combined with sensory loss leads to an increase in the incidence of plantar ulcers (100).

10.3 AIM

Aims The current study aims to investigate changes in Achilles tendon stiffness in patients with DPN and their link to elevated forefoot plantar pressure loading during gait.

Hypothesis:

Patients with DPN will have higher Achilles tendon stiffness compared to controls and reduced ankle and foot joint dorsiflexion; this will be linked with higher forefoot plantar pressures, thereby increasing the risk of diabetic foot ulceration in this foot region.

Specific objectives:

- To assess Achilles tendon stiffness and ankle-foot dorsiflexion between people with diabetes and Controls
- Evaluate factors related to diabetes and Peripheral neuropathy which impact tendon stiffness
- Investigate tendon stiffness impact on plantar pressures.

10.4 METHODS

This research study considered variables related to Achilles tendon morphology and properties, mainly to assess Achilles tendon stiffness. The primary Variables included neuropathy status, muscle physiology, ankle joint anatomy, muscle elongation, tendon cross-sectional area etc., which were ascertained through physical examinations, ultrasonography, magnetic resonance imaging and electromyography, in order to measure morphologic and mechanical features of the tendon.

10.4.1 Ethical Consideration and Informed Consent

The process for NHS ethical submission (IRAS) and approval from ethics bodies (REC and HRA) along with research passport and R&D permissions from participating hospitals (MFT and LTHTR) has been detailed in Chapter 1. MMU ethics (ETHOS) clearance details and participant Informed consent have also been elucidated in aforementioned reference.

10.4.2 Study Population:

The sample size, eligibility criteria (inclusion and exclusion) and process for recruitment of 28 participants in total across the DPN and Control groups have been detailed in Chapter 1. This study was cross-sectional, observational in design conducted among adult participants matched to eligibility criteria of groups.

10.4.3 Testing material, acquisition of mech. properties of Achilles tendon

The key outcome variables of Achilles tendon mechanical properties were to be calculated from a range of measurements including ultrasound and magnetic resonance imaging, anatomical measurements and dynamometry.

Simultaneous measurements of Ultrasound, Dynamometer and an EMG were conducted and synchronised using an external trigger. Raw signals of torque, EMG activity and joint angle were sampled at 1 kHz using a Powerlab acquisition system (AD instruments, New Zealand) and digitally acquired using the Lab chart (Figure 11), AD instruments, New Zealand) data acquisition system. Dynamometer, EMG and Ultrasound were synchronised using an external time-sync switch, which works by sending a pulse to the Powerlab

(recording torque & EMG) and ultrasound (recording muscle fibre lengthening), allowing for retrospective time synchronisation during post-processing.

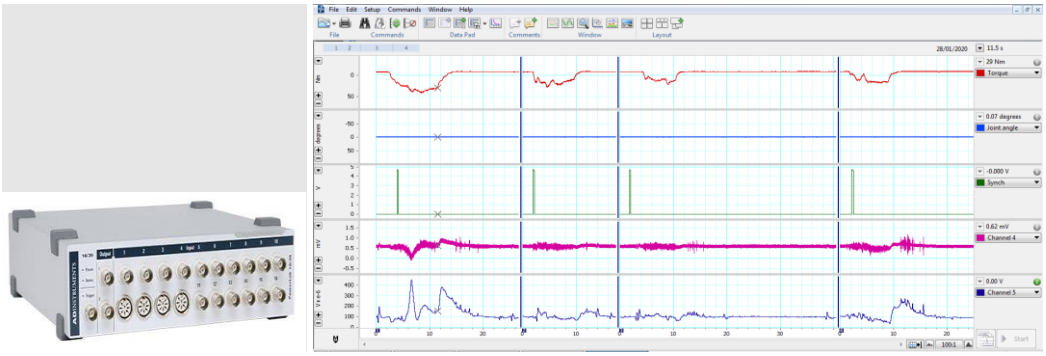


Figure 11- Powerlab device for data acquisition. Labchart synchronized data.

Data collected from varied platforms e.g. Dynamometer (Torque, joint angle), ultrasound and EMG. Lab chart software for acquired data processing and analysis. Source: https://www.adinstruments.com/products/ ADI instruments website. The diagram is a screen shot of current study at MMU Biomechanics research lab. T0.18, Year 2019

10.4.4 Measurement of ankle joint torque

Left foot was considered as a standardised foot for all measurements. To measure ankle joint torque, an isokinetic dynamometer (Cybex NORM; Cybex International, New York, NY) was used. Participants lay prone on bed of dynamometer with foot being fixed into the footplate at a neutral ankle position [i90 deg between foot plate and tibia] and the knee in full extension. The rotational axis of the dynamometer was carefully aligned with the ankle's axis of rotation at rest, using a laser beam aligner for visual guidance. The ankle was flexed at 90 deg to the tibia manually by the investigator, in order to avoid hind-foot flexion/extension as well as inversion/eversion movements. Straps were used around the ankle to prevent extraneous movements during maximal plantarflexions. The following tests were then conducted with a practice before each test to allow participants to become familiarized with the procedures involved.

Maximal voluntary contraction: Participants first performed isometric maximal voluntary contraction (MVC) of the plantar flexors on the dynamometer. To ascertain MVC and as part of a standardised warm-up procedure for conditioning the Achilles tendon, three isometric MVCs were performed (an additional contraction was performed if the MVC torque values were not within 5% of each other).

Figure 12: (1) Dynamometer (2) isometric contraction in prone position

Source:http://www.csmisolutions.com/sites/default/files/humac_norm_brochure_0.pdf. Year 2019.

Ramp Isometric Contraction Protocol: Participants performed a ramped isometric plantarflexion contraction (Figure 12) with gradually increasing torque to a level of 100% of their maximum voluntary contraction (MVC) (assisted via visual feedback on a screen) over 5s and maintained for 2-3s upon reaching MVC.

10.4.5 Co-activation torque measurement

During the development of ankle plantarflexion joint torque measurements, the antagonist co-activation torque of the dorsiflexor muscles were measured. This is because the co-activation torque of the dorsiflexor muscles will oppose the plantarflexion torque and lead to an underestimate of the actual force acting on the Achilles tendon. Thus, to account for reductions in the net plantarflexion torque measured by the dynamometer, the co-activation torque generated by the dorsiflexors, was estimated using measurements of dorsiflexion torque and EMG. For EMG measures, the anterior shank was cleaned over the mid belly of the tibialis anterior muscle with alcohol to reduce skin impedance.

Participants used visual feedback (torque trace was displayed in real time) while performing a maximum dorsiflexor contraction. This assisted in developing torque values corresponding to specific percentages of the maximum dorsiflexion contraction at 20%, 40% and 60% of dorsiflexor MVC and maintain that threshold for 3-5 seconds. As it is known that tibialis anterior is a representative muscle for dorsliflexor co-activation, so during MVCs of plantarflexion, the EMG activity was noted from this region.

10.4.6 Measurement of Achilles tendon elongation

During a ramped isometric plantarflexion MVC of 5 seconds duration, tendon elongation was measured as the proximal displacement of the gastrocnemius myotendinous junction (MTJ) at 10% intervals of joint torque between 0% and 100% Sagittal-plane scanning of the Achilles tendon and myotendinous junction at the medial gastrocnemius was conducted using B-mode ultrasound (My Lab 70, Estate, Italy) while the participant performed a ramped isometric plantarflexion contraction to MVC. Ultrasound scanning was performed using a 100 mm linear-array probe with the sampling frequency maintained at an appropriate level to optimise image quality and ensure adequate availability of ultrasound frames in line with method adopted by Reeves et al (303). The ultrasound probe was secured in position using a custom-made holder to prevent any movement relative to the scanned structure, and an echo-absorptive strip was placed onto the skin casting an echo-absorptive marker on the image to confirm that no movement of the ultrasound probe took place. The longitudinal displacement of the gastrocnemius MTJ was tracked continuously during plantarflexion MVCs.

Videos of the ultrasound scans were processed in VLC media player (Free Software Foundation, Inc., Boston, MA, USA) where they were synchronised with measures of torque and EMG. A timer module (designed in-house) was used along with this software to read timings for image capturing at 10% MVC intervals. The images were then exported to Image J software (National Institutes of Health, Bethesda, MD, USA). Using the measurement function (actual cm length and image cm length synchronized), the displacement of the tendon reference point was quantified by taking images from each point and measuring the distance to reference point (at GM junction)

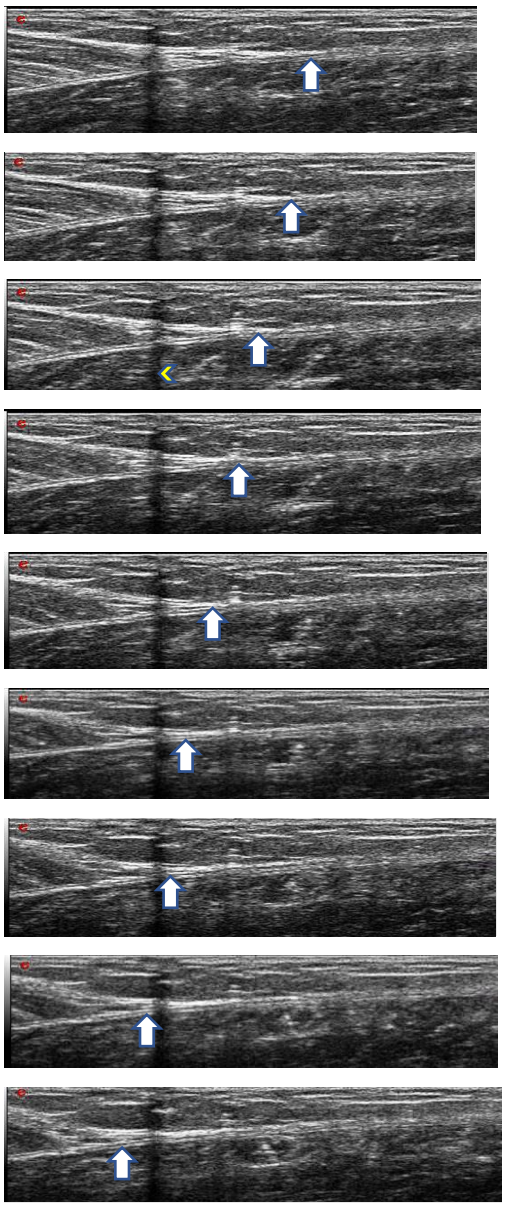


Figure 13: Tendon elongation (US) measurement from Myotendinous Junction.
Representative in-vivo sagittal plane sonograph of the Gastrocnemius Myotendinous Junction (MTJ) during ramped contractions. The white arrow points to a consistently traceable prominent location adjacent to MTJ, which was traced to obtain elongation of Achilles tendon. MTJ monitored in reference to echo-absorptive marker casting a dark line (<) on the ultrasound image through an echo-absorptive strip placed on skin near MTJ. Image from current study participant Ultrasound Scan at Manchester Metropolitan University, Biomechanics Lab (T0.18), February 2019.

10.4.7 Tendon elongation measures correction for heel displacements

In the current study participants were required to perform a ramped maximum voluntary contraction lying prone on dynamometer, where their foot was tightly strapped to the foot plate to control for material deformation and heel displacement (change in distance between malleoli and foot plate) during MVC. Despite best attempts to strap the foot, there is an inevitable ankle rotation and displacement of heel during MVCs. To correct any elongation errors induced by joint rotations during isometric contractions, the calcaneus

was scanned using ultrasound while the ankle joint is passively moved into plantar flexion. A smaller ultrasound linear transducer (Esaote Mylab LA435) was placed on heel, while the participant was lying prone and simultaneously performing a ramped MVC. The heel shift data was collected only from controls, as it was not advisable to bring DPN participant's feet in direct contact with metal plate of the dynamometer without footwear. The heel shift measurement errors will be similar across groups as the heel rise is proportional to the amount of torque (stretching the restraints), so for the regional (absolute) torque measurements the errors will be similar. The heel displacement values were then plotted against tendon force to enable quantification and subsequent correction of tendon elongation errors due to joint rotations and heel shift.

10.4.8 Achilles tendon Moment Arm Measurements

To calculate the tendon force, ankle joint moment arm was measured using MRI (Magnetic Resonance Imaging) where knees were kept fully extended during supine position of study participants. The MRI scanner (0.25 T, G-Scan, Esaote, Italy) acquired sagittal plane scans of participant's left lower leg. The settings were Spin-Echo Fast Fourier sequence. Scanning parameters: 1:59 min scanning time, 18ms echo time, 1020ms repetition time, 1 acquisition, 180×170 mm field of view, 256×256 pixels, 7 mm slice thickness, 1 mm inter-slice gap. MRI compatible wooden wedges were inserted below foot to maintain plantar and dorsiflexion foot position in ± 10 deg of neutral position.

Reuleux method was used for calculation of Moment arm (304). The centre of rotation (CoR) was measured from neutral position scan. The distance from tendon action line to CoR provided MA. First supervisor of study wrote a macro to calculate the MA using above mentioned principal.

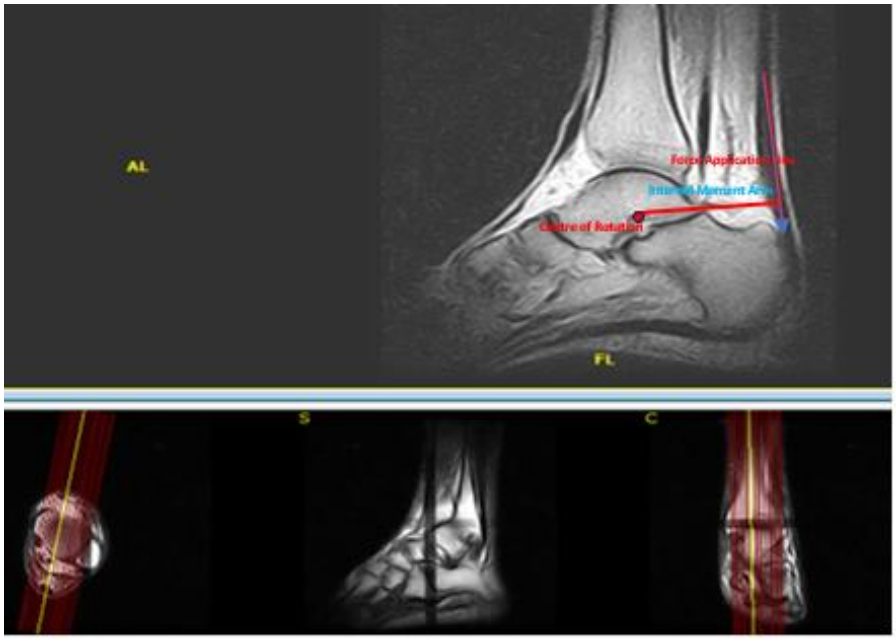


Figure 14 - Internal Moment Arm.

Measured from a sagittal plane MRI scan of the Foot-ankle region. Image shows the Centre of Rotation, Internal moment arm and line of force for Achilles tendon. Image from MRI scan of current study participant at Manchester Metropolitan University, Biomechanics Lab (T0.19), August 2019.

10.4.9 Achilles tendon Length Measurement

The Achilles tendon length was imaged simultaneously using the previously mentioned 0.25-Tesla MRI scanner (G-Scan, Esaote, Italy). The length of the tendon was quantified using sagittal plane MRI scans. T1-weighted sequences was applied with the scanning parameters optimised for image quality whilst minimising scan time. Participants were positioned supine, with the knees fully extended within the MRI scanner and the ankle in the neutral position. The settings were similar to ones used for moment arm calculations.

Using digitising software (VLite, Esaote, Italy) the length of the Achilles tendon was then measured on scan slices (leaving first and last scan slices). Gastrocnemius portion was considered the proximal portion and soleus delineation was considered as end of proximal and till calcaneus insertion it was considered distal.

A small number of participants' tendon length data could not be measured using MRI owing to certain MRI system data extraction issues. Researcher had the advantage of having acquired resting tendon length data of all participants from Ultrasound too. Tendon length measurements from US ultrasound acquired data were avoided not favoured over MRI, as the proximal length of Achilles tendon measurements are sometimes an issue challenge to detect with Ultrasound (305). To acquire Tendon length data from Ultrasound, Principal

supervisor of the research study had developed a novel technique for authentic measurements. Here, two lengths (25 cm each) of medically approved skin adhesive tapes, were kept 5 cm apart (adhesive side facing up) and these lengths were bridged by echo-absorptive flexible markers fixed on them at 3.5 cm intervals; which gave the structure a shape akin to a ladder. This ladder was pasted along complete Achilles tendon length (in excess of myotendinous junction on proximal and beyond calcaneus insertion point at distal). The distance between each step of ladder was designated in such a way that two markers were captured in a single frame captured on US (My Lab 70, Estate, Italy). Participants were lying prone while US scanning was performed using a 100 mm linear-array probe with the sampling frequency maintained at an appropriate level to optimise image quality and ensure adequate availability of ultrasound frames in line with method adopted by Reeves et al (306). Thus, two dark lines (initial and later) were casted on each US image. These US images were ported into Image-J software for measurements of tendon length. The casted dark lines on images acted as guides for seamless continuity of Achilles tendon sections. The later dark line on first image became Initial overlapping dark line for subsequent image. Thus, the complete tendon was imaged in max. 4-5 images. To achieve further assurance, overall Achilles tendon length was also cross-referenced with participant's height multiplied with a constant of 0.00792, calculated from tendon data from Reeves & Cooper (307).

10.4.10Measurement of Cross-sectional Area (CSA) of the Achilles tendon

During the MRI scanning as described above, axial plane scans were acquired starting from the calcaneus and continuing proximally for measurement of the Achilles tendon CSA. Figure 15. T1-weighted sequences was applied with the scanning parameters optimised for image quality whilst minimising scan time (307). The CSA images were exported to Image J software (Image J ver. 1.46r, NIH, USA) for area calculations. Tendon volume was calculated by multiplying individual slice thickness (1 mm gap between slices) with the CSA of the Achilles tendon obtained from that slice. Each slice's volume was summed for the complete Achilles tendon length to obtain total volume of the Achilles tendon.

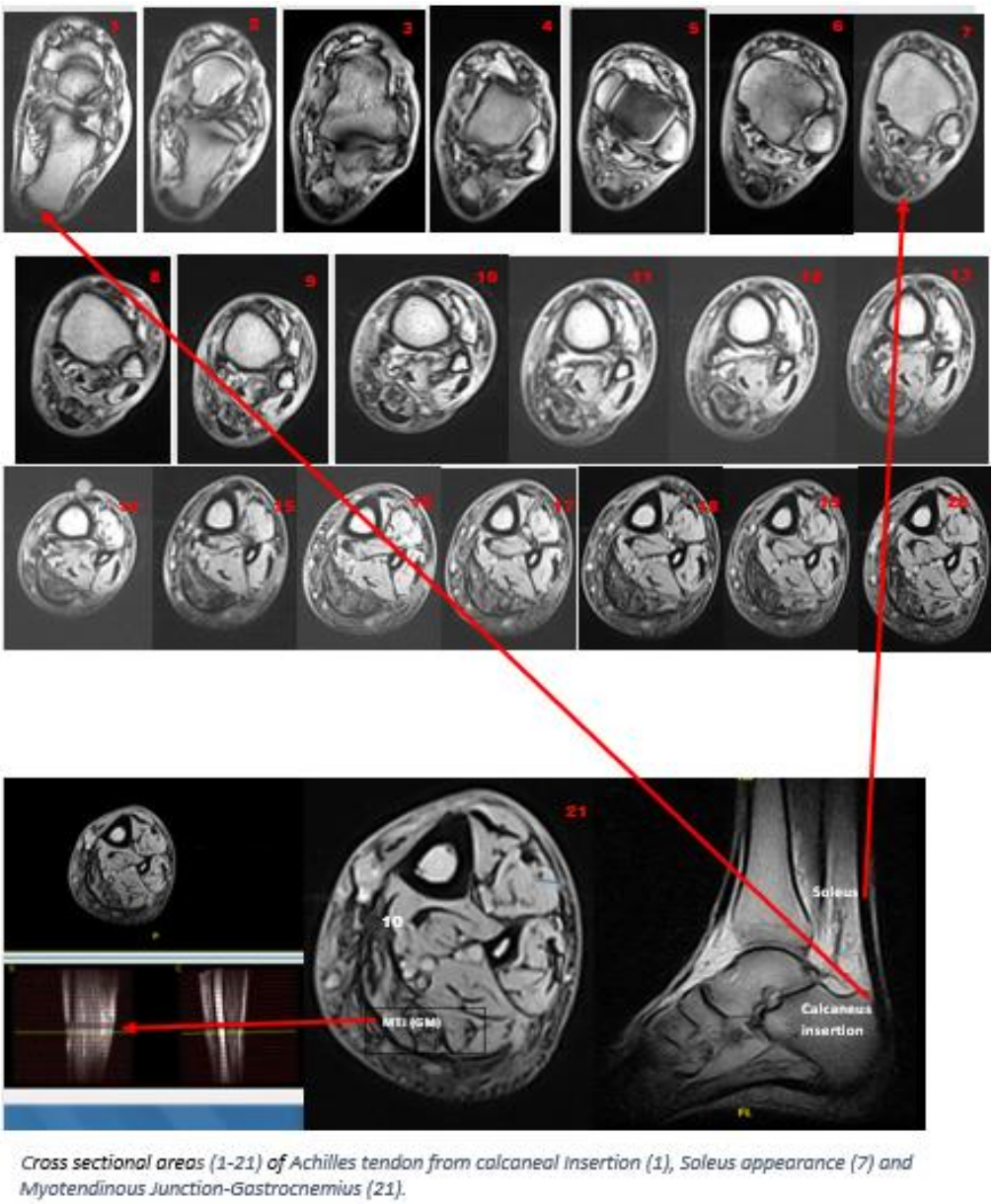


Figure 15 - Achilles tendon cross sectional area measurement from MRI scan.

Magnetic Resonance Images acquired from a study participant in current study at Manchester Metropolitan University, Biomechanics Lab. (T0.18), June 2019.

10.4.11Processing of acquired data on Achilles tendon properties

10.4.11.1 Force–elongation curve

A tendon force-elongation (FE) curve for each participant was generated. The maximal torque value of voluntary contraction was subdivided into equals of 10% incremental values of torque for MVC. These incremental values were divided by the Achilles tendon moment arm values for each individual participant to obtain tendon forces. The elongation

values obtained from ultrasound scans, corresponding to 10% incremental force values were plotted to obtain a force-elongation curve for each participant.

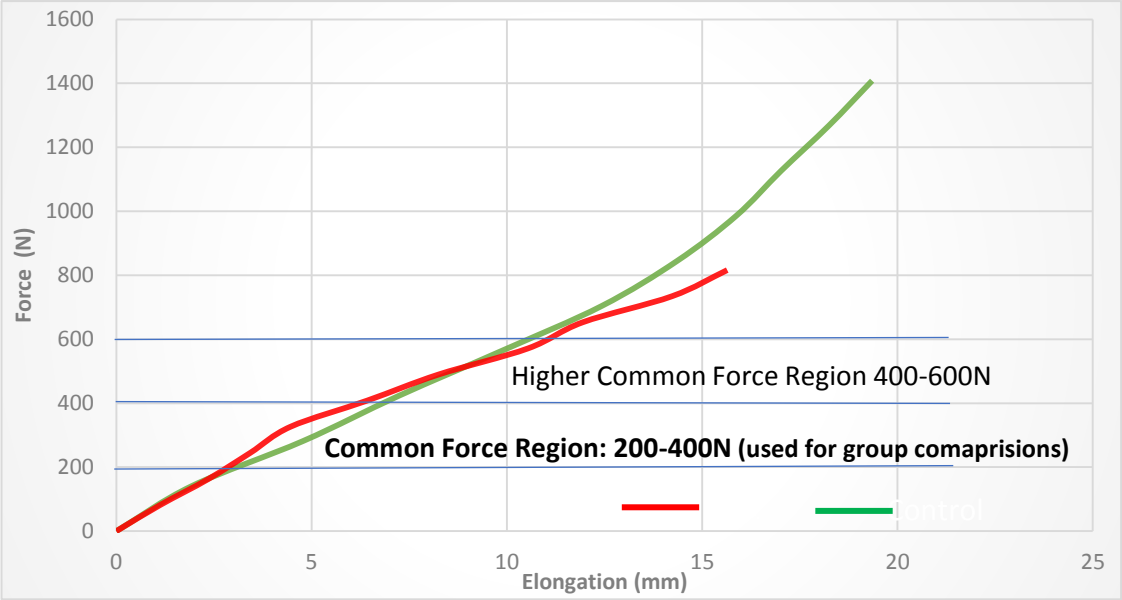


Figure 16: Force-Elongation curve for tendon properties calculation.

A Control and DPN study participant data. The Common Force Areas (CFA) have been used as absolute stiffness study zones. Excel diagram of study participant's data for current study at Manchester Metropolitan University, Biomechanics Lab. (T0.18) May 2019.

10.4.11.2 Heel displacement adjustment for Tendon Elongation

Force-heel displacement curves were generated as described for the tendon force-elongation and was then mapped on the tendon force-elongation curve to correct for these elongation errors.

10.4.11.3 Calculation of Achilles tendon Stiffness

Achilles tendon stiffness was calculated over a common force region (200-400 N; 400-600 N), to enable comparison of stiffness over the same force region and avoid comparison across different parts of the tendon force-elongation curve that would arise with taking a relative force approach. The forces generated by controls during MVC were substantially higher than those generated by DPN patients. Thus, for a valid assessment of tendon properties over the same region of the force-elongation relationship, common force regions were selected, which were representative of forces exerted by both study groups. The highest force of 600N exerted by weakest participant in DPN group was considered the upper limit for absolute force region for comparison of forces between the groups. Although the values of between group comparisons for stiffness ± Std. Dev. are presented for both common force regions, for all detailed comparisons and correlation studies only the stiffness calculated over the 200-400N force region has been considered, as this region

is well within the voluntary force range for all participants and ensured that comparisons did not need to include extrapolated data for any participant.

The tendon force–elongation curve was assumed to be linear over the small force regions where it was measured. This was followed by fitting individual curve with the lowest order polynomial function to yield an r-squared value of 0.95 (typically a second order polynomial). The equation (y =mx + c) generated from this curve and was used to calculate the stiffness values for force and elongation at 200-400 N and 400-600N, where delta values of change in force/change in elongation were used to calculate stiffness within this region for each participant.

10.4.11.4 Calculations for Achilles tendon Forces

10.4.11.5 Torque calculations:

To calculate Achilles tendon force, the first constituent of net torque can be calculated using the equation:

Equation 1

$$Net\ Joint\ Torque = ET + CT$$

Where:

ET- Extensor Torque

CT- Co-contraction Torque (RMS of sEMG-antagonist & agonist muscle activity=EMG during Extensor * Max. Flexor EMG).

10.4.11.6 Co-activation torque calculations

The calculations of the co-activation torque yielded a value of 0.81 ± 1.31 Nm. Some earlier studies using isometric contraction protocols have adjusted the net joint torque based on sEMG (308) while others suggested that this is not required because this seems to be a product of cross-talk, rather than co-contraction (309). In the current study the level of co-activation was felt to be so low that its contribution to influencing the net joint torque was almost negligible for both groups (Ctrls and DPNs) and it was therefore discounted from further analysis.

Renewed equation after discounting CT value.

Equation 2 Total extensor torque equation

$$Net\ Joint\ Torque = ET$$

Achilles tendon force was calculated by dividing the measured plantar flexion joint torque with the Achilles tendon moment arm length.

Equation 3Tendon Force equation

$$Tendon\ forces = \frac{Ankle\ torque}{Achilles\ tendon\ moment\ arm\ length}$$

10.4.11.7 Stress, Strain calculations:

Tendon stress (σ) was calculated by dividing the force exerted by the tendon CSA, while the strain (ε) was obtained through dividing the change in tendon length during isometric contraction by the initial resting length of the Achilles tendon (where Ln is the original tendon section resting length), in the longitudinal direction (equation 3).

Equation 4: Stress and Strain equation

$$Stress\ (\varepsilon) = Force\ (F)/Cross\ Sectional\ Area\ (CSA)$$

$$Achilles\ Tendon\ Force\ (F) = Net\ Joint\ Torque/Moment\ Arm\ (MA)$$

$$Strain\ (\varepsilon) = Change\ in\ Length\ of\ AT\ during\ ramped\text{^}\ MVC\ (\Delta L) - Heel\ Displacement\ during\ MVC/Original\ Length\ of\ AT\ (Ln).$$

^ This value is drawn from multiple different points during a ramped MVC and not only at the max of the MVC

10.4.11.8 Calculation of Young's Modulus

The Young's modulus of the gastrocnemius tendon was estimated by multiplying the stiffness value by the ratio of tendon length to tendon CSA (306,310)

10.4.12Measurement of ankle joint range of motion

The dynamometry values of the ankle joint RoM was obtained by using the isokinetic dynamometer (Cybex Norm, NY, USA). Here the participant was asked to lie in prone position. The standard foot was fixed into the footplate at a neutral ankle position. Participants were encouraged to plantar and dorsiflex as much as they could without any external assistance (voluntary dorsiflexion).

To measure assisted RoM, participants were again asked to plantarflex and dorsiflex to their maximum capacity (after a few minutes rest on completion of the voluntary contraction activity), and the moment they reached their maximum range, the

experimenter gently applied further pressure in the intended direction, until the foot would not dorsiflex any further.

10.4.13Measurement of Metatarsophalangeal range of motion

The range of motion of the first metatarsophalangeal joint was measured with the help of a goniometer as also done in other MTPJ RoM studies (311,312). The range of motion testing at the first MPJ was performed by taking one hand to stabilize the first metatarsal, just proximal to the metatarsal head with the other hand taken to the proximal phalanx of the hallux and by moving the joint into dorsiflexion and plantarflexion. The participant was lying supine in non-weight-bearing position, and the researcher set one arm of goniometer on the first metatarsal bone and the other arm was set on the hallux, while maintaining ankle in neutral position. The experimenter established the test position by stabilizing the 1st metatarsal in plantarflexion and demonstrated to the participant their expected action by dorsiflexing their Metatarsophalangeal joint. Again, goniometer alignment was confirmed before starting the testing by maintaining its axis medial to the centre of the metatarsal head and stationary arm was realigned to metatarsal, while the moving arm was aligned with proximal phalange Fig_-). Participants were encouraged to undertake maximum plantar flexion of the hallux, followed by maximum dorsiflexion. These measurements were undertaken three times and then averaged (313).

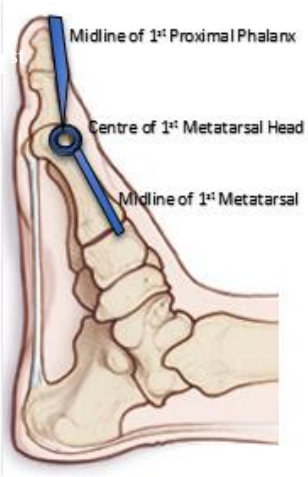


Figure 17:-Goniometer placement on the metatarsal head
its alignment with metatarsal and phalanx for measurement of RoM for MTPJ. Source: Self-designed image for illustration purpose only.

10.4.14Advanced Glycated End-products (AGEs) Level Assessment

AGEs levels were measured non-invasively by placing the forearm of participant on an AGE reader (DiagnOptics B.V., Groningen, The Netherlands) (Figure 18). It makes use of the characteristic fluorescent properties of AGEs when illuminated with UV light, referred to as skin autofluorescence. The AGE Reader shows the result as a number (Skin autofluorescence graded between 0-5). These measurements reflect the glycometabolic memory. Another part of reader output is a colour coded (green, red, yellow) graph comparing results to pre-fed reference values (healthy age matched individuals). If the measurement result is in the orange or red area, this indicates an increased accumulation of AGEs in the tissue.

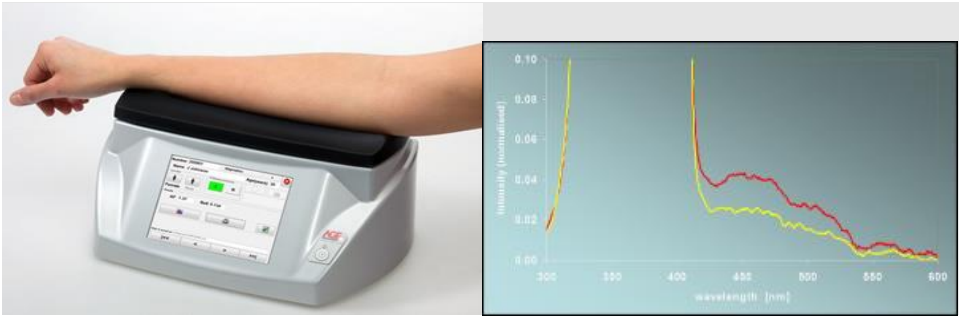


Figure 18: Age reader. Used band-fluorescence from fluorescent AGEs.
Source: Images, Graphs and technical information has been reproduced from https://www.diagnoptics.com/age-reader Diagnoptics Technologies B.V @diagnoptics 2019.

10.4.15Statistical analysis

Statistical analyses included descriptive statistics, ANOVA and ANCOVA. Differences between the groups (DPN and Controls) regarding the plantar pressure analysis were calculated using Independent T-test. The correlations between socio-clinical and pressure variables were calculated with SPSS correlation bivariate analysis. The collected data was tabulated and analysed using software SPSS statistical software (Statistical Package for Social Sciences, IBM SPSS statistics Ver. 26, IBM corp.) for windows. All data and values have been expressed and presented as group mean ± SD.

10.4.15.1 Test of Normality

A test of normality was performed on the data. As a statistical test to confirm hypothesis Shapiro Wilk test was used. As majority of data was not normal, so it was decided to use a nonparametric version of the test, which does not assume normality. As normality was

tested and Stiffness was non-parametric, a spearman's correlation test was used for all correlations with plantar pressures (non-parametric).

The data were explored for normality of distribution prior to inferential analysis. This test is used to determine whether sample data has been drawn from a normally distributed population (within some tolerance). In this case, independent samples Student's *t*-test requires a normally distributed sample population. As a statistical test to confirm hypothesis Shapiro Wilk test was used. As the majority of data was not normally distributed, it was decided to use a nonparametric version of the test, which does not assume normality. Spearman correlation method computes the correlation between the rank of x and the rank of y variables. The output table used Shapiro-Wilk Test results, which is more appropriate for small sample sizes (< 50 samples) for numerical means of assessing normality. As the test suggests, If the Sig. value of the Shapiro-Wilk Test is greater than 0.05, the data is normal, and If it is below 0.05, the data significantly deviate from a normal distribution. Spearman correlation method computes the correlation between the rank of x and the rank of y variables.

10.4.15.2 Independent samples Student's t-test

Differences between controls and DPN group were determined using the SPSS function of independent samples Student *t*-test which reflects the difference between the means of two groups, which may be correlated in certain features. All data and values have been expressed and presented as group mean ± SD and analysed with p value ≤ 0.05 was taken as statistically significant difference between groups. Despite non-normal distribution a T-test was used as it is less likely to generate type 1 errors.

All data and values have been expressed and presented as group mean ± SD and analysed with p value ≤ 0.05 was taken as statistically significant difference between groups. Despite non-normal distribution an independent samples Student's *t*-test was used as it is less likely to generate type 1 errors.

10.4.15.3 Correlations

Correlation analysis was used to evaluate the strength of relationship between stiffness, ankle joint range of motion, pressure variables and the clinical measures. As study variables are non-parametric, a Spearman's rank-order correlation was used.

10.4.15.4 ANCOVA

ANCOVA (Analysis of covariance) has been used in the study to assess for the effect of age as a covariate on all major variables.

10.5 RESULTS:

The main dependent variables assessed in this study were Achilles tendon mechanical properties and morphology and ankle and foot joint dorsiflexion range of motion. These variables were considered in relation to the development of foot pressures in specific foot regions established from chapter 1.

10.5.1 Demographics and clinical data

The key demographic variables recorded included height, weight, BMI, whose methods for acquisition and assessment have been presented in Chapter 1. Age was significantly higher in the controls (Ctrls 39±6 years) compared to the DPN group (71±9 years), p<0.001); Body-mass was higher in the DPN group as compared to controls (Ctrls 84±6, DPN 92±15 kg, p<.05) and also BMI (Ctrls 27±2, DPN_PU 29±4. P<.05). No significant differences were seen between groups in height.

Table 4- Demographic and clinical characteristics of participants.
*Mean values ±SD (Std. Dev.) of all variables by group. CTRL: Healthy Controls, DPN. * Variable that shows significant (p>0.05) group effect when adjusted for Age as a covariate using an ANCOVA. ‡ Non-Parametric (Shapiro-Wilk). AGEs level is a graded value of 0-5 risk factor based on calendar age/skin autofluorescence value.*

Variable	CTRL			DPN			SD	T-test p-value
Anthropometry								
Age (Years)	39.0	±	6.0	71.0	±	9.0		<0.001‡
Body mass (kg)	84.0	±	6.0	92.0	±	15.0		<.05*
Height (m)	1.8	±	0.3	1.8	±	0.2		0.36‡
BMI (kg/m2) *	27.0	±	2.0	29.0	±	4.0		<.05*‡
Clinical								
Neuropathy Disability Score (score/10)	1.0	±	1.0	8.0	±	1.3		<.001*‡
VPT (Volts)	3.0	±	1.5	27.0	±	11.0		<.001*‡
Tactile Sense- LOPS	0.0	±	0.0	1.0	±	0.4		<.001*‡
Diabetes Duration (Years)	0.0	±	0.0	15.0	±	5.0		<.001*‡
AGEs Score (Auto-florescence level: 0-5)	1.9	±	0.2	3.0	±	0.4		<.001*‡

The clinical data showed significant differences (p<.001) within study groups for NDS, VPT, LOPS and Diabetes duration. The details of the methodology relating to these variables has been presented in Chapter 1. Advanced Glycated Products showed a remarkable difference within study groups, where the auto-fluorescence levels were significantly elevated in case of DPNs (Ctrls 1.9±0.2 vs DPNs 3.0±0.4, p<.001).

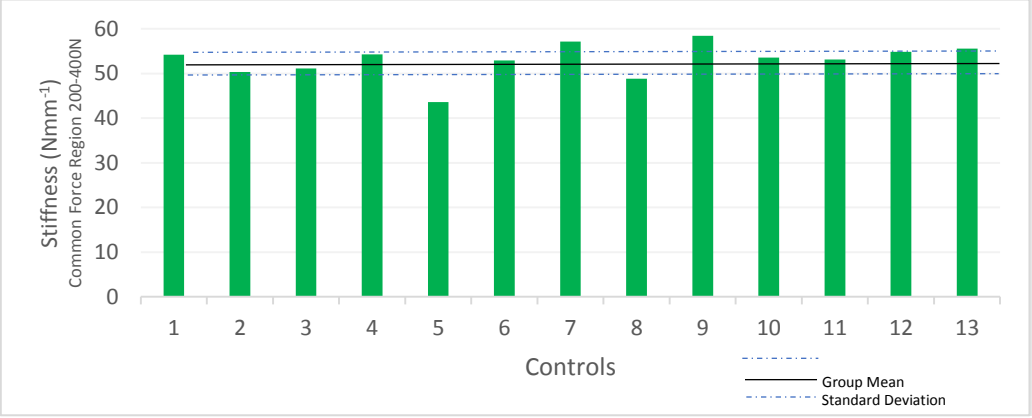
10.5.2 Tendon properties:

Mean heel displacement from controls was 0.30±0.06mm. This value was accordingly deducted from final values of the tendon elongation.

Table 5 – Tendon properties data (Force region 200-400N) of two study groups.
Achilles tendon's mechanical, material, and morphological property values for study groups. Mean values ±SD (Std. Dev.) of all variables by group. CTRL: Healthy Controls, DPN: Diabetic Peripheral Neuropathy, CSA: cross-sectional area. Values are means ± SD and depict group averages of data averaged across 3 isometric plantar-flexion trials for each individual. See methods for calculations.

Variable	CTRL		SD	DPN		SD	p value
Achilles Tendon properties							
Resting Tendon Length (mm)	177.1	±	5.9	181.4	±	6.4	
Achilles tendon Moment Arm (mm)	52.5	±	1.2	51.6	±	0.7	<0.05
Tendon Cross Sectional Area (mm^2)	70.8	±	4.2	84.3	±	4	<0.001
Volume (mm^3)	12.5	±	0.8	15.3	±	1	<0.001
Achilles Tendon Properties- Common Force Regions (200-400 N)							
Tendon Elongation (mm)	5.6	±	0.7	3.5	±	0.9	<0.001
Torque (N·m)	15.9	±	1.3	15.1	±	1.6	
Tendon Force (N)	303.7	±	24.7	297.5	±	28.9	
Tendon Stiffness (Nmm^{-1})	52.9	±	3.8	80.1	±	14.7	<0.001
Stress (MPa)	4.3	±	0.4	3.5	±	0.3	<0.001
Strain, (%)	3.2	±	0.5	1.9	±	0.5	<0.001
Young's modulus (MPa)	133.2	±	16.3	172.9	±	33.2	<0.001

The mechanical, material and morphological properties of Achilles tendon have been presented in Table 3 (for common force region of 200-400N). The cross-sectional area and volume have been presented for the complete Achilles tendon region, which showed highly significant differences of p<.001. All other parameters viz. tendon elongation, stiffness, stress, strain and young's modulus presented highly significant (p<.001) differences between Ctrls and DPNs



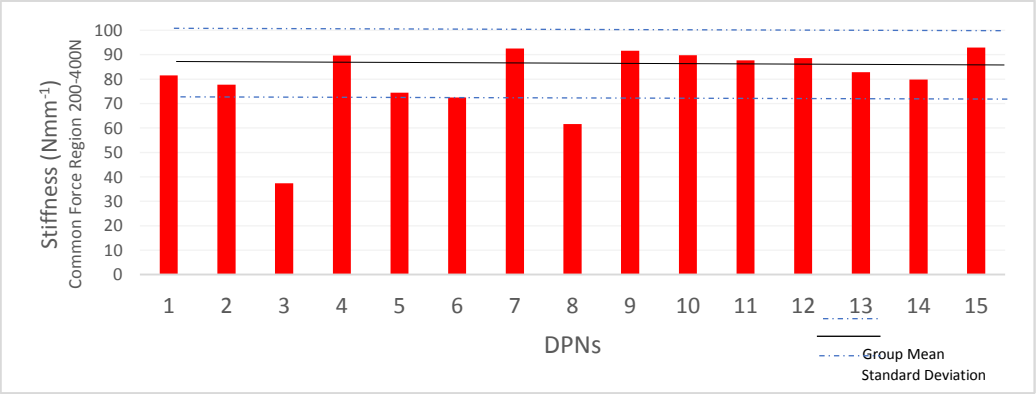


Figure 19: stiffness values for individual study participants

10.5.3 Ankle and Foot Joint Complex range of motion

Table 6: Foot-Ankle Joint range of motion.
*Values are means ± SD. * denotes significantly (P<0.05) different from controls. MTPJ: Metatarsophalangeal joint.*

Variable	CTRL		SD	DPN		SD	p value
Metatarsophalangeal Joint (deg)							
MTPJ Dorsiflexion	50.4	±	7.4	24.1	±	9.9	<.05
MTPJ total Range of Motion	88.7	±	6.9	53.1	±	8.8	<.05
Ankle Joint Complex (deg)							
Voluntary Dorsiflexion RoM	28.9	±	2.7	16.5	±	4.7	<.001
Voluntary total Range of Motion	73.6	±	8.9	41.9	±	8.6	<.001
Assisted Dorsiflexion RoM	34.8	±	3.6	20.2	±	5.2	<.001
Assisted total Range of Motion	86.5	±	9.9	50.4	±	9.3	<.001

10.5.4 Effect of Age (Analysis of Covariance - ANCOVA)

Because age was significantly different between groups, (Ctrls 39±6 vs DPN 71±9 years, p<0.001) an ANCOVA was used to test the effect of age as a covariate. The primary outcomes measures that were identified as different using the initial ANOVA: Achilles tendon stiffness, ankle RoM and peak pressures remained significant when using the ANCOVA with age as the covariate (p<0.05). Similarly, the majority of variables e.g. body

mass (p<.05), BMI (p<.05) relevant to the study remained significantly different with the ANCOVA when comparing between group differences and considering age as a covariate.

10.5.5 Correlations

10.5.5.1 Correlation of tendon stiffness with demographic and clinical variables.

*Table 7 Achilles tendon stiffness correlation with anthropometric and Clinical variables. Significant correlations are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient.*

Variable	Spearman's Rho values
	Stiffness
Demographic	
Body Mass (kg)	0.428*
Body Mass Index-BMI (Score)	0.488**
Age (Years)	0.542**
Wellbeing (Score out of 100)	-0.302
Lifestyle (Score out of 100)	-0.544**
Clinical	
Diabetes Duration (Years)	0.637**
Vibration Perception Threshold-VPT (V)	0.667**
Modified Neuropathy Disability Score-mNDS (Score out of 10)	0.704**
Advances Glycated End-products-AGEs level (Calendar age/Risk intensity. Graded 0-5)	0.670**

The study tested correlations between tendon stiffness and clinical factors of VPT, mNDS, diabetes duration and AGEs Table 7 and found highly significant correlations (p<0.001) with all parameters Figure 20 with the exception of wellbeing. AGEs and diabetes duration showed a strong correlation of rho=0.853(Figure 21).

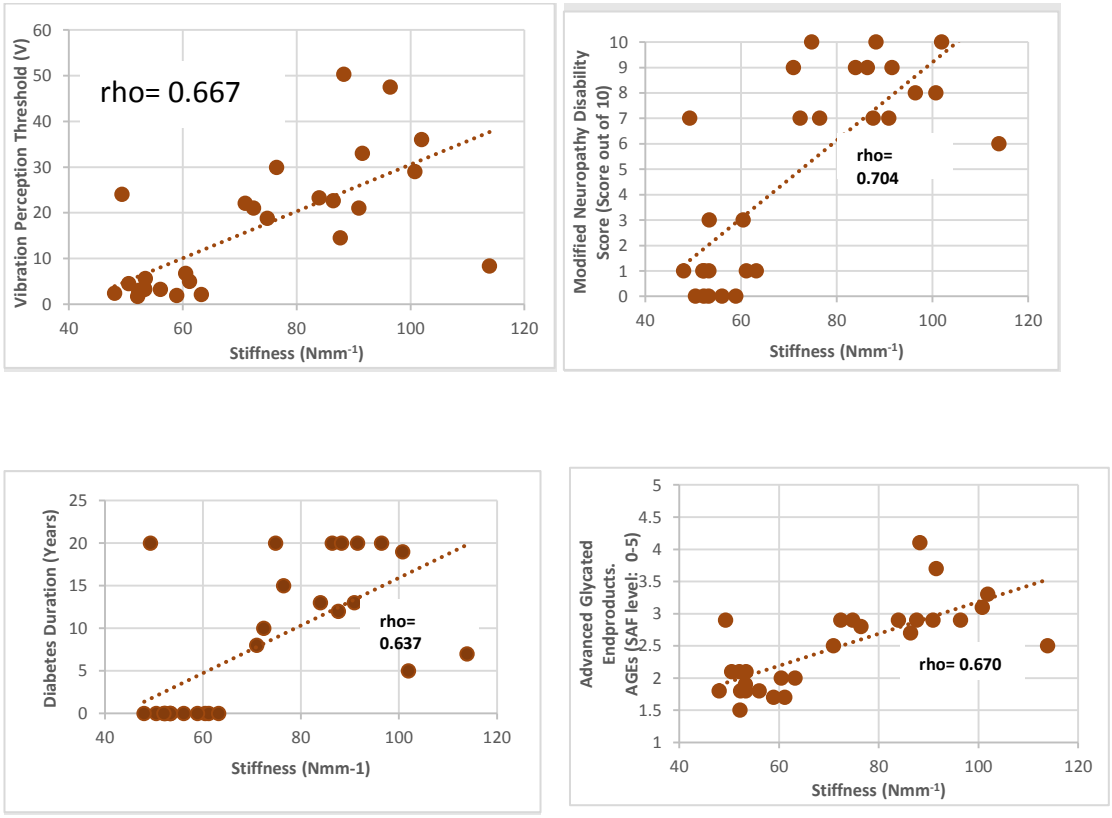


Figure 20: Achilles tendon stiffness correlation with Clinical parameters.

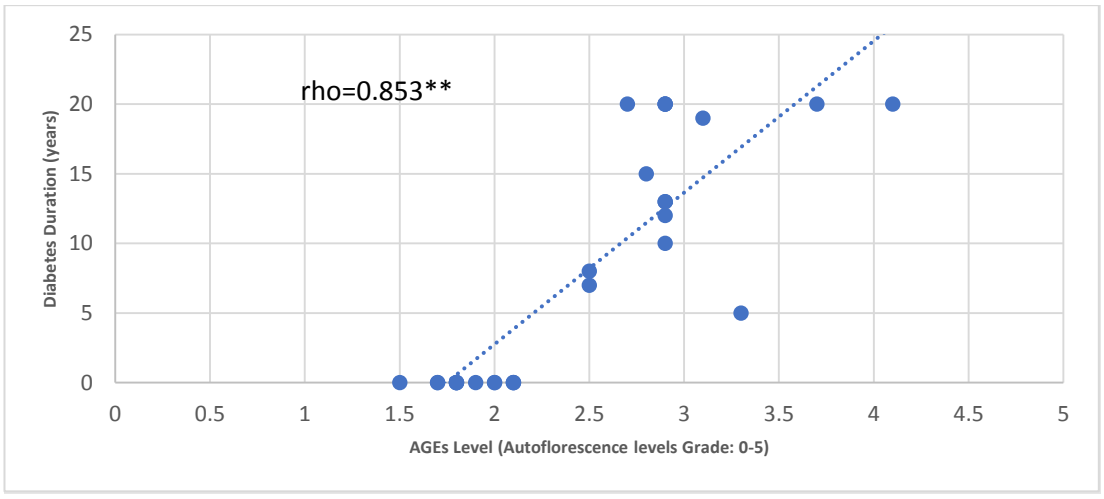


*Figure 21: Correlation of Advanced Glycated Endproducts with Diabetes Duration Studied among DPN and Ctrls. Strong correlation observed**.*

10.5.5.2 Stiffness correlation with ankle joint range of motion

Table 8 - Achilles tendon stiffness correlations with Foot ankle joint range of motion.

*Significant correlations are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient.*

	Spearman's Rho values
Range of Motion (RoM)	Stiffness
MTPJ RoM (Deg)	
MTPJ Dorsiflexion RoM	-0.653**
MTPJ total Range of Motion	-0.648**
Ankle Complex (Deg)	
Voluntary Dorsiflexion RoM	-0.456*
Voluntary Range of Motion	-0.513**
Assisted Dorsiflexion RoM	-0.489**
Assisted Range of Motion	-0.516**

10.5.5.3 Plantar pressure correlations with tendon properties, clinical and range of motion.

Table 9: Achilles tendon Stiffness correlation with plantar pressures.

*Significant spearman's correlations are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient.*

	Spearman's Rho values			
Region	Stiffness	Stress (MPa)	Strain (%)	Young's Modulus (MPa)
Peak Plantar Pressure (k.Pa)				
Total Foot	0.404*	-0.501**	-0.438*	-0.512**
Hallux	0.284	-0.088	-0.046	0.132
2nd Toe	0.255	-0.235	-0.299	0.215
First Metatarsal	0.371*	-0.469*	-0.215	-0.561**
Second Metatarsal	0.222	-0.138	0.057	-0.125
Forefoot	0.387*	-0.479**	-0.256	-0.513**
Rear foot	-0.026	0.03	0.077	-0.05
Pressure Time Integrals (kPa·s)				
Total Foot	0.25	-0.512**	-0.386*	-0.563**
Hallux	-0.02	-0.163	-0.027	-0.219
Second Toe	0.373*	-0.298	-.401*	0.339
First Metatarsal	-0.208	-0.407*	-0.253	-0.488**
Second Metatarsal	-0.511**	0.074	0.314	-.402*
Fore foot	-0.182	-0.249	0.02	-0.188
Rear foot	0.373*	-0.004	-0.138	0.229
Force Time Integrals (N·s)				
Hallux	0.490**	.476*	0.570**	-0.371*
First Metatarsal	-0.375*	-0.046	0.182	-0.352
Second Metatarsal	-0.651**	0.373*	.589**	-.528**
Fore Foot	-0.631**	0.152	.437*	-.447*

Table 10: Plantar pressures correlation with Advanced Glycated End Products.

*(Skin Autofluorescence level 0-5). Significant correlations are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient.*

	Spearman's rho values
Variable	AGEs (AF level 0-5)
Peak Plantar Pressure (kPa)	
Total foot	0.516**
First Metatarsal	0.431*
Fore Foot	0.414*
Pressure Time Integral (kPa·s)	
Total Foot	0.413*
First Toe	0.214
First Metatarsal	0.265
Fore Foot	0.154
Achilles Tendon Stiffness (Nmm^{-1})	
Stiffness	0.670**

Table 11: Plantar pressure correlations with Joint Range of Motion.

*Significant correlations are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient.*

	Spearman's rho		
Regions	Metatarsophalangeal Joint Dorsiflexion (Deg)	Ankle Joint Voluntary Dorsiflexion (Deg)	Ankle Joint Assisted Dorsiflexion (Deg)
Peak Plantar Pressure (kPa)			
Total Foot	-0.23	-0.418*	-0.385*
First Metatarsal	-0.374*	-0.495**	-0.483**
Fore foot	-0.379*	-0.477*	-0.369*
Pressure Time Integral (kPa.s)			
Total Foot	-0.209	-0.430*	-0.375*
Hallux	-0.177	-0.303	-0.243
First Metatarsal	-0.135	-0.465*	-0.396*
Fore foot	-0.261	-0.266	-0.129

10.6 DISCUSSION

This study showed key differences in Achilles tendon mechanical properties and foot/ankle joint dorsiflexion range, which correlated with forefoot pressure development with implications for ulcer risk in people with DPN. Measured over absolute force regions, tendon stiffness was higher in patients with DPN compared to controls. Ankle and Metatarsophalangeal joint (MTPJ) dorsiflexion range of motion was significantly smaller in DPN patients compared to controls. Key correlations between Achilles tendon stiffness and ankle joint dorsiflexion and MTPJ dorsiflexion were observed, implying that elevated tendon stiffness and limited ankle and MTP joint dorsiflexion could be contributory factors for elevated forefoot pressures (314). This increased forefoot pressure could lead to tissue breakdown and the progression towards diabetic foot ulcers in the DPN population (315,316).

10.6.1 Tendon Mechanical Properties

The current study observed increased tendon stiffness in people with DPN (Table 5) compared to controls ($p<.001$), which can be attributed to non-enzymatic glycation of tendon collagen and the gradual build-up of short and long-lived advanced glycation Endproducts (AGEs) in material constituents of tendons (253).

As stated earlier, the study has chosen common force region of 200-400 N for detailed measurement of tendon stiffness. This approach was adopted to ensure appropriate comparison between groups notwithstanding the differences in maximal tendon force between controls and DPNs, the comparative stiffness values in the two study groups will therefore relate to the same absolute forces.

The clinical parameters expectedly showed highly elevated values in DPNs in comparison with controls for duration of diabetes, neuropathy, vibration perception and AGEs. The implications of these clinical aspects (except AGEs) have been discussed in an earlier chapter. The formation of advanced glycation end products (AGEs) has been recognized as an important pathophysiological mechanism in the pathogenesis of micro and macrovascular complications of diabetes and diabetic neuropathy (253). The consequential foot ulcers via diabetes and peripheral neuropathy route has major role played by non-enzymatic glycation and accumulation of AGEs in the pathway. The assessed clinical variables in the current study, demonstrate significant correlations with Achilles

tendon properties and therefore indicate that tendon properties may be associated with risk of foot ulceration. The AGEs level demonstrated significant correlations with peak pressure variables of whole foot (0.516**), first Metatarsal (0.431*) and forefoot (0.414*). The AGEs have previously been suggested as an independent marker of foot ulceration risk (317) and peak pressures suggested as a surrogate for foot ulcer risk (318)., The correlation between AGEs and Achilles tendon stiffness (0.670**) in the current study, may also imply that tendon stiffness is associated with diabetic foot ulceration risk.

It has been theorised mostly through previous animal model studies (319,320) that diabetes is associated with Achilles tendon morphological changes including increased stiffness in rabbit Achilles tendon due to glycation-induced collagen cross-linking (321). Recent limited in vivo human studies in diabetes patients showed that Achilles tendon stiffness and skin connective tissue cross-linking were greater in diabetic patients compared with controls (322). Another study by Grant et al (323) found morphologic abnormalities (tigheting) in the Achilles tendons in line with clinical observations of alrming reduction of Achilles tendon-gastrocnemius-soleus complex in DPNs with severe conditions, that may precipitate serious foot ulceration. The findings of the present study are therefore in line with these previous animal studies and the very limited data from human studies, all showing an increased stiffening of tendons with diabetes.

Earlier researches have established that tendon composing collagen fiber's geometrical provisions are associated with mechanical properties of tendons (324). The current study has found higher levels of AGEs in the DPN group compared to controls and shown moderate-strong correlations between AGEs level and severity of neuropathy. This suggests that disturbance of the collagen fiber structure and arrangement owing to non-enzymatic glycation, accompanied by an increase in mechanically stiffer collagen as a result of DPN, may alter the tendon's mechanical and material properties increasing tendon stiffness and modulus (314)

The important constituent of tendon mechanical properties i.e. stiffness (the tendon's resistance to tensile elongation) has a significant influence on force transmission to the skeleton, muscle power, and energy absorption and release during human movement (325–327). Young's modulus represents stiffness normalised to the geometric dimensions of the tendon. In the present study the Young's modulus of the Achilles tendon was higher in the DPN group compared to controls, despite a larger tendon CSA in the DPN group. This

indicates not only the mechanical properties are altered in diabetes with a stiffer tendon, but also the material of the tendon is intrinsically stiffer. Tendon cross sectional area is observed to be larger in DPNs. An in vivo study by Couppe et al. (303) comparing controlled diabetes vs uncontrolled sugar diabetics, has found larger tendon CSA, and has linked this alteration to non-enzymatic glycation and AGEs. Tendon stiffness showed moderate-strong positive correlations with duration of diabetes (0.637), the level of AGEs (0.670) and neuropathy assessments: the vibration perception threshold (0.667) and neuropathy disability score (0.704). This strongly suggests that tendon stiffness increases with increasing severity of diabetic peripheral neuropathy, which may also be linked with duration of diabetes and AGE levels. This seems intuitive since diabetic neuropathy and poor glycaemic control increase non-enzymatic glycation and AGE development in collagenous tissues including tendon.

10.6.2 Limited ankle and MTP joint Dorsiflexion

This study showed a marked limitation to ankle and foot joint dorsiflexion in patients with DPN compared to controls (Table 6). Voluntary ankle dorsiflexion in the DPN group was 57% of that found in the control group (Ctrl 29 deg vs DPN 17 deg, p<.001). A systematic review and meta-analysis by Hazari et al. (328) reported references of studies with similar values as reported in current study for the ankle DF in DPN vs control, in their meta-analysis. Research studies by Anita (329) showed Ctrl $27.7^{o} \pm 5.1$ vs DPN $18.5^{o} \pm 8.8$), Saura et al. (330) reported Ctrl $29.01^{o} \pm 3.29$ vs DPN $20.24^{o} \pm 4.08$) and Zinny et. al (331) demonstrated Ctrl $31^{o} \pm 8.76$ vs DPN $17.9^{o} \pm 4.14$ values on ankle dorsiflexion. In the current study total range of motion at ankle joint was 57% in DPN in comparison to controls. The lower Ankle DF and RoM values found in current study are steadily consistent with previous reports recording reduced ankle DF ROM (332,333) and augmented stiffness in people with diabetes (334). Researchers have reported that deviations in ankle dorsiflexion range of motion and stiffness in people with DPN can be ascribed to process which dependent on pathology viz. metabolic disorder driven mechanisms of non-enzymatic glycation of collagen and accumulation of AGEs (335) and usage mechanisms alluding to adaptive fiber shortening within the triceps surae (336)

At the MTP joint, dorsiflexion in the DPN group was 47% of that in controls (Ctrls 50.4±7.4deg vs DPN 24.1±9.9deg, p<.001). The MTPJ total RoM in the DPN group was 60%

of that achieved by the controls. The 1st MTPJ range of motion of between 65 to 75 deg is the minimum required for normal gait (337), whereas in the current study the DPN group could only achieve a mean MTPJ of 53 deg. Studies also suggest that diabetic foot has lower capability to absorb shocks with limited motion at the metatarsophalangeal joints and may cause an aberrant distribution of PP (331). Consistent with the present study, D'Ambrogi et al.(338) found that range of motion of the first metatarsophalangeal joint was significantly reduced in DPNs (54% of controls) and proposed that this reduced range of motion may play an important role of the increased PPP in the forefoot regions (first toe and first metatarsal head).

In the current study ankle joint voluntary/assisted dorsiflexion range of motion and peak plantar pressure showed significant inverse correlations with whole foot, forefoot and first metatarsal (Table 11), while MTPJ dorsiflexion showed a significant inverse correlation with peak plantar pressures at the first metatarsal and forefoot (Table 11). These inverse correlations between peak pressure and ankle/MTPJ dorsiflexion range of motion indicate that as dorsiflexion decreases, plantar pressure increases. These findings are novel in highlighting the important relationship between increased Achilles tendon stiffness, reduced ankle-foot dorsiflexion and increased plantar pressures. These results support the notion that increased Achilles tendon stiffness and limited ankle-foot dorsiflexion are major contributory factors in elevated forefoot plantar pressures and therefore increased DFU risk.

In the current study, Young's modulus's was significantly higher in the DPN group (Ctrls 133.2MPa vs. DPN 172.9MPa) indicating a change to the tendon material; this mechanism is a major factor in the increase of tendon stiffness (339). Similarly the cross sectional area was increased in DPNs when compared with controls (Ctrls 70.8mm^2 vs DPNs 84.3mm^2); indicating a change of the tendon morphological properties, which may also be responsible for enhanced tendon stiffness (340). Studies have indicated that such morphological and material changes are not a result of increased collagen synthesis but also from morphology of collagen fibril and collagen molecular cross-linking levels(341). Mechanical stimuli can influence tendon adaptations, thus timely and quantitative information on Achilles tendon mechanical properties might be part of a preventive strategy in rehabilitation regimes for people at risk of DFU.

10.6.3 Limitations

The outcomes of current study must be interpreted in light of its limitations. The main limitation of the study is that being cross-sectional design and the potential role of other factors e.g. joint deformities, foot posture, measurement accuracies and such relevant variables; that may intercede the association between plantar loading and employed foot movement modifications/strategy, depending on the underlying pathology and its severity.

There may be differences in the stiffness at different points along the tendon, whereas the current study assessed differences at same place for both groups, whereby it is assumed that increases in stiffness are homogenous throughout the tendon. The influence of changes in the cross-sectional area throughout the length of Achilles tendon, was controlled to some extent by also involving Young's modulus alongside stiffness.

The current study has only considered Achilles tendon stiffness and not the complete Muscle Tendon Unit (MTU) stiffness. Although, it is quite possible that MTU stiffness and not only the AT stiffness is causing limited DF (249) and it has also been established that that there is no lengthening of the proximal aponeurosis of the medial gastrocnemius by passive ankle dorsiflexion up to –30° of dorsiflexion (342) and in the current study ankle DF for DPNs was well below this limit.

It is normal to assume during plantarflexion measurements of torque using dynamometric that the triceps-surae muscle-tendon complex works completely in the sagittal plane. This can lead to a lower estimation of the finally calculated tendon force, especially when the other component for force calculation i.e. moment arm is only calculated from one DF and one PF position, whereas MA changes values from rest to maximal isometric contractions. Similarly, ultrasound imaging used for tendon elongation is also two-dimensional and has limitation of capability to incorporate tendon movement in the transverse plane.

10.7 Conclusions

Thus the in vivo human Achilles tendon mechanical and material characteristics investigated during the current experimental study do support the hypothesis that patients with DPN will have higher tendon stiffness compared to controls and thus reduced ankle-foot dorsiflexion; this is linked to higher forefoot plantar pressures, thereby increasing the risk of diabetic foot ulceration in this foot region.

11 EXPERIMENTAL CHAPTER 3: RELATIONSHIP BETWEEN DYNAMIC GAIT BIOMECHANICS, ANKLE STIFFNESS AND PLANTAR PRESSURES

11.1 ABSTRACT

Human foot-ankle complex has a multifaceted task of movement and constantly balancing during bipedal ambulation against gravity besides negotiating uneven surfaces through gait mechanisms. This study hypothesized that alterations to gait strategy will vary between people with diabetic peripheral neuropathy (DPN) and healthy controls and would be associated with changes in ankle joint and foot kinematics. A cross-sectional study was set-up to investigate the relationship of Achilles tendon stiffness upon dynamic gait variables and plantar pressures during walking in people with diabetic peripheral neuropathy. Participants were n=15 people with diabetic peripheral neuropathy and n=13 healthy controls. Kinematic and kinetic data were recorded for over ground walking. Ground reaction forces, and ankle angles, moments and powers calculated. Heel rise was 10% earlier in case of DPN patients as compared to Controls ($p<0.05$). During walking reduced dorsiflexion of 3.5 deg. was observed in DPNs when compared with controls ($p<0.05$). People with diabetic peripheral neuropathy walked with a slower speed ($p<0.001$) and wider base ($p<0.001$) as compared to controls. Heel rise correlated with all major risk factors of ulceration including AGEs (rho=-0.375), reduced ankle dorsiflexion (rho=0.391), forefoot plantar pressure (rho=-0.375) and tissue stiffness (rho=0.391). Other key associations identified included: stiffness and Gait velocity (rho=-0.479), stiffness and peak dorsiflexion (rho=-0.427), vertical peak ground reaction force and stiffness (rho=0.644), Peak plantar Pressure at 2nd Toe and ankle dorsiflexion (rho=-0.576) while walking. These marked gait differences between DPN and control groups, followed by highly demonstrable correlations with at-risk foot parameters, suggest that increased Achilles tendon stiffness and limited ankle dorsiflexion in patients with DPN cause an earlier heel rise, contributing to earlier and more prolonged forefoot loading, predisposing a higher risk of diabetic foot ulcers.

11.2 INTRODUCTION

11.2.1 GAIT: A General Overview

Understanding of bipedal movement ability granted to humans is vital to further recognise issues occurring during human locomotion. Gait is typically considered the method by which movement from one spatially location to another occurs. Gait analysis can inform on the alterations, impairments and anomalies in the way humans move. The study of ground reaction forces force and spatial-temporal parameters can be done through identified measurement techniques.

11.2.2 Biomechanical alterations to gait strategy alter force/pressure application

Altered plantar pressure distribution during gait is an important etiopathogenesis risk factor for the development of foot ulcers. Thus, a review on aspects of foot kinematic and kinetic characteristics in diabetes is important to understand the biomechanical changes. Gait changes alter the mechanical loading of the foot and thus may have implications upon the formation of ulcers by mechanical damage. To understand altered foot loading, one needs to understand the normal mechanical loading of the foot.

The systematic review by Hazari et al. (328) with meta-analysis reported significant differences in Gait variables among diabetic. As described by Deursen et al. (343) regarding role of Ground Reaction Forces (GRFs) during weight-bearing activities, GRFs manage to expose the plantar side of the foot. Such forces lead to tissue deformation. GRFs represent the forces applied to the foot, which determine in a simple representation the manner by which the foot is being mechanically loaded. This has implications for whether and where under diabetic foot ulcers may form by altering the pressure magnitudes and distribution.

11.2.2.1 Altered Gait in people with DPN and history of ulceration

Functional abnormalities and structural alterations could be major reasons for development of diabetic foot ulcers which have a role of Gait mechanisms. Studies have shown that loading of the mechanical component of the foot is observed to be elevated during walking (67). Gait characteristics differ in individuals with diabetes compared with those without diabetes (344). Altered walking patterns may be due to decrease in ankle strength and mobility (283). How the gait patterns effect the onset of ulcer is still not clear (345).

Gait changes in patients with diabetes along with advanced glycosylation end products (AGEs) affect the soft tissues of the foot tendons and ankle joint. People with gait deficits tend to walk slower than their healthy counterparts. Severity of the disease is directly correlated with level of abnormalities (346). The ankle strength in people with diabetes is drastically reduced. People with high body weight also have issues with speed and have increased double limb support.

Because such gait characteristics are also found frequently in diabetes patients without sensation loss, however, researchers are raising questions about the extent to which these and other changes are associated with neuropathy per se or are indicative of a more general diabetes-related syndrome. This is important, because whether they have neuropathy or not, patients with diabetes are at higher risk of gait-related problems, including foot ulcers and falling; neuropathy merely increases that risk (347,348)

The above studies and relevant literature have amply informed that these changes in gait (strategy, compensation, pattern etc.) exist, it is now further important to understand whether and how any of these factors impact diabetic foot ulcer formation. I.e. which changes create increases to plantar pressure? This study will provide important observations regarding the association between gait characteristics and diabetic foot ulcer risk.

11.2.2.2 Gait variables investigated:

The key gait parameters investigated in this study included the spatiotemporal, kinematic and kinetics of Dynamic Gait. Muscles and tendons about the ankle, knee and hip are typically considered the main mechanical power producers during human gait. Using inverse dynamics to estimate net power generated about these joints has become ubiquitous in human gait analysis studies (349).

11.2.2.3 Spatiotemporal variables:

As the name signifies, these variables are the Spatial attributes (Distance parameters) of a gait e.g. step, stride length etc. and Temporal attributes (Time parameters) e.g. step time, cadence (number of steps per unit time), walking speed (velocity) of gait, single limb support (amount of time spent on a limb expressed as a percentage of the gait cycle).

11.2.2.4 Kinematic variables:

Kinematic parameters describe body segment positions, their speeds and angles beside others. Examples of human lower limb kinematic parameters could be joint angles, joint velocity and joint accelerations.

11.2.2.5 Kinetic Variables

Gait kinetics studies the cause of motion during walking. This will include the ground reaction forces , the power, the moments and pressure underneath the foot, besides the muscle involved in ensuring locomotion (350,351). Foot kinetics provide an insight into the non-vector components of GAIT. Therefore, it was important to study the vertical ground reaction forces and shear forces.

Vertical Ground Reaction Forces

The parameters assessed in the present study included vertical peak Ground reaction force GRF (N), Left foot-LGRF Integral, and right foot-RGRF Integral.

Ankle Power & Moment

The variables considered in the present study for ankle maximum power (Watts), were concentric peak power & Eccentric peak power and the Ankle moments.

11.2.2.6 Shear Forces

Our study investigated the shear forces in the forefoot plantar region. The studied Shear forces (N.kg) included LGRF Integral X & LGRF Integral Y and corresponding RGRF Integral X & Y. Shear stress has been studied as one of the major reasons for diabetic foot ulcers but this component has been less studied (352). Delbridge et al. (353) stressed upon shear as the main reasons for deep tissue breakdowns. Research so far has underestimated the significance of shear stress probably due to lack of technical measurements systems (354). Shear is slowly gaining traction in the research environment with advent of new devices and systems to measure it (355).

11.2.2.7 Foot-Ankle Joint complex (FAJC)

FAJC is major area of interest in the current study as it is where the Achilles tendon stiffness was measured along determining the segmental angles and RoM. The ankle joint complex bears a force majority of the force during walking and running activities (356).

11.2.2.8 Hallux Valgus and Metatarsophalangeal Range of Motion

Hallux (the big toe) has a critical role in maintaining balance. The Hallux must also bear the most weight when standing. Hallux is connected to first ray via 1st metatarsal joint. The first ray is the segment of the foot composed of the first metatarsal and first cuneiform bones (357).

11.2.2.9 Ankle Angle

Ankle angle is a Joint angle (also called inter-segmental angle), thus a joint angle is an angle between the two segments on either side of the joint, is measured in degrees. As the orientation of the body changes, so does the ankle angle. Ankle angle measurements were done in reference of foot and shank angle in the current study. Ankle motion is primarily in sagittal plane. Studies have defined Range of motions for these planes (358).

11.2.2.9.1.1 Foot progression angle (FPA)

The FPA is a Segment angle. Segment angle is quite different from Joint angle, as it is the angle of the segment with respect to the right-hand horizontal. As the orientation of the body changes, so does the foot progression angle (Figure 26). It has been observed that progression angle of foot in people with conditions of diabetes have a greater tendency to out-toe, which can be adding additional load on the forefoot region (359).

1.4.4.3. Heel Rise Time

Forefoot has to tolerate more weight and for longer duration if there is an early heel rise (360). Also, the inability of the tibia to move forward probably to reduced ankle dorsiflexion, when joins with early heel rise, it results into joint pronation and increased load on midtarsal (361). Therefore, measurements predicting the heel-rise time are considered to be particularly meaningful in the current study.

11.2.3 Aim:

To investigate the relationship between Achilles tendon (ankle region) Stiffness and dynamic Gait variables in people with diabetic peripheral neuropathy.

Secondary aim:

Investigate the relationship between dynamic gait variables and plantar pressures during walking in people with diabetic peripheral neuropathy.

Hypothesis

It is hypothesized that gait strategy will be altered due to changes in stiffness at ankle joint complex in people with risk of diabetic foot as compared to non-diabetic control group.

11.3 METHODS

This research study considered variables related to Gait properties including Spatiotemporal, Kinematics and Kinetics of gait to understand gait variability, adjustments and compensations within two study groups. The primary outcome Variables included Ankle Joint Complex (Achilles Tendon) Stiffness, Dynamic ankle RoM, GAIT kinematics and kinetics, metatarsal joint angles and plantar pressures which were ascertained through dynamic gait patterns, walking speed, forces exerted during movements, electromyography, and other measurements methods explained in stiffness and plantar pressure assessments.

11.3.1 Ethical Consideration and Informed Consent

Appropriate ethical approvals were obtained from the relevant bodies as detailed in Chapter 1, for recruitment of participants. Testing and research activities were carried out at Manchester Metropolitan University (MMU) research labs. University ethics (ETHOS) clearance was also obtained to commence the recruitment. Eligible participants gave written informed consent after studying the supplied Patient Information Sheet (PIS) Appendix 4. Confidentiality of the information was assured as per NHS ethics norms. Further details on Ethical consideration have been mentioned in Chapter 1.

11.3.2 Study Population:

Details of full inclusion/exclusion criteria for each group and subgroup are provided in the Chapter 1.

11.3.3 Study Design & outcome variables:

This study was cross-sectional, observational in design conducted among adult participants. The outcome variables of the study included:

- Ankle Joint Complex (Achilles Tendon) Stiffness
- Dynamic ankle Range-of-Motion
- Gait spatiotemporal parameters
- Gait kinematics
- Gait kinetics (Vertical and Shear forces)
- Ankle joint moment and pressure
- Dynamic Metatarsophalangeal joint angles and range-of-motion.
- Peak Plantar pressures & Pressure Time Integrals

11.3.4 Gait data collection, processing and analysis

The spatiotemporal, Kinematic and Kinetic data acquisition was undertaken in the Manchester Metropolitan University gait analysis unit of biomechanics and musculoskeletal lab. (T0.18). Researcher has also used the plantar pressures and Achilles tendon morphology, material and mechanical properties results from earlier chapters for evaluation of correlations. In plantar pressure data, only peak plantar pressure and pressure time integral data of total foot, forefoot and correlating plantar sites at Toe and metatarsal regions have been utilized for association studies, as minimal/no significant differences were observed at the heel region.

Assessment of walking was performed on a 10 meter even surface within a laboratory setting. Floor markings at different locations within and at the ends of walkway, were placed to facilitate recognizing start and end of walking trials. The dynamic movements on this walkway were monitored by a movement tracking system (Vicon, Oxford, UK) and the forces generated during these dynamic gait movements were recorded by surface embedded force plates (Kistler, Winterthur, Switzerland). The participants were wearing body hugging sports shorts and vests, with florescent reflective markers placed on them. These reflective markers were placed on their body for the motion analysis cameras to monitor their movements. The study participants were provided standard medical shoes (DARCO, U.K.) as per their foot size. To simultaneously collect plantar pressures during GAIT movements, an in-shoe pressure mapping system known as Tekscan (Tekscan Inc., Boston, MA) was placed inside their Shoe. Please See Chapter 1 for details on the plantar pressure mapping system. Once ready, participants were asked to walk on walkway at self-selected speed. This was done to collect Spatiotemporal, Kinematics and kinetics data for foot and lower limb.

11.3.4.1 Kinematic data collection

Marked gait differences between DPN and Control groups, followed by highly demonstrable correlations with at risk foot parameters suggest that increased Achilles tendon stiffness and limited ankle dorsiflexion in patients with DPN cause an earlier heel rise, contributing to earlier and more prolonged forefoot loading, predisposing a higher risk of DFU

Figure 22 - Marker positions (anterior and posterior) during level walking.

The numbered positions refer to wireless EMG sensors. B. Participant standing on lab. walkway set-up for Gait analysis trial (force plate can be viewed below participant's feet) with standard shoes, positioned reflective markers and EMG sensors, wearing in-sole pressure sensor and their transmitters with wired USB connection, and abdomen belt for carrying wires to avoid tripping during walking. Courtesy: A study participant at Manchester Metropolitan University biomechanics lab.

Study required two types of markers: calibration markers (for defining the segments) and tracking markers (for computing the movements). Tracking markers on the segments were placed in such a way that it ensures lower movement. Skin movement artefacts are more susceptible in markers near extreme distal or proximal end of segments. During gait, the upper-body is considered as the 'passenger' unit whereas the lower-body is the locomotor unit (362).

Table 12: Six degree of freedom marker set (Thorax & pelvic region)

Waist markers are the key markers in modelling the pelvis bone, which is the major segment governing the other subsequent skeleton segments.			
Labels	Related Segment	Anatomical Location	Additional Description
Upper limb			
CLA	Clavicle bone	bone that connects the breastplate (sternum) to the shoulder	
C7	7th cervical vertebra	Most inferior vertebra in the neck region	
LSHO	Shoulder	Extreme top of Left Shoulder Blade	
RSHO	Shoulder	Extreme top of Right Shoulder Blade	
Rscapula	Shoulder Blade	Middle of Right Shoulder Blade	It is a positioning marker
STRN	Breastbone	Front side of the thorax	
T10	tenth thoracic vertebra	one of twelve vertebrae that make up the central section of the vertebral column	T10 has a complete articular facet
Pelvic Region			
LASI	Pelvis	left anterior superior iliac spine	Placed the marker on the protruding bones located on the left and right side of the pelvis front.
RASI	Pelvis	right anterior superior iliac spine	
L_ILcrest	Pelvis	Left Femoral greater Trochanter	Placed the markers on left and right side of the hip, where one can palpate the hip joint or the most lateral prominence of the greater trochanter.
R_ILcrest	Pelvis	Right Femoral greater Trochanter	
LIPSI	Pelvis	Left Iliac Posterior Spine	Placed each marker on the two dimples, which can be palpated near the spine right above the hips.
RIPSI		Right Iliac Posterior Spine	
SACR	Pelvis	It is at the end of fused vertebra of spine	

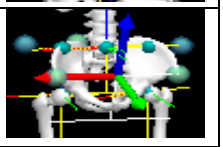
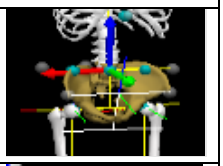
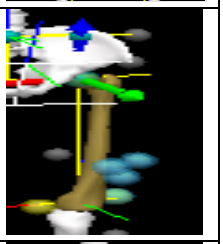
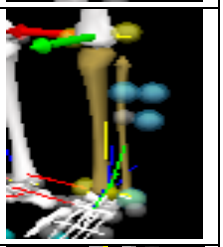
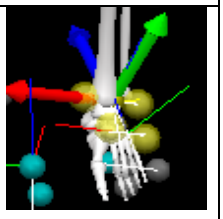
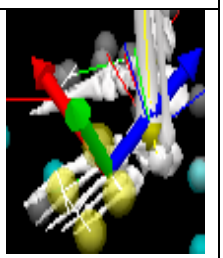
Table 13: Placement for 6 DOF lower limb marker set (Leg region)

The joint centre of the knee and the ankle is modelled at the midpoint of the lateral and medial joint markers. Assuming that centre of the femoral head aligns with the centre of the acetabulum, its virtual location is modelled using markers on the pelvis segment (posterior and anterior iliac spine markers). The lower extremity segments are modelled along these three virtual locations.			
Leg Markers			
LTH1, LTH2, LTH3, LTHB	Upper Leg	Left Thigh	Placed the markers at the front center of the thigh near the midline. These markers are placed for distinguishing left and right side of the skeleton. For best results, slightly offset the height of right and left marker to introduce an asymmetry.
RTH1, RTH2, RTH3, RTHB	Upper Leg	Right Thigh	
LKNEE	Upper Leg	Left Femur Lateral Epicondyle	Place the marker on the lateral prominence of the knee joint axis. More specifically, the marker was placed on the femur epicondyle. Needed to ask the subject to flex and extend the knee few times to locate the axis.
RKNEE		Right Femur Lateral Epicondyle	
LKNEE_M	Upper Leg	Left Femur Medial Epicondyle	Place the marker on the Medial prominence of the knee joint axis. Asked the subject to flex and extend the knee few times to locate the knee axis.
RKNEE_M	Upper Leg	Right Femur Medial Epicondyle	
LSHA1, LSHA2, LSHA3, LSHA4	Lower leg	Left fibula	Placed four markers in a clockwise manner on each shin bone near the midline of the lower leg.
RSHA1, RSHA2, RSHA3, RSHA4	Lower Leg	Right Fibula	

Table 14 - Placement for 6 DOF lower limb marker set (Foot & Toe Leg region)

Labels	Related Segment	Anatomical Location
LANK	Lower Leg/Foot	Left Fibula Ankle Lateral
RANK	Lower Leg/Foot	Right Fibula Ankle Lateral
LANK_M	Lower Leg/Foot	Left Talus Ankle Medial
RANK_M	Lower Leg/Foot	Right Talus Ankle Medial
LHEEL	Foot (Heel)	Left Foot Calcaneus
RHEEL	Foot (Heel)	Right Foot Calcaneus
LMB1	Foot (Cuboid)	Left Foot Metatarsal Base
LMB5	Foot (Medial Cuneiform)	Left Foot Metatarsal Base
RMB1	Foot (Cuboid)	Right Foot Metatarsal Base
RMB5	Foot (Medial Cuneiform)	Right Foot Metatarsal Base
LMH5	Foot	Left Foot Fifth Metatarsal
RMH5	Foot	Right Foot Fifth Metatarsal
LMH1	Foot	Left Foot First Metatarsal
RMH1	Foot	Right Foot First Metatarsal
LTOE2	Toes	Left Second Distal Phalanx
RTOE2	Toes	Right Second Distal Phalanx

Table 15 - Segmental definitions for 3D Gait model.

Segment Name		Lateral	Joint Centre	Medial	Position
Thorax/Ab	Proximal	None	Pelvis Origin	None	
	Distal	RSHO	None	LSHO	
Pelvis	Proximal	L_ILIAC	None	None	
	Distal	RKNE	None	RKNE-M	
RPV_2	Proximal	R_ILIAC	None	L_ILIAC	
	Distal	R_HIP	None	L_HIP	
Left Thigh	Proximal	None	L_Hip	None	
	Distal	LKNE	None	LKNE_M	
Left Shank	Proximal	LKNE	None	LKNE_M	
	Distal	LANK	None	LANK_M	
Left Foot	Proximal	LANK	None	LANK_M	
	Distal	None	LMH1	None	
	Distal				
Left Midfoot	Proximal	LANK	None	LANK_M	
	Distal	LMB5	None	LMB1	
Left Fore foot	Proximal	LMB5	None	LMB1	
	Distal	LMH5	None	LMH1	

11.3.4.1.1 Foot Mobility Measurements: Hallux Valgus and 1st MTPJ RoM measurements (non-dynamic):

Identification of prominent issues like hypermobility of the first ray were assessed by measuring hallux valgus and RoM of hallux. The hallux valgus angle and range of motion of the first metatarsophalangeal joint were measured with the help of a goniometer (311,312). Participants were encouraged to undertake maximum plantar flexion of the hallux, followed by attempt to undertake maximum dorsiflexion for recording the range of motion of the hallux. These measurements were undertaken three time and then averaged (313). As per literature In the literature, a HVA of 0 to 15 degrees is considered to be normal (363,364). Methods for other static RoM measurements at Ankle Joint complex have been described in chapter 2.

11.3.4.2 Kinetic data collection

A 10-metre walkway was designed, Kinetic data was collected using three force platforms (Kistler, Winterthur, Switzerland) embedded in the walkway. These ground reaction forces were measured at 1,000 Hz from the force plates and synchronized with the kinematic data. Both external and internal forces interaction and responses were quantified. Ground reaction force vectors were studied for vertical, mediolateral and anteroposterior forces. The study focussed on observing the pathway of ground reaction force in stance phase in sagittal plane at initial contact, loading response, terminal stance and pre-swing. Participants were wearing close fitting, but non-restrictive, above knee, shorts. Standardised shoes were also provided across all participants.

Participants were asked to walk the length of the walkway at their self-selected speed. To avoid modulating the participant's natural gait but to ensure that their foot lands onto the force platform, researcher moved the position where participants start their walk until optimal. Walking trials with recording on both limbs were repeated until five good (defined as 'clean' strikes with the foot inside the borders of the force plates) trials were collected. These kinetic data were measured at 1,000 Hz from these force plates and synchronized with the kinematic data simultaneously collected through Vicon. The current study quantified both external and internal forces interaction and responses, data processing in visual 3D (C-motion Inc, Maryland, USA).

11.3.5 Data processing

Study used Vicon Nexus software and its existing pipelines to run sequences of frequently used processing operations. Modifications to existing pipelines were also made at occasions for our customised operations including Core Processing, Subject Calibration, fill Gaps & Filter Data Operations for automating the post-processing of data, such as gap-filling and data-filtering and Data Processing Operations for automating the production of model outputs (forces and moments, joint angles, etc). Gap filling was done using spline fill (for gap lengths of 100 frames) and pattern fill if suitable marker was available. This process was followed by filtering and further using the automatic gait events generation capability of the Gait data processing software.

11.3.5.1 Kinematic Variables

The C3D files (calibration and motion files) from Vicon were exported to Visual 3D C-Motion, Rockville, Maryland). Majority of spatiotemporal gait events were automatically detected by Visual 3D. A pipeline was used for generation of events. An automated report was generated, whose data was exported to MS excel sheets for further processing and later statistical analysis in SPSS software.

The measured variables included Stance width (m), Gait Cycle (s), Step length (m), Step time (s), Stance time (s), Swing time (s), Initial Double support (s), Stride length (m), double limb Support (s), Cadence (Step/minute), Gait velocity and Step W: L Ratio (Figure 23). To assess these parameters Visual3D, force plates were set-up as described in earlier section.

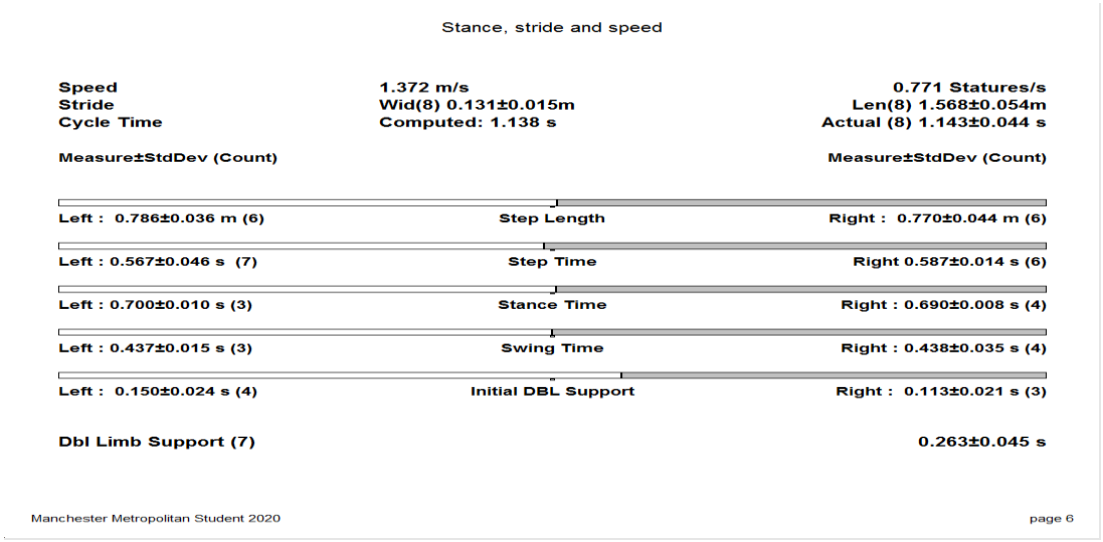


Figure 23: Spatiotemporal data report generated in Visual 3D.

11.3.5.1.1 Ankle Angle

The variables of kinematics which were studied included Dynamic Ankle ROM, Dynamic Peak Dorsiflexion and Dynamic Peak Plantar flexion, Peak MTJ ROM (deg) and Foot Progression Angle (deg). The ankle angle (Figure 24) was measured at the sagittal plane at Heel strike. Additionally, dynamic ankle RoM during walking was measured by difference between peak dorsiflexion and peak plantarflexion during each gait cycle.

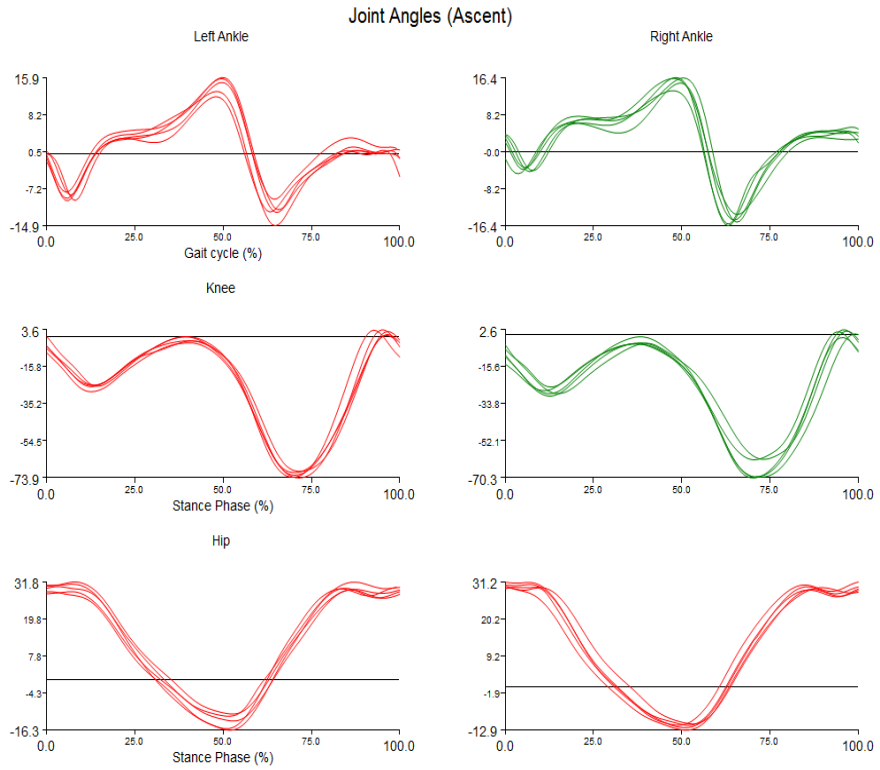


Figure 24: A control participant's Joint angles report for Ankle, Knee and Hip region.

11.3.5.1.2 Heel Rise Time

Heel rise/End of foot flat was defined as the instance when proximal end of left foot moved upward (velocity >0.08m/s) in the vertical direction. Heel rise timings were assessed by detection of the instant of vertical velocity of the proximal end (heel marker) of the foot segment beginning to start to move upward (vertical velocity >0.08m/s) following foot flat. Heel rise time was then normalised as a percentage of stance time. Sagittal ankle angle at the instant of heel rise was also recorded.

11.3.5.1.3 Peak flexion during stance phase (Mid and foot forefoot segments)

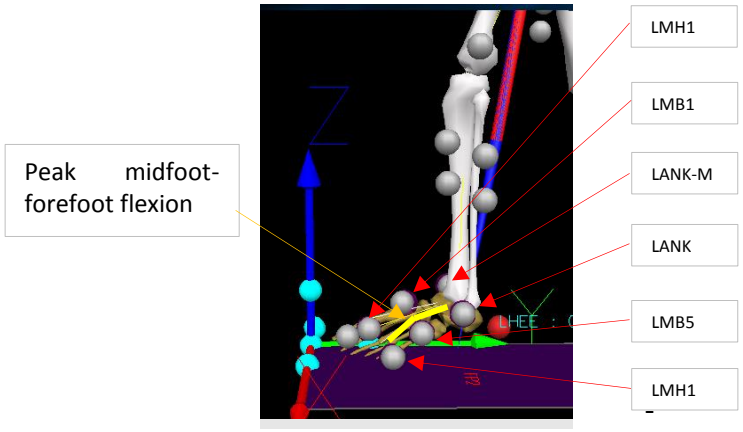


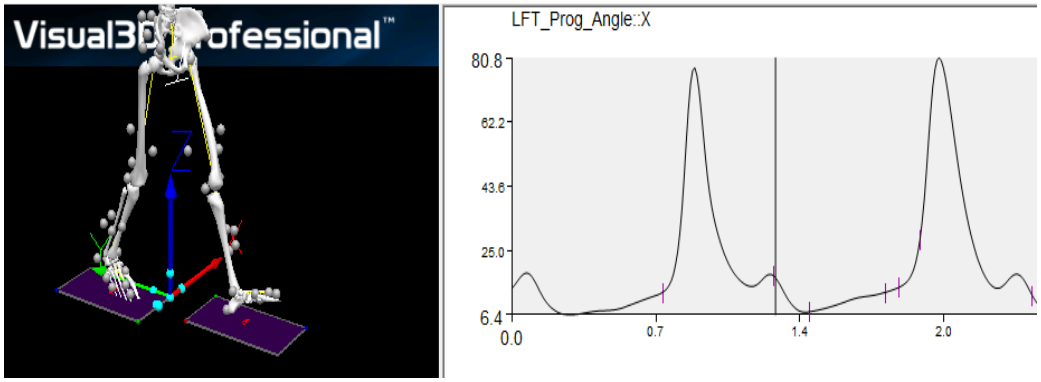
Figure 25: Peak Midfoot-forefoot flexion

Segment definition: Left Midfoot (Ankle to Metatarsal Base)- Proximal position markers viz. Ankle Lateral malleolus (LANK) & Ankle medial malleolus (LANK-M) and distal position markers viz. Lateral 5th metatarsal base (LMB5) and Medial 1st Metatarsal base medial (LMB1). Forefoot (Metatarsal base to metatarsal head)- Proximal position markers viz. Lateral 5th metatarsal base (LMB5) and Medial 1st Metatarsal base (LMB1) and distal position marker viz. Lateral 5th metatarsal Head (LMH5) and Medial 1st Metatarsal Head (LMH1). Next step was to calculate angle between mid and forefoot segments. A pipeline was designed for determining midfoot-forefoot angle. In link model X-axis graph (sagittal plane) was plotted with labels marked for events of Heel strike and Toe off to determine complete stance phase. The measurements required adjustments through neutral reference angle of the forefoot midfoot segments, which was drawn from static standing position calibrations file, which was called into motion files for this purpose. Flexion and extension values were then reported relative to the neutral position. This led to peak flexion calculations during stance phase (Figure 25).

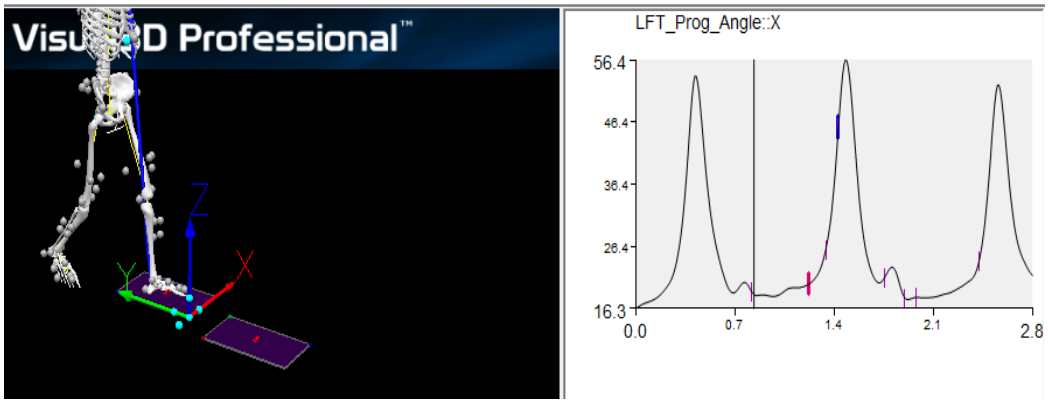
11.3.5.1.4 Foot progression angle (FPA)

Foot Progression angle was the value of transverse plane rotation of the foot segment around the local superior-inferior axis (angle away from the axis i.e. direction of walking) at the mid-stance of the gait (365). In the case of the current study, with DPN patients participating, the foot flat definition was critical, so in the majority of cases FPA pipeline was created considering foot flat event as occurring between heel-strike and heel-rise (3 frames before heel rise). The reason for considering pre-heel rise event for measurement was that the foot was flat and no change to foot angle should occur whilst placed on the ground. Start of foot flat was defined as the instance when distal and proximal end of left

foot where touching the floor. End of foot flat was defined as the instance when proximal end of left foot moved upward (velocity >0.08m/s) in the vertical direction in certain cases, event threshold was shifted by some frames as identified by visual observation of actual foot flat. Foot flat in this study was considered as the mid-stance.



a. Foot progression by a DPN participant



b. Foot Progression by a Control participant

Figure 26: Gait events during foot progression in study groups

image a) & b) have been captured using 3D gait analysis system (VICON), after exporting to V3D biomechanics analytics software. The experiments were conducted at MMU Gait lab, while participants walked on a level walkway at self-selected speeds.

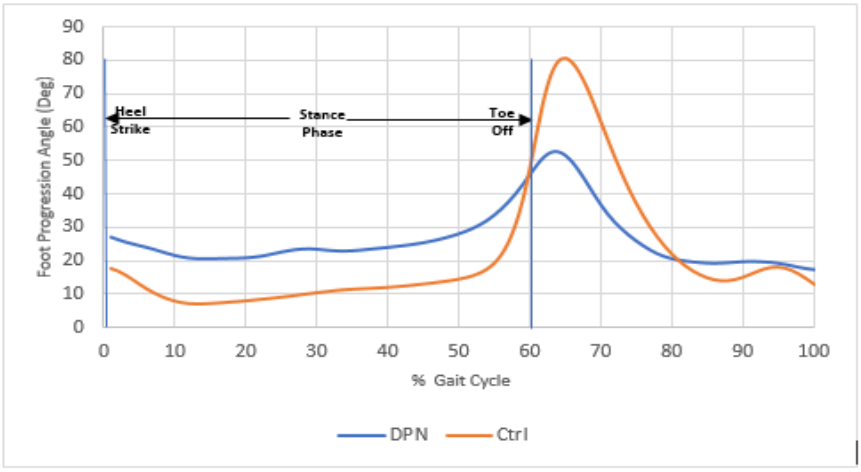


Figure 27: Foot progression angle during a normalized Gait cycle.

11.3.5.2 Kinetic Variables analysis

Foot kinetics provide an insight into causes of the forces and thus the vertical ground reaction forces and Shear forces were measured.

11.3.5.2.1 Vertical Ground Reaction Forces (GRF)

The parameters studied included Vertical Peak GRF (N), LGRF Integral, and RGRF Integral Z.

11.3.5.2.2 Shear Forces

The studied Shear forces (N·kg) included LGRF Integral X & LGRF Integral Y and corresponding RGRF Integral X & Y.

11.3.5.2.3 Ankle Maximum Power

In sagittal plane, the peak power (Watts) were calculated for both Concentric Peak Power and Eccentric Peak Power during a gait cycle.

11.3.5.2.4 Ankle max moment

In sagittal plane, the peak dorsiflexion and plantarflexion moments (Nm) at ankle were calculated in both DF and PF directions by measuring Peak PF Moment and Peak DF Moment during a gait cycle.

11.3.6 Statistical analyses

Statistical analyses included descriptive statistics, ANOVA and ANCOVA. Differences between the groups regarding the GAIT analysis were calculated using Independent T-Test. The correlations between variables were calculated with SPSS correlation bivariate analysis. The collected data was tabulated and analysed using software SPSS (Statistical Package for Social Sciences, SPSS statistical software (IBM SPSS statistics Ver. 26, IBM corp.) for windows.

11.3.6.1 Test of Normality

Overall normality was tested in SPSS using “Explore” function. As Stiffness and Plantar Pressure were non-parametric, a Spearman’s (rho) correlation test was used for all correlation’s studies of variables with Stiffness and plantar pressures. A test of normality was performed on the data.

11.3.6.2 Independent samples Student's t-test

In this study differences of variables between controls and DPN groups were assessed using an independent samples Student's *t*-test to determine, if there is a significant difference between the means of two groups, which may be correlated in certain features.

11.3.6.3 ANCOVA

There was a significant difference in GAIT velocity between groups, therefore it was decided to use ANCOVA (Analysis of covariance) to assess for the effect of GAIT velocity as a covariate on variables of GAIT. ANCOVA is used to test the main and interaction effects of categorical variables on a continuous dependent variable, controlling for the effects of selected other continuous variables, which co-vary with the dependent.

11.3.6.4 Gait correlations with Stiffness

As one of the main aims of this study was to investigate the relationship between Stiffness and GAIT Variables, so using SPSS Correlation function, GAIT variables and their correlation with stiffness was calculated. As the data was non-parametric in nature, so Spearman's rho was used to study the correlations.

11.3.6.4.1 Spatiotemporal variables correlations with Stiffness

The parameters of spatiotemporal variable included Stance Width, Gait Cycle, Step Length, Step Time, Stance Time, Swing Time, Double Support Time, and Gait Velocity. While Heel Rise parameters whose correlation with stiffness was calculated included Heel Rise Percent Stance, Heel Rise Time, Ankle Angle @Heel Rise. Correlations between stiffness and kinematic and kinetic variables were assessed. Additionally, stiffness was correlated against measurements of foot mobility.

11.3.6.4.2 Heel Rise time correlation with GAIT Kinetics & Plantar Pressure

In SPSS the bivariate analysis was undertaken to ascertain the correlations between Heel rise time, Kinetic Gait parameters and the forefoot peak plantar pressure. As the variables were non-parametric, thus Spearman's rho was used.

11.3.6.4.3 Dynamic RoM correlation with Static Voluntary and Assisted RoM.

The intent was to investigate relationship between static and dynamic RoM at Ankle joint complex and MTPJ to visualize association of joint mobility during resting and walking phase of study groups. This was assessed by studying correlation in RoM at these regions in resting and movement stage. During this study static RoM (Voluntary and Assisted) at

ankle joint complex and MTPJ RoM were already measured during earlier testing, thus this static data was used for benchmarking. Now obtained dynamic RoM (PF, DF and Overall RoM) during walking trials, was used for correlation study. In SPSS the bivariate analysis was undertaken to ascertain the correlation. As the variables were non-parametric, thus Spearman's rho was used for depiction of correlations while also ascertaining the Significance (2-Tailed).

11.3.6.4.4 Pressure Time Integrals correlation with GAIT variables

Spatiotemporal parameters of Step time and Gait Velocity were correlated with plantar pressure and force time Integrals. In SPSS the bivariate analysis was undertaken to ascertain the correlation. As the variables were non-parametric, thus Spearman's rho was used for depiction of correlations while also ascertaining the Significance (2-Tailed).

11.4 RESULTS

An extensive range of gait variables were analysed and are presented in showing multiple between group differences between controls and the DPN group including wider stance, shorter step lengths, longer stance times, and double support times in the DPN group. Cadence and gait velocity were lower in the DPN group and heel rise occurred earlier in the stance phase in the DPN group compared to the controls.

Table 16 - Gait Kinematics data showing difference in DPN and Control participants.

Mean values ± SD of all variables by group. Bold values denote t-test p-value is less than 0.05.

	Control			DPN			t-test
Spatiotemporal Variables	Mean		SD	Mean		SD	p value
Stance Width (m)	0.11	±	0.02	0.13	±	0.03	<.050
Gait Cycle (s)	1.17	±	0.06	1.19	±	0.09	0.535
Step Length(m)	0.74	±	0.05	0.63	±	0.07	<.001
Gait Efficiency*	0.16	±	0.02	0.2	±	0.05	<.05
Step Time (s)	0.54	±	0.06	0.57	±	0.05	0.105
Stance time (s)	0.64	±	0.06	0.69	±	0.04	<.05
Swing Time (s)	0.44	±	0.11	0.48	±	0.03	0.234
Initial Double support (s)	0.16	±	0.06	0.11	±	0.03	<.05
Double limb Support (s)	0.17	±	0.05	0.35	±	0.41	<.001
Cadence (Step/minute)	113	±	10	108	±	10	<.05
Gait Velocity (m/s)	1.29	±	0.11	1.08	±	0.12	<.001
Heel Rise							
Heel Rise Time (s)	0.5	±	0.03	0.47	±	0.04	<.05
% of stance	0.74	±	0.06	0.68	±	0.05	0.01
Ankle Angle at Heel Rise (deg)	9.91	±	0.79	10.86	±	2.06	0.05

**Gait efficiency: stance width to steplength ratio*

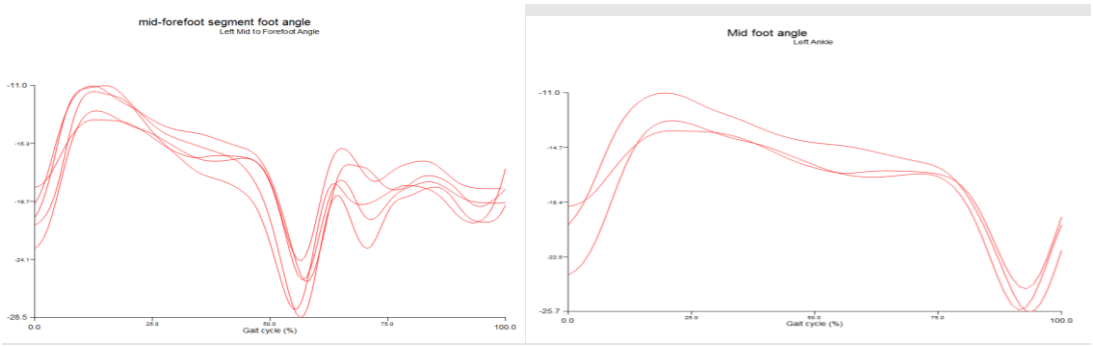


Figure 28- Peak flexion determination for Mid-forefoot segment.

Along with angles in region to define foot mobility. Angles observed at mid-forefoot and mid-foot for a DPN participant, demonstrating reduced peak flexion.

Table 17 -Kinematic alterations in study group.

In the diabetes neuropathic group in comparison to the controls during walking.

	CTRL			DPN			t-test
Range of Motion	Mean		SD	Mean		SD	p value
Left Ankle ROM (deg)	29.8	±	2.3	23.6	±	3.3	<0.001
Right Ankle ROM (deg)	27.8	±	2.3	24.1	±	2.8	<0.001
Left Ankle Peak DF (deg)	15.4	±	1.9	11.9	±	3.1	<0.05
Right Ankle Peak DF (deg)	12.4	±	2.8	11.7	±	2.9	0.566
Left Ankle Peak PF (deg)	14.3	±	2.2	11.3	±	4.2	<0.05
Right Ankle Peak PF (deg)	15.2	±	2.5	12.2	±	2.2	<0.05
Foot Mobility							
Foot Progression Angle (deg)	-7.7	±	1.6	-13.3	±	4.2	<0.001
Peak flexion Midfoot forefoot (deg)	-14.06	±	3.29	-10.41	±	4.41	<.05

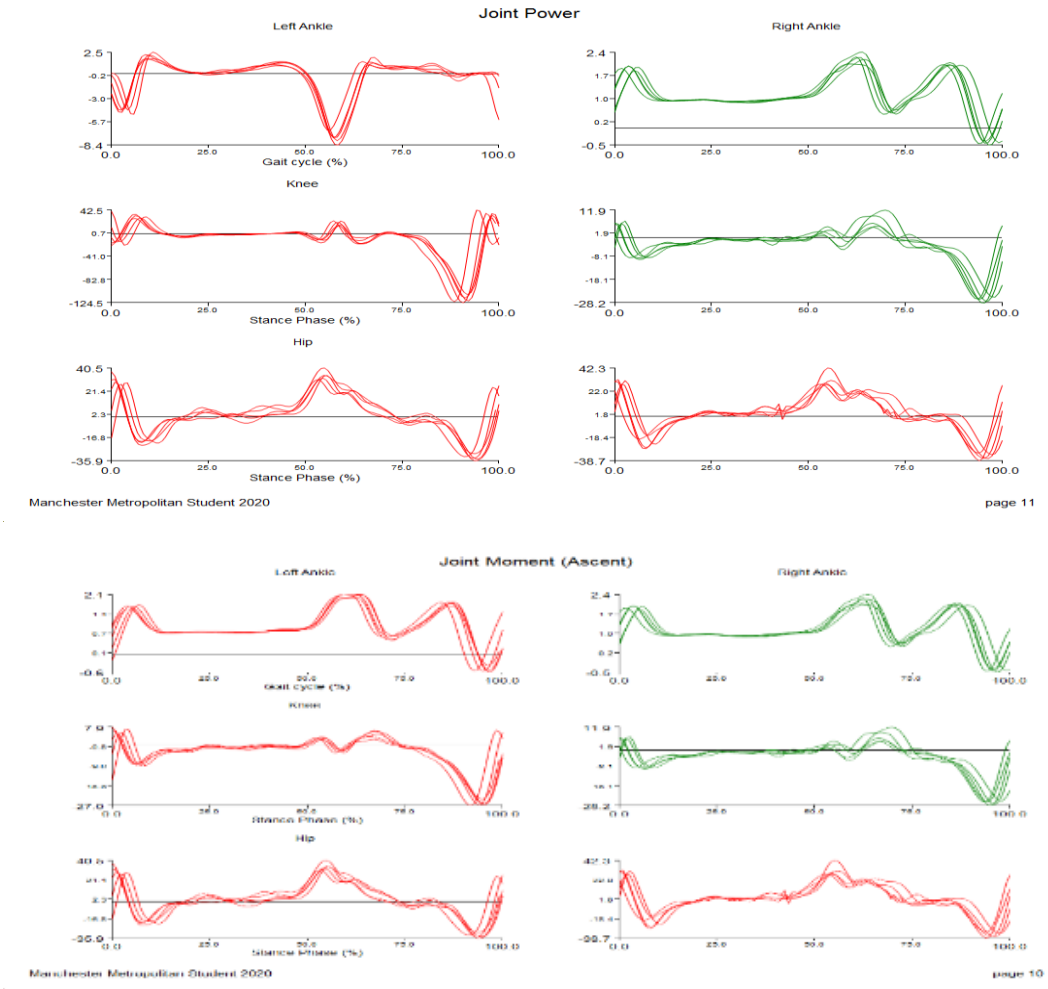


Figure 29: V3D report with joint power and moments

Table 18: Kinetic gait alterations in study groups.

Mean values (±SD) of all variables by group. Bold values denote t-test p-value is less than 0.05. LGRF: Left foot Ground Reaction Force. RGRF: Right Foot Ground Reaction Force. ANCOVA was run with Gait Velocity as Covariate.

	CTRL			DPN			t-test	ANCOVA
Kinetic Variables	Mean		SD	Mean		SD	p-value	p-value
Vertical Peak Left (L) and Right (R) Ground Reaction Force (GRF) (N)								
LGRF Integral Z	324.5	±	74.4	551.9	±	94.6	<.001	
RGRF Integral Z	344.2	±	39.2	557.4	±	107.6	<.001	
Shear (N·kg)								
LGRF Integral X	22.3	±	3.4	29.6	±	9.5	<.05	
LGRF Integral Y	36.1	±	3.9	52.1	±	16.7	<.05	
RGRF Integral X	18.5	±	2.2	27	±	7.8	<.001	
RGRF Integral Y	42.6	±	5.4	50.9	±	17.9	0.115	
Ankle maximum Power (W)								
Concentric Peak Power	221.9	±	46.5	160.3	±	70.9	<.05	0.266
Eccentric Peak Power	88	±	28.8	81.4	±	41.1	0.388	0.234
Ankle maximum moment (Nm)								
Plantar flexion Peak Moment	112.8	±	26.9	90.3	±	33.2	<.05	0.271
Dorsiflexion Peak Moment	15.6	±	2.7	12.1	±	5.3	<.05	0.684

Table 19: Heel rise correlation with tendon properties, RoM, & GRF
*Range of Motion (measured during gait and isolated dynamometry tests), Plantar Pressures and other GAIT variables across all participants in the DPN and control groups. Correlations (spearman's rho) are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient for significance, also denoted by bold values.*

	Heel Rise	
Variables	Time	Percent Stance
Clinical and Achilles Tendon Morphological Variables		
Achilles Tendon Stiffness	-0.441*	-0.497**
Achilles Tendon elongation	0.511**	0.451*
Advanced Glycated End products (AGEs) level	-0.375*	-0.447*
Plantar Pressures		
Pressure Time Integral-Total Foot	-0.278	-0.157
Pressure Time Integral-Hallux	-0.175	-0.112
Pressure Time Integral-Forefoot	-0.247	-0.232
Peak Plantar Pressure-Total Foot	-0.369*	-0.197
Peak Plantar Pressure-Hallux	-0.229	-0.245
Peak Plantar Pressure-3rd Toe	-0.458*	-0.339
Peak Plantar Pressure-4th & 5th Toe	-0.452*	-0.373*
Peak Plantar Pressure-2nd Metatarsal	-0.146	-0.221
Peak Plantar Pressure- Forefoot	-0.375*	-0.216
Range of Motion (deg)		
Ankle Range of Motion (Gait)	0.368*	0.379*
Peak Dorsiflexion (Gait)	0.391*	.564**
Voluntary Peak Dorsiflexion (Dynamometry)	0.24	0.29
Assisted Peak Dorsiflexion (Dynamometry)	0.235	0.369*
Metatarsophalangeal Joint Dorsiflexion (Dynamometry)	0.391*	0.415*
Foot mobility		
Hallux Valgus Angle (deg)	-0.373*	-0.547**
Peak Power		
Dynamic Concentric Peak Power (Gait)	0.375*	
Dynamic Eccentric Peak Power (Gait)	0.558**	

Static values observed with knee extended.

Table 20: Gait variables and Achilles Tendon Stiffness correlations
*Shown across all participants in the DPN and control groups. Correlations (spearman's rho) are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient for significance, also denoted by bold values.*

Variables	Achilles Tendon Stiffness
Spatiotemporal	
Stance Width (m)	-0.028
Gait Cycle (s)	-0.181
Step Length (m)	-0.579**
Step Time (s)	0.131
Stance Time (s)	0.201
Swing Time (s)	-0.114
Double Support Time (s)	-0.041
Gait Velocity (m/s)	-0.479**
Gait efficiency (SW:SL ratio)	0.369*
Kinematics (deg)	
Ankle Range of Motion (Dynamic)	-0.484**
Peak Ankle Dorsiflexion (Dynamic)	-0.427*
Peak Ankle Plantarflexion (Dynamic)	-0.263
Peak flexion @ Midfoot-Forefoot	0.553**
Ankle Angle @ Heel Rise (deg)	0.497**
Kinetics	
LGRF Integral Z	0.644**
RGRF Integral Z	0.672**
Shear (N·kg)	
LGRF Integral X	0.264
LGRF Integral Y	0.457*
RGRF Integral X	0.446*
RGRF Integral Y	0.406*
Ankle max. Power (Watts)	
Concentric Peak Power	-0.292
Eccentric Peak Power	-0.234
Ankle max moment (Nm)	
DF Peak Moment	-.579**
PF Peak Moment	-0.226

Vertical Left (L) Right (R) Ground Reaction Force (GRF)

Table 21: Dynamic RoM correlation with dynamometry RoM
*Showing measurements of Voluntary and Assisted Range of Motion across all participants in the DPN and control groups. Correlations (spearman's rho) are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient for significance also denoted by bold values.*

Variables	Dynamic Ankle Range of Motion (Gait) (deg)		
Ankle Range of Motion (deg)	Peak Dorsiflexion	Peak Plantarflexion	Range of Motion
Voluntary Peak Dorsiflexion (Dynamometry)	0.475*	0.288	0.598**
Voluntary Range of Motion (Dynamometry)	0.490**	0.249	0.612**
Assisted Peak Dorsiflexion (Dynamometry)	0.520**	0.295	0.634**
Assisted Range of Motion (Dynamometry)	0.497**	0.262	0.619**

All static range of motion in extended knee

Table 22: Plantar Pressure correlations with Gait variables
*Correlation between Plantar pressure variables for the Total Foot and Forefoot against Gait parameters across all participants in the DPN and control groups. Correlations (spearman's rho) are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient for significance, also denoted by bold values. PPP: Peak Plantar Pressure, PTI: Pressure Time Integral, X and Y represent geometrical planes, GRF: Ground Reaction Force*

	Correlation Coefficient			
Gait Variable	Peak Plantar Pressure (PPP)			
	Hallux	2nd Toe	1st Metatarsal	2nd Metatarsal
Stance Width (m)	-0.082	0.191	-0.078	0.094
Gait Cycle	0.007	-0.199	0.168	0.342
Step Length (m)	-0.021	-0.335	-0.262	-0.076
Step Time (s)	-0.119	0.234	0.302	0.119
Stance Time (s)	0.061	0.091	0.205	0.085
Swing Time (s)	-0.216	0.097	0.005	-.384*
Double Support Time (s)	0.091	-0.315	-0.207	0.013
Gait Velocity (m/s)	-0.098	-0.373*	-0.369*	-0.034
Ankle Range of Motion (deg)	-0.327	-.479**	-0.442*	-0.274
Ankle Peak Dorsiflexion(deg)	-0.093	-.576**	-0.369*	-0.224
Ankle Peak Plantarflexion(deg)	-.460*	-0.131	-0.322	-0.272
Ankle Angle at Heel Rise(deg)	0.153	0.229	0.244	0.093
Shear-GRF Integral X	0.407*	.604**	0.606**	0.385*
Shear-GRF Integral Y	0.222	0.447*	0.411*	0.038
Vertical GRF Integral Z	-0.136	-0.186	-0.233	-0.423*
Concentric Peak Power	0.145	-0.054	-0.086	0.217
Dorsiflexion Peak Moment	-0.125	-0.008	-0.248	-0.379*
Eccentric Peak Power	-0.165	-0.107	-0.19	-.384*
Plantarflexion Peak Moment	0.308	-0.166	0.222	0.1

*Table 23: Correlation between Peak Plantar pressure regions correlation and Gait parameters across all participants in the DPN and control groups Correlations (spearman's rho) are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient for significance, also denoted by bold values.*

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	Correlation Coefficient			
Gait Variable	Peak Plantar Pressure (PPP)			
	Hallux	2nd Toe	1st Metatarsal	2nd Metatarsal
Stance Width (m)	-0.08	0.191	-0.078	0.094
Gait Cycle	0.007	-0.199	0.168	0.342
Step Length (m)	-0.02	-0.335	-0.262	-0.076
Step Time (s)	-0.12	0.234	0.302	0.119
Stance Time (s)	0.061	0.091	0.205	0.085
Swing Time (s)	-0.22	0.097	0.005	-.384*
Double Support Time (s)	0.091	-0.315	-0.207	0.013
Gait Velocity (m/s)	-0.1	-0.373*	-0.369*	-0.034
Ankle Range of Motion (deg)	-0.33	-.479**	-0.442*	-0.274
Ankle Peak Dorsiflexion(deg)	-0.09	-.576**	-0.369*	-0.224
Ankle Peak Plantarflexion(deg)	-.460*	-0.131	-0.322	-0.272
Ankle Angle at Heel Rise(deg)	0.153	0.229	0.244	0.093
Shear-GRF Integral X	0.407*	.604**	0.606**	0.385*
Shear-GRF Integral Y	0.222	0.447*	0.411*	0.038
Vertical GRF Integral Z	-0.14	-0.186	-0.233	-0.423*
Concentric Peak Power	0.145	-0.054	-0.086	0.217
Dorsiflexion Peak Moment	-0.13	-0.008	-0.248	-0.379*
Eccentric Peak Power	-0.17	-0.107	-0.19	-.384*
Plantarflexion Peak Moment	0.308	-0.166	0.222	0.1

Gait velocity also showed significant correlation with Peak Plantar Pressure in 3rd Toe (rho=-0.4338), 4&5th Toe (rho=-0.485**) and 3rd Metatarsal region (rho=0.423*) and also with Pressure Time Integral at 2nd Toe (rho=-.514**), 3rd Toe (rho=-.515**), 4&5th Toe (rho=-.392*) and 3rd Metatarsal (rho=.420*). PPP: Peak Plantar Pressure, PTI: Pressure Time Integral, X and Y represent geometrical planes, GRF: Ground Reaction Force

Table 24: Correlations between Dynamic Gait variables and isolated dynamometry.
*Measurements of Range of Motion, Clinical and Foot Deformity, across all participants in the DPN and control groups. Correlations (spearman's rho) are indicated by a * (p<0.05) or ** (p<0.01) after the correlation coefficient for significance. Moderate to strong correlation* (366) *denoted by bold values.*

X and Y represent geometrical planes, GRF: Ground Reaction Force

	Correlation Coefficient				
Gait Variable					
	MTPJ_DF	Voluntary Ankle DF	Assisted Ankle DF	AGEs Score	Hallux Valgus Angle
Stance Width (m)	-0.075	-0.216	-0.235	0.232	0.115
Gait Cycle	0.198	-0.222	-0.197	0.006	0.141
Step Length (m)	.583**	.660**	.686**	-.662**	-.624**
Step Time (s)	-.403*	-.455*	-.492**	.521**	.494**
Stance Time (s)	-0.238	-0.35	-.400*	.392*	.429*
Swing Time (s)	0.095	-0.186	-0.172	0.126	0.234
Double Support Time (s)	0.266	0.186	0.213	-0.344	-0.275
Gait Velocity (m/s)	.648**	.735**	.752**	-.662**	-.691**
Ankle Range of Motion (deg)	.638 **	.598**	.634**	-.613**	-.613**
Ankle Peak Dorsiflexion(deg)	.463*	.475*	.520**	-.565**	-.635**
Ankle Peak Plantarflexion(deg)	0.367	0.222	0.248	-0.229	-0.237
Ankle Angle at Heel Rise(deg)	-0.197	-0.197	-0.198	0.232	0.131
Shear-GRF Integral X	-0.208	-.397*	-.507**	.484**	.507**
Shear-GRF Integral Y	-.465*	-.508**	-.546**	.542**	.671**
Vertical GRF Integral Z	-.603**	-.635**	-.673**	.668**	.733**
Concentric Peak Power	.376*	.504**	.493**	-.485**	-.389*
Dorsiflexion Peak Moment	0.298	0.321	0.361	-.531**	-.437*
Eccentric Peak Power	0.277	0.288	0.273	-0.317	-0.372

11.5 Discussion

The current study is unique in identifying a number of key gait differences in patients with DPN and associations with Achilles tendon stiffness, limited ankle/foot joint dorsiflexion and plantar pressures, which are proposed as novel mechanisms underpinning the risk of diabetic foot ulcers.

Key finding: Heel-rise timings and relationship to the ankle/foot mechanics

The key focus of the current study was to assess associations between changes in gait, and the mechanics of loading around the ankle-foot complex, enabling assessment of how different gait alterations impact diabetic foot ulcer risk. The study showed that patients with DPNs raised their heel earlier in the gait cycle (DPN 0.47s vs Ctrl 0.50s), which resulted in earlier and therefore prolonged forefoot loading. The DPN group showed earlier heel rise timings, despite longer stance durations, further elucidating how the pressure on the forefoot would be higher for longer as demonstrated in Chapter 1. In Chapter 2, it was shown that DPN patients have a stiffer Achilles tendon and reduced ankle dorsiflexion, leading to the hypothesis that a stiffer ankle-foot joint complex would result in earlier heel-rise and therefore prolonged forefoot loading during gait. This study confirms the hypothesis of an earlier heel rise, which is underpinned by the factors described above resulting in the heel being pulled off the ground as the tibia rotates forward over the ankle during mid-stance gait. This pulling of the heel off the ground causes earlier transition of weight onto the forefoot, as demonstrated by greater forefoot pressures, shown by increased peak pressures and pressure-time integrals (Chapter 1), in addition to and furthermore by the correlations between forefoot pressure-time integral and earlier heel-rise timings.

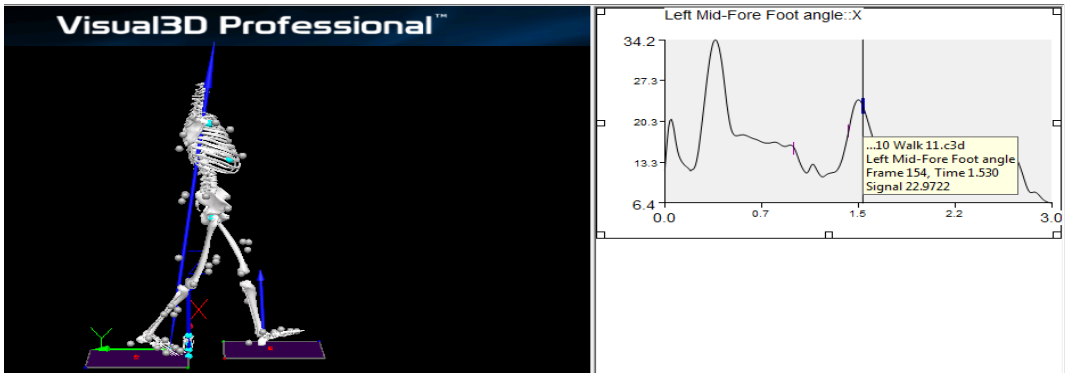

Figure 30 –Heel rise event demonstrated in Visual 3D.
X-axis graph shown to highlight gait events during Heel rise. A current study DPN participant demonstrating early heel rise.

Heel rise demonstrated moderate-to-strong correlations (-0.441) with Achilles tendon stiffness and (0.391, 0.369) ankle dorsiflexion (measured during gait and isolated dynamometry testing) supporting the link between increased Achilles tendon stiffness, limited ankle-foot dorsiflexion and an earlier heel rise. Following from this, the study found moderate correlations (-0.375) between heel rise and forefoot plantar pressures, supporting the hypothesis that increased Achilles tendon stiffness, limited ankle-foot dorsiflexion leads to an earlier heel rise and elevated forefoot plantar pressure, increasing the risk of diabetic foot ulceration.

Therefore, the theory proposed here for the occurrence of earlier heel-rise is that of stiffer soft-tissue around the ankle joint decreasing the dorsiflexion range-of-motion of the joint, resulting in the shank pulling the heel off the ground sooner, as the tibia cannot rotate as far forward over the ankle during stance. The present study has shown an association between isolated dynamometry measures of assisted ankle dorsiflexion and Heel Rise (rho=0.369*) and peak dynamic ankle dorsiflexion and early heel rise (EHR) component of time (rho=0.391*) and % stance (rho=0.564**), pointing towards limited ankle dorsiflexion as a key factor in the early heel rise during the stance phase of gait.

It has been demonstrated (in Chapter 2) that when compared with Controls, DPNs showed higher Achilles tendon stiffness , which indicated association with increased non-enzymatic glycation reactions culminating into higher levels of AGEs , which is an independent risk factor for Diabetic foot ulcers (367). Significant correlations of early heel rise elements have been observed with AGEs level (rho= -0.497**), tendon stiffness (rho= 0.497**) and tendon elongation (0.511**). Also, ankle angle during heel rise showed a significant correlation (rho= 0.490**) with Achilles tendon Stiffness. These factors indicate a strong association between Achilles tendon stiffness and early heel rise during the stance phase of gait. Both of these variables have marked association with other established independent markers (PPP, Hallux Valgus angle, Vertical GRF, Gait velocity) of DFU risk.

In chapter 1, higher peak plantar pressures at the forefoot in DPN patients were observed in comparison to Controls. The current study found significant inverse correlations between heel rise time and Peak plantar pressure at total foot (rho=-0.363*) and forefoot (rho= -0.375*). Also, significant correlations of Heel Rise have been observed with plantar shear forces (rho= -0.376*) and concentric peak power (rho=0.375*). These results indicate

a role for early heel rise during the stance phase of gait in elevated forefoot plantar pressure experienced by patients with DPN.

These significant differences in between group comparisons and substantial correlations of Heel rise with stiffness, ankle dorsiflexion and forefoot pressures, amply indicate possibility of earlier and prolonged forefoot loading in DPNs, which is a proposed risk for DFU.

Role of Achilles tendon stiffness in altered Gait

The present study has shown significant correlations between Achilles tendon stiffness and Gait parameters of dynamic ankle dorsiflexion (rho= -0.484**), peak flexion at midfoot-forefoot (rho= -0.553**), Shear forces at forefoot (rho=0.456*), vertical ground reaction force (rho=0.644**) and Gait velocity (rho= -0.479*). As discussed in earlier chapters' (Chapter 2) Achilles tendon stiffness has a strong correlation with plantar pressures and other demographic variables like diabetes duration, AGEs, VPT and mNDS. Therefore, these results further reinforce the link between increased Achilles tendon stiffness, limited ankle dorsiflexion, early heel rise and elevated plantar pressures increasing the risk of DFU. If the Muscle activity at dorsiflexors and plantarflexors is also assessed while walking, there is a possibility that co-contraction will stiffen joints. If tendon and muscle stiffness is increased, this may mean DPN also alter their co-contraction levels. e.g. given the Achilles tendon is stiffer, do DPN patients activate the agonist (tibialis anterior) more during initial contact to control pronation and compensate for the stiffer Achilles tendon and Gastrocnemius. This can be a useful input for further research on this matter.

Gait alterations role in forefoot plantar pressure

In the current study Gait Speed showed significant correlations with pressure time integral at Total foot* and Toes (T2**, T3**, T45*), and also significant correlations were observed with peak plantar pressures at total foot*, Toes (T2*, T3**, T45**) and 1^{st*} and 3^{Rd*} Metatarsal. Loading method of medialisation is when faster speeds reduce pressure under the foot (368–370). In experimental chapter 1 on plantar pressures, the current study found, the peak plantar pressure in the DPN group was similar to previous studies using insoles for measurements of pressure, where participants walked at natural self-selected speeds (371).

Gait variables as independent risk factors of diabetic foot

The spatiotemporal investigations in current study revealed that as regards propulsion parameters, DPNs had a lower Gait velocity, a wider stance width (Ctrls 0.11m vs DPN

0.13m) and a longer double limb support time when compared with controls. Mostly all previous studies dealing with Gait in DPNs have reported similar results of decreased gait velocity and an increased base of gait (372–376). Altered ankle stiffness will have impacts upon the range of motion, which may impact gait speed and step length. Because, a lower range of motion is required for shorter step lengths. Therefore, patients with DPN may shorten step length to keep within their ability. Increasing the base of gait is thought to be a compensatory strategy adopted by DPNs to increase stability and balance during ambulation (374,377). Nevertheless, the results of gait analysis in general and spatiotemporal investigations in particular, with diabetic peripheral neuropathy participants at risk of diabetic foot and impaired/altered gait, can fluctuate as per inclusion criteria, severity of neuropathy, methods of data acquisition and the placement/precision of sensors technology utilised. Previous studies have reported that the conservative gait strategies adopted by DPNs resulting in decreased walking speed, increased base of gait and longer duration of double limb support, serve as red flag warnings for advancing neuropathy and foot ulcer risk (374,378).

In the current study, the findings on spatiotemporal differences between groups can be possibly explained through the assumption that the DPN group during gait progression, experienced a reduction in stability during single limb support (379). Previous studies have identified similar impairments in elderly with DPN, which is the issue of difficulty maintaining balance when transferring from bipedal to unipedal stance and during the termination of gait (380), which is evident in the current study, where DPNs were spending a longer time in double limb support (DPNs: 0.35s vs Controls: 0.17s). Therefore, from the current study outcomes, it seems that patients with DPN may be needing to take a series of shorter and wider steps due to experiencing postural instability, adjusting by spending longer durations in stance and double limb support; this results in decreased gait velocity along with less time to adjust trajectories of progression and disturbed frontal plane foot placement in foot progression. This condition when visualised with a reduced ability to ankle dorsiflex at loading response and diminished peak flexion at stance, leads to early and prolonged forefoot loading with elevated vertical GRF and shear forces. The increased peak pressure on medial forefoot regions renders DPN patients at higher risk of DFU.

In the current study, the DPN group have shown lower dynamic peak flexion during walking at midfoot-forefoot (Figure 28). Investigations revealed a difference in Peak flexion at midfoot-forefoot difference during stance phase between controls and DPNs by up to 4

deg. This is a novel finding, as midfoot-forefoot angle peaking ability despite being a critical conduit of stance to swing phase transition, has been overlooked in previous studies. A previous study by Jeong et al. (381) with different foot inter-segment definition, had suggested that DPN patients have lower flexion ability (mean difference of 3 deg) than controls in the midfoot region during heel rise, associating midfoot dysfunction of gait with "foot at risk" in people with DPN. Akin to stiffer ankle, stiffer mid-foot anatomy may alter loading on the entire foot including the forefoot.

Gait Speed and lifestyle choices

Lifestyle included self-reporting on adherence levels to daily routines on exercise, diet, and other daily activities. In the current study, a highly significant correlation between Gait speed and lifestyle was observed, which corroborates with other studies reporting similar results (382,383). DPNs self-reported lower adherence to lifestyle choices as compared to controls and this has been reported earlier in Chapter 1. Previous studies suggest that that DPNs are less likely to get the recommended amount of exercise per week and also tend to walk slower and less (384). The current study has shown that DPN patients have a slower speed and different lifestyle choices, as compared to controls. Retrospective studies involving people with previous diabetic foot ulcers concluded that this tendency of slower steps, inadequate exercise, reduced daily activities could represent a high-risk biomechanical characteristic leading to foot ulceration (385,386). This provides current study another reinforcement to fact of higher foot risk for DPN individuals.

In the current study, gait variables like gait velocity showed a strong correlation (0.648) with MTPJ dorsiflexion, dynamometry Voluntary ankle dorsiflexion (0.735), AGEs score (-0.662) and Hallux valgus angle (-0.691), which meant that gait velocity will be positively associated with increased ankle and MTPJ dorsiflexion, while inversely associated with AGEs level and hallux valgus angle. AGEs and foot deformity are known independent markers of risk for diabetic foot ulcer. Lowered foot ankle complex DF is associated with DFU risks. Therefore, all the independent variables when correlated with gait speed refer to its further association with diabetic foot risks, by this analogy. Concentric peak power (leading to shortening of plantar flexors). Other gait parameters like Vertical GRF, stride length, ankle dorsiflexion and overall range of motion at ankle during walking showed associations with AGEs Hallux Valgus angle, indicating that substantial number of Gait parameters can indicate risks of foot ulceration.

Between groups comparisons (DPN vs Controls) have revealed that DPNs showed reduced ankle dorsiflexion, both during dynamometry assessments (Table 24) as well while walking. The correlation was even stronger when passive limits of range of motion were induced during dynamometry assessment. As in the current and a substantial number of previous studies, reduced dorsiflexion has been shown as factor enhancing DFU risks. This current study also indicates that either of three methods of assessment of dorsiflexion are independently associated with each other and they can be interchangeably used as risk indicators for diabetic foot risks.

In the current study, between groups comparisons showed significantly lower Peak Dorsiflexion and overall Range of motion in DPNs while walking, as compared to controls. This assessment is quite in line with previous studies with DPN (387,388) indicating lower dorsiflexion as a foot at risk, indicator. This study also investigated the association between isolated dynamometry-based measurements and dynamic gait measures of ankle joint sagittal plane motions. Isolated dynamometry-based measurements data of dorsiflexion and overall range of motion from Voluntary and Assisted efforts was linked with same components dynamic data. Significant correlations were observed between voluntary dorsiflexion (rho=0.475*) and corresponding peak dorsiflexion, while walking. Isolated dynamometry-based assisted dorsiflexion, as expected showed higher correlation (rho=0.520**) with dynamic peak dorsiflexion.

Ankle and MTPJ Range of Motion

In current study, DPNs had shown a lower static Voluntary ankle Dorsiflexion ($p<.001$) and diminished ability of Dorsiflexion at Metatarsophalangeal joint. Reduced ankle joint mobility could be an aberrant functioning of the collagen materials in locomotion tendons and muscles. Studies have reported reduced MTPJ RoM in DFU populations (389). DPNs have shown pattern of gait changes including decreased great toe dorsiflexion during propulsion (390). The association between reduced ankle joint mobility and higher neuropathic foot ulceration was demonstrated by Delbridge et al. (391).

The current study through its experimental chapters on plantar pressures and tendon stiffness, found significant correlation in AGEs accumulation, ankle (Achilles tendon) stiffness, static ankle dorsiflexion and plantar pressure.

Foot mobility parameters showed considerably lower angles of movement in DPNs during between groups comparisons. These angles include Ankle angle, Foot progression angle

(Figure26) and mid-foot forefoot angle. This indicates that foot mobility is constrained in case of DPNs. Lower foot mobility is an indicative of diabetic foot ulceration risk (392)

Role of plantar forces, moments and power

DPNs have impaired foot mobility and reduced maximal muscle strength in foot ankle joint complex (393). A previous study demonstrated that Dorsiflexion strength was ∼60% less in DPN than controls (394). The current study has shown DPNs have lower peak powers and lower moments (<.05). This means that DPNs may be positioning their foot differently i.e. maybe they land with a flatter foot to reduce the demand! The current study has shown that DPN have a gait strategy different than Controls viz. slow speed, wider base, and higher toe-out angle during foot progression and also from landing at HS (Heel strike) with a lower foot angle and finally the decreased dorsiflexion at heel strike. These conservative gait strategies of DPNs might be resulting in uneven plantar pressure distribution in a progressively detrimental mien during walking, thus leading to higher foot loading and ulceration risk.

A change in stride length alters both ankle joint moment and foot pressure (395). Gait modifications are generally observed for compensation in DPNs (396). Distribution of plantar pressure within plantar region also becomes significant (397). Increased forces especially shear is linked to ulcer risk (398) as also verified to certain extent in current study by higher mediolateral (XY) ground reaction force integrals observed in DPNs ($p<.05$) as compared to controls. Similarly, vertical GRF differences were substantially higher in DPN when compared to controls (Ctrl 324N vs DPN 551N, $p<.001$) in current study. Vertical forces cause increased and longer duration pressure on forefoot (399). It is worth to note the fact that there is a difference observed in current study between moments of DPNs vs Ctrls, while when ANCOVA was carried out with Gait velocity as a covariate, it showed no difference between groups (Table 18). I.e. which means if they walked at the same speed there wouldn't be a difference? So, the difference in groups for moments is explained by the fact they were walking slower. In kinetic variables of present study, the Ankle concentric peak power was significantly lower in DPNs when compared to Controls. Similarly, plantar flexion and dorsiflexion peak moment were significantly lower in DPNs, when compared with Ctrls. (Table 18), which indicate towards higher diabetic foot ulcer risks.

It has been observed that when compared with controls the DPNs have shown characteristically reduced ability in kinetic aspects of the gait concerning with moments,

mobility in foot ankle complex (400). Citing from earlier chapter 1 on clinical factors of sensory impairment (presence of severe neuropathy, impaired vibration and protective sensation) and this experimental chapter's Gait analysis showing decreased lower-extremity strength (force-producing capacity), are believed to contribute to impaired gait in DPNs (401). Gait alterations need to studied in more details to device mechanisms to have minimum impact on ulcerated foot (402)

11.6 CONCLUSION

Marked gait differences between DPN and Control groups, followed by highly demonstrable correlations with at risk foot parameters suggest that increased Achilles tendon stiffness and limited ankle dorsiflexion in patients with DPN cause an earlier heel rise, contributing to earlier and more prolonged forefoot loading, predisposing a higher risk of DFU.

12 OVERALL DISCUSSION

The work of this thesis aimed to investigate the effect of novel biomechanical and neuropathic factors underpinning diabetic foot ulcer development. Specifically, the association between Achilles tendon stiffness, limited ankle-foot dorsiflexion and altered loading of the foot during gait were examined for their effects on plantar pressure development as a proxy for diabetic foot ulcer risk. The thesis presents a series of cross-sectional studies conducted in with people with severe diabetic peripheral neuropathy and healthy controls.

This thesis considered contributory factors to altered foot loading, foot deformities, gait disturbances, soft tissue alterations that produce abnormally high forces of pressure as further risk factors for diabetic foot ulcer. Achilles tendon function in humans can influence foot function and is particularly relevant in people with diabetes for the development of diabetic foot ulcers. Linked to Achilles tendon stiffness, the study also examined the relationship between ankle dorsiflexion range of motion, and ankle joint passive tendon stiffness, with plantar foot pressure loading and ulceration risk during gait. The study attempted to address some key questions for the at-risk patient population viz. peak dorsiflexion range of motion at the foot-ankle complex, Achilles tendon stiffness, altered plantar forefoot pressure loading during gait, thereby attempting to combine information and key theories suggested by past work regarding the effect of diabetes upon stiffening tissues and its role in development of ulceration.

Establishing appropriate plantar pressure parameters

Diabetic peripheral neuropathy (DPN) a major complication of diabetes impairs sensory and motor functions of the foot and ankle region causing musculoskeletal limitations that result in altered plantar pressures. It was hypothesised that DPN patients will have elevated foot pressure distribution compared to controls and this will be reflected more clearly in certain pressure variables than others. This experimental chapter aimed to establish the most relevant pressure parameter(s) to act as a proxy for diabetic foot ulcer risk. Four key pressure variables were investigated: peak pressures, pressure-time integrals (force and pressure), force-time integral and a stance averaged peak pressure. Peak plantar pressure and pressure-time integrals significantly correlated with established markers of diabetic foot ulceration including duration of diabetes, advanced glycated end products and severity of peripheral neuropathy (neuropathy disability score and vibration perception

threshold) identifying them as the most appropriate proxy measures for DFU risk and taken forwards to be used in the subsequent chapters of the thesis.

Foot at risk demonstration

Socio-demographic parameters showed significant variations between controls and DPN groups. Anthropometric parameters e.g. body mass and BMI were higher in DPNs, while lifestyle habits and wellbeing scores were lower in the DPN population compared to controls. Clinical variables including neuropathy status, were assessed during the current study, as factors along with plantar pressures, associated with the likelihood of ulceration (182). A high mean (modified) Neuropathic Disability Score (8/10) in the DPN population under study, was also a significant risk factor for diabetic foot ulceration (185). The LOPS variables of monofilament testing and IpTT showed marked differences ($p<.001$) between controls and DPNs. Wellbeing and lifestyle scores were lower in the DPN group as compared to controls. People with low daily activity have alteration in foot morphology and material properties making them susceptible to plantar soft tissue disruptions (191). Therefore, all major clinical variables demonstrated that the DPN population represent a higher risk of foot ulceration compared to the control group, providing a suitable comparison for identifying differences in pressures relevant to ulcer risk.

This study suggests that individuals with DPN and DFUs have elevated PPP and PTI, and their risk of ulceration was highly associated with the severity of neuropathy and high PPP variables especially the forefoot peak plantar pressure.

The PPP across the total foot and forefoot regions (Metatarsals and Toes) showed correlations with key risk factors of diabetic foot (204) which can lead to ulcerations (141,205). In comparison to PPP, whilst PTI correlated with the duration of diabetes, the lack of correlation with sensory loss variables identified it as a less useful predictor of DFU. Similarly, the inverse associations seen with FTI again, whilst of potential further interest regarding loading strategy alterations, it is unlikely to show a route to DFU, limiting the potential of FTI as an indicator of DFU risk. Most previous studies have studied PPP as a general parameter for associations with small fibre neuropathy, and this study has attempted to visualize specific plantar regions also to define regional PPP relationships with neuropathy severity.

Role of Achilles tendon stiffness in forefoot pressure development

People with diabetes are known to have high collagen cross-linking and Advanced Glycated Endproducts (AGEs) accumulation leading to stiffer tissues including increased Achilles

tendon stiffness. This change in tissue mechanical properties is likely to exert an impact upon biomechanical behaviour during movements. One key suggestion is that stiffer ankle joint complexes may alter the loading of the foot and therefore the pressures experienced under the foot. This thesis presents data showing that Achilles tendon stiffness in people with diabetic peripheral neuropathy was significantly higher than controls (DPN 80 Nmm-1 vs Ctrl 53Nmm^{-1}).

Achilles tendon stiffness was moderately correlated with forefoot peak plantar pressure (rho=0.387). Patients with DPN were seen to have higher tendon stiffness compared to controls and thus reduced ankle-foot dorsiflexion; additionally this was linked to higher forefoot plantar pressures, thereby indicating the increased stiffness of tissues in people with diabetes as a potential risk factor for foot ulceration. Ankle and Metatarsophalangeal joint (MTPJ) dorsiflexion range of motion was significantly smaller in DPN patients compared to controls. Key correlations between Achilles tendon stiffness and ankle joint dorsiflexion and MTPJ dorsiflexion were observed, implying that elevated tendon stiffness and limited ankle and MTP joint dorsiflexion could be contributory factors for elevated forefoot pressures (314). This increased forefoot pressure could lead to tissue breakdown and the progression towards diabetic foot ulcers in the DPN population (315,316).

In the current study, ankle joint voluntary/assisted dorsiflexion range of motion and peak plantar pressure showed significant inverse correlations with whole foot, forefoot and first metatarsal (Table 11), while MTPJ dorsiflexion showed a significant inverse correlation with peak plantar pressures at the first metatarsal and forefoot. These inverse correlations between peak pressure and ankle/MTPJ dorsiflexion range of motion indicate that as dorsiflexion decreases, plantar pressure increases. These findings are novel in highlighting the important relationship between increased Achilles tendon stiffness, reduced ankle-foot dorsiflexion and increased plantar pressures. These results support the notion that increased Achilles tendon stiffness and limited ankle-foot dorsiflexion are major contributory factors in elevated forefoot plantar pressures and therefore increased DFU risk.

In the current study, Young's modulus's was significantly higher in the DPN group (Ctrls 133 MPa vs. DPN 173 MPa) indicating a change to the tendon material; this mechanism is a major factor in the increase of tendon stiffness (339). Similarly the cross sectional area was increased in DPNs when compared with controls (Ctrls 71mm^2 vs DPNs 84 mm^2); indicating a change of the tendon morphological properties, which may also be responsible for

enhanced tendon stiffness (340). Thus the in vivo human Achilles tendon mechanical and material characteristics investigated during the current experimental study do support the hypothesis that patients with DPN will have higher tendon stiffness compared to controls and thus reduced ankle-foot dorsiflexion; this is linked to higher forefoot plantar pressures, thereby increasing the risk of diabetic foot ulceration in this foot region.

Relationship between dynamic gait biomechanics, ankle stiffness and plantar pressures

The final objective of the current thesis was to investigate the hypothesis that alterations to gait strategy will vary between people with diabetic peripheral neuropathy (DPN) and healthy controls and would be associated with the changes to mechanical pressure and ankle joint stiffness properties reported.

Heel rise occurred 10% earlier during the stance phase in the case of DPN patients as compared to Controls, resulting in earlier and therefore prolonged forefoot loading. This was despite longer stance durations in the DPN group, further elucidating how the pressure on the forefoot would be higher for longer as demonstrated in earlier plantar pressure study. This confirmed the hypothesis generated following assessment of Achilles tendon stiffness that DPN patients have a stiffer Achilles tendon and reduced ankle dorsiflexion, leading to the hypothesis that a stiffer ankle-foot joint complex would result in earlier heel-rise and therefore prolonged forefoot loading during gait. This study confirms the hypothesis of an earlier heel rise, in addition to and further supported by the correlations between forefoot pressure-time integral and earlier heel-rise timings. Therefore, the theory proposed here for the occurrence of earlier heel-rise is that of stiffer soft-tissue around the ankle joint decreasing the dorsiflexion range-of-motion of the joint, resulting in the shank pulling the heel off the ground sooner, as the tibia cannot rotate as far forward over the ankle during stance.

The present study has shown an association between isolated dynamometry measures of assisted ankle dorsiflexion and heel rise (rho=0.369*) and peak dynamic ankle dorsiflexion and early heel rise (EHR) component of time (rho=0.391*) and % stance (rho=0.564**), pointing towards limited ankle dorsiflexion as a key factor in the early heel rise during the stance phase of gait.

It has been demonstrated (in Chapter 2) that when compared with Controls, DPNs showed higher Achilles tendon stiffness , which indicated association with increased non-enzymatic glycation reactions culminating into higher levels of AGEs , which is an independent risk

factor for DFU (367). Significant correlations of early heel rise elements have been observed with AGEs level (rho= -0.497**), tendon stiffness (rho= 0.497**) and tendon elongation (0.511**). Also, ankle angle during heel rise showed a significant correlation (rho= 0.490**) with Achilles tendon Stiffness. These factors indicate a strong association between Achilles tendon stiffness and early heel rise during the stance phase of gait. Both of these variables have marked association with other established independent markers (PPP, Hallux Valgus angle, vertical GRF, gait velocity) of DFU risk.

In chapter 1, higher peak plantar pressures at the forefoot in DPN patients were observed in comparison to Controls. The current study found significant inverse correlations between heel rise time and Peak plantar pressure at total foot (rho=-0.363*) and forefoot (rho= -0.375*). Also, significant correlations of heel rise have been observed with plantar shear forces (rho= -0.376*) and concentric peak power (rho=0.375*). These results indicate a role for early heel rise during the stance phase of gait in elevated forefoot plantar pressure experienced by patients with DPN. These significant differences in between group comparisons and substantial correlations of Heel rise with stiffness, ankle dorsiflexion and forefoot pressures, amply indicate possibility of earlier and prolonged forefoot loading in DPNs, which has been proposed as a risk for DFU.

In the current study, the DPN group have shown lower dynamic peak flexion during walking at midfoot-forefoot. Investigations revealed a difference in Peak flexion at midfoot-forefoot difference during stance phase between controls and DPNs by up to 4 deg. This is a novel finding, as midfoot-forefoot angle peaking ability despite being a critical conduit of stance to swing phase transition, has been overlooked in previous studies. A previous study by Jeong et al. (381) with different foot inter-segment definition, had suggested that DPN patients have lower flexion ability (mean difference of 3 deg) than controls in the midfoot region during heel rise, associating midfoot dysfunction of gait with “foot at risk” in people with DPN. Akin to stiffer ankle, stiffer mid-foot anatomy may alter loading on the entire foot including the forefoot.

The present study has shown significant correlations between Achilles tendon stiffness and gait parameters of dynamic ankle dorsiflexion (rho= -0.484**), peak flexion at midfoot-forefoot (rho= -0.553**), Shear forces at forefoot (rho=0.456*), vertical ground reaction force (rho=0.644**) and gait velocity (rho= -0.479*). As discussed in earlier chapters’ (Chapter 2) Achilles tendon stiffness demonstrated a strong correlation with plantar

pressures and other demographic variables like diabetes duration, AGEs, VPT and mNDS. Therefore, these results further reinforce the link between increased Achilles tendon stiffness, limited ankle dorsiflexion, early heel rise and elevated plantar pressures increasing the risk of DFU.

The spatiotemporal investigations in the current study revealed that as regards propulsion parameters, DPNs had a lower gait velocity, a wider stance width (Ctrls 0.11m vs DPN 0.13m) and a longer double limb support time when compared with controls. Previous studies dealing with gait in DPNs have reported similar results of decreased gait velocity and an increased base of gait (372–376). Altered ankle stiffness will have impacts upon the range of motion, which may impact gait speed and step length. Because, a lower range of motion is required for shorter step lengths. Therefore, patients with DPN may shorten step length to keep within their ability. Increasing the base of gait is thought to be a compensatory strategy adopted by DPNs to increase stability and balance during ambulation (374,377).

Previous studies (382,383) suggest that that DPNs are less likely to get the recommended amount of exercise per week and also tend to walk slower and less (384). The current study has shown that DPN patients have a slower speed and different lifestyle choices, as compared to controls. Retrospective studies involving people with previous diabetic foot ulcers concluded that this tendency of slower steps, inadequate exercise, reduced daily activities could represent a high-risk biomechanical characteristic leading to foot ulceration (385,386). This provides current study another reinforcement to fact of higher foot risk for DPN individuals.

In the current study, gait variables like gait velocity showed a strong correlation (0.648) with MTPJ dorsiflexion, dynamometry Voluntary ankle dorsiflexion (0.735), AGEs score (-0.662) and Hallux valgus angle (-0.691), which meant that gait velocity will be positively associated with increased ankle and MTPJ dorsiflexion, while inversely associated with AGEs level and Hallux valgus angle. AGEs and foot deformity are known independent markers of risk for diabetic foot ulcer. Lowered foot ankle complex Dorsiflexion is associated with DFU risks. Therefore, all of the independent variables when correlated with gait speed refer to its further association with diabetic foot risks, by this analogy. Other gait parameters like Vertical GRF, stride length, ankle dorsiflexion and overall range of motion

at ankle during walking showed associations with AGEs Hallux Valgus angle, indicating that substantial number of Gait parameters can indicate risks of foot ulceration.

Between groups comparisons (DPN vs Controls) have revealed that DPNs showed reduced ankle dorsiflexion, both during dynamometry assessments as well while walking. The correlation was even stronger when passive limits of range of motion were induced during dynamometry assessment. The current study also indicates that either of three methods of assessment of dorsiflexion are independently associated with each other and they can be interchangeably used as risk indicators for diabetic foot risks.

In the current study, between groups comparisons showed significantly lower Peak Dorsiflexion and overall Range of motion in DPNs while walking, as compared to controls. This assessment is quite in line with previous studies with DPN (387,388) indicating lower dorsiflexion as a foot at risk, indicator. In current study, DPNs had shown a lower static Voluntary ankle Dorsiflexion ($p<.001$) and diminished ability of Dorsiflexion at Metatarsophalangeal joint. Reduced ankle joint mobility could be an aberrant functioning of the collagen materials in locomotion tendons and muscles. Studies have reported reduced MTPJ RoM in DFU populations (389). The association between reduced ankle joint mobility and higher neuropathic foot ulceration was demonstrated by Delbridge et al. (391).

The current study through its experimental chapters on plantar pressures and tendon stiffness, found significant correlation in AGEs accumulation, ankle (Achilles tendon) stiffness, static ankle dorsiflexion and plantar pressure. Foot mobility parameters showed considerably lower angles of movement in DPNs during between groups comparisons. These angles include Ankle angle, Foot progression angle and mid-foot forefoot angle. This indicates that foot mobility is constrained in case of DPNs. Lower foot mobility is an indicative of diabetic foot ulceration risk (392). DPNs have impaired foot mobility and reduced maximal muscle strength in foot ankle joint complex (393). A previous study demonstrated that Dorsiflexion strength was ∼60% less in DPN than controls (394). The current study has shown DPNs have lower peak powers and lower moments ($<.05$). This means that DPNs may be positioning their foot differently i.e. maybe they land with a flatter foot to reduce the demand! The current study has shown that DPN have a gait strategy different than Controls viz. slow speed, wider base, and higher toe-out angle during foot progression and also from landing at HS (Heel strike) with a lower foot angle and finally the

decreased dorsiflexion at heel strike. These conservative gait strategies of DPNs might be resulting in uneven plantar pressure distribution in a progressively detrimental mien during walking, thus leading to higher foot loading and ulceration risk.

Distribution of plantar pressure within plantar region also becomes significant (397). Increased forces especially shear is linked to ulcer risk (398) as also verified to certain extent in current study by higher mediolateral (XY) ground reaction force integrals observed in DPNs ($p<.05$) as compared to controls. Similarly, vertical GRF differences were substantially higher in DPN when compared to controls (Ctrl 324N vs DPN 551N, $p<.001$) in current study. Vertical forces cause increased and longer duration pressure on forefoot (399).

It has been observed that when compared with controls the DPNs have shown characteristically reduced ability in kinetic aspects of the gait concerning with moments, mobility in foot ankle complex (400). Citing from earlier chapter 1 on clinical factors of sensory impairment (presence of severe neuropathy, impaired vibration and protective sensation) and this experimental chapter's Gait analysis showing decreased lower-extremity strength (force-producing capacity), are believed to contribute to impaired gait in DPNs (401). Gait alterations need to studied in more details to device mechanisms to have minimum impact on ulcerated foot (402).

Marked gait differences between DPN and Control groups, followed by highly demonstrable correlations with at risk foot parameters suggest that increased Achilles tendon stiffness and limited ankle dorsiflexion in patients with DPN cause an earlier heel rise, contributing to earlier and more prolonged forefoot loading, predisposing to a higher risk of diabetic foot ulcers.

12.1 FUTURE RESEARCH AND WAY FORWARD

Prediction algorithms for diabetic foot ulcer risks

A multiple regression can be carried out among all the study outcomes/variables to prioritise significant variables based on their strength of correlations with diabetic foot ulcer risk. A comprehensive list of established and novel variables of interest identified during course of this study (along with substantial number of other experiments carried out during this study e.g. foot geometry, deformities, proprioception, balance & posture etc. but were not elucidated in present study to maintain remit/scope of present study)

can be statistically analysed and a significance cut-off can be decided for inclusion of variables in algorithm. This can lead to a DFU risk prediction algorithm design.

Decision Support System for Non-invasive, minimally invasive and surgical interventions. In basic clinical settings, utilization of few devices and visual observations can support decision tree algorithms during routine screenings during patient visits.

The study outcomes have indicated tendon stiffness, among others, as a factor leading to earlier and higher forefoot loading, which is assessible in clinical settings Similarly, the present study has also indicated on impaired ankle dorsiflexion range of motion as a factor contributing to higher and earlier loading of forefoot. Therefore, a simple goniometer can be used to assess ankle DF RoM. Other factors like hallux valgus angle, Arch height, proprioception etc. can also be assessed using basic measurement devices. A simple walk examination can also reveal factors of gait speed, foot drop, stride length and width etc. Severity level cut-offs for each variable can be worked out and a logical decision tree can be used by clinicians/podiatrists to ascertain risk prevention suggestions. Besides, in early stage of people at risk of DFU, tendon stretching exercise regimes can be introduced as preventive measure in cases of people with stiffer tendons e.g. bespoke eccentric heel drop protocol can be designed. As per IWGDF recommendations (403) for people with DFU risk (IWGDF risk category low to moderate) should perform foot and mobility-related exercises aimed at reducing risk factors of ulceration, i.e. decreasing peak pressure and increasing foot and ankle range of motion, and with the aim of improving neuropathy symptoms. It has been observed that inducing mechanical load is therapeutic regardless of how tendon is loaded during stretching exercises. Lower limb exercises are preventive and conservative measures, where clinicians don't have to change the pathology to have a good outcome, as exercises can improve functioning, despite the pathology remaining. These exercises can help Achilles tendon and the foot-ankle muscle-tendon complex biomechanically robust, which can induce increased capacity to tolerate raised demands owing to foot deformities and uneven plantar pressure distribution. Other interventions include Corticosteroid injections (which demonstrate short-term pain relief), Sclerosering injection (the research suggests a clinical role for sclerosis therapy for those who fail with eccentric exercises), & Platelet-rich plasma injections or PRP (which demonstrated no positive effect aside from tendon thickening when compared to placebo). A systematic review and meta-analysis by Sarah et al. (404) has discussed utility of surgical interventions like tendon lengthening and fascia release for healing and preventing diabetic foot ulcers, which suggested that Achilles

tendon lengthening and gastrocnemius recession appear to be effective surgical treatments for healing diabetic foot ulcers. Other operative interventions include percutaneous longitudinal tenotomies, minimally invasive tendon stripping, open tenosynovectomies, open debridement and tubularization, and tendon augmentation with flexor hallucis longus. Thus, the study outcomes along with other decision tools can assist clinicians in making treatment plans which may include considering exercise regimens for tendon stretching, delaying invasive interventions or referring for earliest invasive interventions.

Neuromodulation, Motor and sensory nerve stimulation

Extensive research is already underway in the field of peripheral nerve stimulation for assistive locomotive movements. Miniature rechargeable implantable pulse generator (RIPG) are being used in other cases of impaired movements. Other sensors e.g. accelerometers that allow the RIPG to sense whether the patient is sitting or lying on his or her back or side and to automatically adjust programmes that have been preselected in each position or activity. Other new technique (with regulatory approvals) going through clinical trials may allow more precise and even stimulation, where fine electrodes with 4 contacts are threaded through the epidural space and allowed to lie up against tissue known as the sensory dorsal root ganglia. The technique can selectively stimulate different areas, which allows focussing of stimulation onto specific nerve roots or parts of nerve roots. Due to the local anatomy, the stimulation remains relatively even when the patient moves. Such low power is required that a non-RIPG will suffice with excellent device longevity. The UK Health policymaking advisory group, the National Institute of Clinical Excellence (NICE), issued guidance that neurostimulators should be used for refractory neuropathic pain, finding it both effective and cost-effective, with lower lifetime healthcare cost and better long-term outcomes (405). It is likely that in the future, medical implants that provide neurostimulation will be as commonplace as heart pacemakers are today. This study has taken a small step in advising that gait alterations (reduced ankle DF, early heel rise etc.) may be responsible for earlier and elevated loading of forefoot and uneven distribution of plantar pressures, thus, similar neuromodulations can be used for prevention and treatment for people at higher risk of DFUs.

Future Research possibilities and financial impact of present study

The present study can serve as a way forward for the future direction of research in early clinical screening for risk profiling of the diabetic foot. Early detection and prevention of a DFU can impact pathways of delivering foot care to patients with diabetes. The proposed study outcomes will potentially play a role in the 80% of lower limb amputations that are considered avoidable and a substantial share in reducing the present financial burden of £1 billion pounds on diabetic foot spent by healthcare system of U.K.

12.2 Overall conclusion

The interlinked series of experiments presented in this thesis have proposed significant factors affecting foot loading and ulceration in people with diabetic peripheral neuropathy. The proposed thesis hypothesis that increased Achilles tendon stiffness would be associated with an increased ulcer risk, through the development of elevated forefoot pressures, was confirmed by the phenomenon of Achilles tendon stiffening (effected by excessive accumulation of AGEs by collagen glycation) causing reduced ankle dorsiflexion and early heel rise, which was also related to hastened and higher plantar loads in frontal region. These significant differences observed in DPNs as compared to Controls, were found to be highly correlating with recognised risk factors of diabetic foot Ulcers.

The outcomes of the above experimentation results can be extrapolated with diabetic foot ulcers aetiology by associating them with established previous researches on diabetic foot ulcers. It can be reasoned that these patterns of abnormal plantar loading while walking in DPNs with repetitive stress at same plantar sites, can be causing tissue inflammation and formation of hyperkeratotic, hard skin or callus (406). The 'normal' foot has the 'gifts' of Mother Nature in the form of proprioception for navigation and spatial management and sense of pain when interacting with adverse situation or trauma. Both these mechanisms are altered or impaired in DPNs besides the protective sensation is lost, thus the component of even distribution of pressures becomes the main cause for aberrant foot loading(407), although gait balancing adjustments are made during progression(408). Such Chronic irritations over a longer period of time also caused by events explained in current study (and sometimes hastened by sudden external trauma) causes enzymatic autolysis with tissue breakdown and ulceration (409).

12.3 SALIENT POINTS OF STUDY

- The thesis presents a series of cross-sectional studies conducted in people with diabetic peripheral neuropathy (DPN, n=13) and healthy controls (Ctrls, n=13).
- Total foot pressure was greater in people with DPN for both peak plantar pressure and pressure time integral, when compared to Ctrls.
- People with diabetes and peripheral neuropathy showed significantly higher peak plantar pressure and pressure time integral in DPNs at Hallux, 1st & 2nd Toes, metatarsals and overall forefoot region.
- Key plantar pressure variables correlated with measures of neuropathy, reinforcing the link between pressure and neuropathic foot status.
- Achilles tendon stiffness is higher in people with DPN than controls.
- Strong correlations were identified between Achilles tendon stiffness and key clinical characteristics including neuropathy severity and advanced glycation end-products.
- Increasing tendon stiffness was related to increasing forefoot peak plantar pressure variables.
- When walking, people with DPN alter their gait strategy, due to limited ankle-joint mobility, which is related to ankle joint stiffness.
- The current study is unique in identifying a number of key gait differences in patients with DPN and associations with Achilles tendon stiffness, limited ankle/foot joint dorsiflexion and plantar pressures, which are proposed as novel mechanisms underpinning the risk of diabetic foot ulcers.
- In summary, stiffening of the ankle-joint complex was associated with increases in pressure variables under the forefoot in DPN patients. This was linked to an earlier heel rise also causing earlier and prolonged loading of the forefoot area.
- Increased stiffness of the ankle-foot joint complex is a key factor underpinning elevated plantar pressures and therefore increased diabetic foot ulcer risk.
- The proposed early DFU risk assessments tools can impact pathways of delivering foot care to patients with diabetes.

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14 APPENDICES

Appendix 1 REC favourable opinion letter

North West - Greater Manchester East Research Ethics Committee
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8009

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

09 July 2018

Prof. Neil Reeves
Professor of Musculoskeletal Biomechanics
Manchester Metropolitan University
School of Healthcare Science, Faculty of Science & Engineering
John Dalton Building, Manchester Metropolitan University
Oxford Road, Manchester
M1 5GD

Dear Prof. Reeves

Study title:	Factors affecting foot loading and ulcer risk in diabetes patients
REC reference:	18/NW/0274
IRAS project ID:	239893

Thank you for your letter of 04 July 2018 responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.
Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).
Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Copies of advertisement materials for research participants [Control advertisement]	1.0	11 March 2018
Copies of advertisement materials for research participants [DFU]	1.1	21 May 2018
Copies of advertisement materials for research participants [Neuropathy]	1.0	21 May 2018
Covering letter on headed paper [Covering letter]	1	16 March 2018
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [insurance evidence letter]	1.0	28 November 2017
GP/consultant information sheets or letters [GP letter]	1.0	11 March 2018
IRAS Application Form [IRAS_Form_22032018]		22 March 2018
Letter from sponsor [MMU sponsorship letter]	1.0	16 March 2018
Letters of invitation to participant [Recruitment letter]	1.0	11 March 2018
Other [MRI Screening form]	1	19 March 2018
Other [Directions to MMU]	1.0	21 May 2018
Other [Email contact template]	1.0	21 May 2018
Other [Participant letter]	1.0	21 May 2018
Other [telephone conversation template]	1.0	21 May 2018
Participant consent form	1.1	21 May 2018
Participant information sheet (PIS)	1.1	01 June 2018
Research protocol or project proposal	1.1	01 June 2018
Response to Request for Further Information [email response]		04 July 2018
Summary CV for Chief Investigator (CI) [CI CV]		
Summary CV for student [CV Neeraj]	1.0	12 March 2018
Summary CV for supervisor (student research) [Bowling CV]	1	19 March 2018

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.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high-quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

18/NW/0274 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

Signed on behalf of Mr Simon Jones Chair

Email: nrescommittee.northwest-gmeast@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Alison Lloyd
Dr. Lynne Webster, Manchester University NHS Foundation Trust

Appendix 2: HRA approval letter for research study

Professor Neil Reeves
Professor of Musculoskeletal Biomechanics Email: hra.approval@nhs.net
Manchester Metropolitan University
School of Healthcare Science, Faculty of Science &Engineering
John Dalton Building, Manchester Metropolitan University
Oxford Road, Manchester
M1 5GD
n.reeves@mmu.ac.uk
30 August 2018

Dear Professor Reeves,

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	Factors affecting foot loading and ulcer risk in diabetes patients
IRAS project ID:	239893
REC reference:	18/NW/0274
Sponsor	Manchester Metropolitan University

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Participating NHS organisations in England and Wales **will not** be required to formally confirm capacity and capability before you may commence research activity at site. As such, you may commence the research at each organisation 35 days following sponsor provision to the site of the local information pack, so long as:

 You have contacted participating NHS organisations (see below for details) The NHS organisation has not provided a reason as to why they cannot participate The NHS organisation has not requested additional time to confirm.

You may start the research prior to the above deadline if the site positively confirms that the research may proceed.

If not already done so, you should now provide the local information pack for your study to your participating NHS organisations. A current list of R&D contacts is accessible at the NHS RD Forum website and these contacts MUST be used for this purpose. After entering your IRAS ID you will be able to access a password protected document (password: **White22**). The password is updated on a monthly basis so please obtain the relevant contact information as soon as possible; please do not hesitate to contact me should you encounter any issues.

Commencing research activities at any NHS organisation before providing them with the full local information pack and allowing them the agreed duration to opt-out, or to request additional time (unless you have received from their R&D department notification that you may commence), is a breach of the terms of HRA and HCRW Approval. Further information is provided in the "*summary of assessment*" section towards the end of this document.

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your nonNHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Ms Alison Lloyd
Tel: 0161 247 2836
Email: ethics@mmu.ac.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **239893**. Please quote this on all correspondence.

Yours sincerely

Gemma Oakes
Assessor

Email: hra.approval@nhs.net

Copy to: Ms Alison Lloyd, Manchester Metropolitan University [Sponsor Contact] ethics@mmu.ac.uk
Dr Lynne Webster, Manchester University NHS Foundation Trust [Lead NHS R&D *Contact]* lynne.webster@cmft.nhs.uk **List of Documents**

Health Research Authority

HRA Statement of Activities

for Participating NHS Organisations in England (template version 4.2)

For non-commercial studies, one Statement of Activities should be completed as a template for each site-type in the study. Each Statement of Activities should be accompanied by a completed HRA Schedule of Events, as part of the submission via IRAS for HRA Approval.

Blue shaded fields (also marked with an asterisk*) should be completed by the sponsor/applicant prior to submission to the HRA.
Where appropriate, for the purpose of confirming capacity and capability, green shaded fields (also marked with a caret^) should be completed by the participating organisation before returning the document to the sponsor.
Other questions may be completed either by the sponsor/applicant or participating organisation (or collaboratively between both parties), as appropriate.

For participating organisations in Northern Ireland, Scotland or Wales, the sponsor should transfer a Site Specific Information Form to each local research team for completion and submission to their research management support function.

To provide an answer in the form, click in a box with the blue text and over-write this text, or select the relevant option if presented with drop-down text. A separate guidance document is provided and should be consulted prior to completion of this template. Please also read the question specific guidance where present.

IRAS ID*	239893
Short Study Title*	Foot loading and ulcer risk in diabetes patients
Full Study Title*	Factors affecting foot loading and ulcer risk in diabetes patients
Contact details of sponsor, or sponsor's delegated point of contact (e.g. Study Manager), for questions relating to study set-up*	Alison Lloyd ethics@mmu.ac.uk 0161 247 2836
Site Type*	Other Select one option. If 'Other', give details. Identification Site

1 applicable law (including, without limitation, the Human Tissue Act 2004).

Factors affecting Foot Loading and Ulcer Risk in Diabetes Patients
PIS Version 1.3, 23 August, 2018
IRAS Project ID: 239893
Your participation, your choice
Before you decide whether to take part, it is important to understand the purpose of the study and what it involves. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Please take your time to decide whether you wish to take part.

Why have you been selected?

You qualify to participate in this study as you belong to one of the following categories:

1. You have been diagnosed with Type 2 diabetes
2. You have been diagnosed with Type 2 diabetes and had a history of previous foot ulcer
3. You are a 'control' participant who does not have diabetes

What is the purpose of the study?

Some people with diabetes experience a wound on the bottom of their feet known as a foot ulcer. Foot ulcers can be difficult to heal and cause a number of problems. The most ideal situation is to try and stop them from developing in the first place. To be able to stop them we need to fully understand how they develop. We know that developing high pressure on the bottom of the feet is a major risk factor for a foot ulcer. We know a number of things that make the pressure on the foot high, but there are some other things that we do not understand very well, such as how does the tendon on the back of your lower leg contribute to the development of ulcers. This study aims to understand these things in more detail so that we might be better able to prevent foot ulcers in the future.

Do I have to take part?

No, your participation is entirely voluntary and it's up to you to decide whether to take part. If you decide to take part, you can choose to withdraw from the study at any time without giving a reason. This will not affect the care you receive now or in the future.

What will I have to do if I take part?

Prior to your visit we will ask you some simple questions about your health; these questions will check that you are eligible to participate in the study. If you are eligible, the researcher will confirm that you have received and understood the participant information sheet and will give you the opportunity to ask any questions you have. When you are satisfied with all the information you have received, you will be asked to sign a consent form to confirm that you are happy to take part in the study. You will be given a copy of the consent form to keep for your records.

You will then be given an appointment time and date convenient for you, to visit our research lab at the Manchester Metropolitan University, Manchester. During your visit, you will have four different test sessions with our research team. These can all be performed on the same day or spread across different days depending on your preference. If you choose to spread the tests across different days, they will all need to be completed within a month. The researchers will fully explain all procedures. It will be entirely your choice to complete the assessments during a single visit of at least four hours. These sessions can be combined into 2 or more visits according to your preference. At the end of the first visit you will be provided with a wrist based activity monitor to wear for 4 weeks, this will subsequentially be collected from you at one of the following sessions which will take place at least 3 weeks from the date of the first session.

Session 1: This session lasts around 45 minutes. We will do simple non-invasive tests to check for nerve damage (peripheral neuropathy) in your feet. This will involve assessing whether you can feel a selection of sensations (vibration, temperature and sharp/blunt). We will also test the reflex of the tendon on the back of your ankle by 'tapping' on the back of your ankle. We will take the temperature of your feet using a non-contact thermometer. We will ask you to place your arm on a device that will non-invasively measure specific enzymes in your body (AGEs).

If you are a control participant and during testing, we identify that you may have peripheral neuropathy, we will withdraw you from the study, and also with your consent, will notify your GP of the results.

Session 2: This session will be of approximately one hour. Here we will assess the strength in your ankle and lower leg using a testing machine called a dynamometer. You will lie comfortably on a bed facing down. We will ask you to apply some force on the foot plate which will be in contact with your foot (using your calf muscles for 5-second periods) - in a similar way as if you were applying your foot forcefully to the brake pedal in a car. You will be asked to exert force only for very short periods of time and will be given plenty of rest in between. You may feel more comfortable doing this test in shorts (which we can provide if needed), but you can also wear trousers to do this. During this test we will place an ultrasound probe on the tendon at the back of your calf. This experience will be like a short gym session for your calf muscles. After these tests we will also place the ultrasound probe on the bottom of your foot to take an image of the fat pad on the bottom of your foot.

Session 3: This session will assess your gait (a person's manner of walking) and will last for approximately an hour. We ask that you bring non-restrictive, but tight-fitting clothing with you (we have some available for you to change into if you have nothing appropriate) because we will place small reflective markers on your legs and feet. The movement of these markers will be accurately followed by a camera system. This camera does not 'see' you; it only sees the movement of markers placed on your body. From the movement of these markers we are able to understand how your legs and feet are moving. Thin, flexible insole sensors will then be inserted into your shoes to measure the pressures on the bottom of your foot during walking. We will provide you with standard footwear for this test and will simply observe and measure as you walk naturally along a 10-metre section of floor in our testing area. While you stand, we will also ask you to attempt to rise upon your tiptoes, to hold this position, and then lower your heels back down onto the ground (feet flat) standing position. We will ask you to repeat this a few times.

Session 4: This session will take around one hour. In this final session, we will take an image of the back of your ankle area and calf muscles using a Magnetic Resonance Imaging (MRI) scanner. You will just relax and lie comfortably on the bed of the MRI scanner while it takes images of the back of your ankle area and calf muscles.

What happens if I lose mental capacity whilst I am involved in the study?

If you lose mental capacity following providing consent to participation in this study, we will withdraw you from the study. Any identifiable information already collected will be destroyed; however, any anonymised information already collected will be retained and used in the study.

What are the potential risks or discomfort?

It is not expected that any of the laboratory assessments will cause discomfort. The assessment of your calf muscle strength will involve some level of effort but just in short bursts and we will give you plenty of rest between efforts. This experience will be like a short gym session for your calf muscles. There is a very small risk that you may feel some discomfort in your calf muscle after this test, but in the unlikely event you do, it will go away very quickly when you get up and stretch your leg. The risks of the assessments are essentially the same as the potential risks in daily life. During

assessment of your normal walking pattern, there is a risk of falling. However, this risk is much lower than in normal daily life because research staff will monitor you very closely. Your visit to our research centre will require 4 hours at least during the tests, which can be undertaken by you in a single or more visits.

Preparing your skin for attachment of Electromyography EMGs (to measure muscle activity) may require shaving small areas of hair to improve the electrode contact with your skin.

Are there any possible benefits?

1. You will have a detailed assessment on the level of nerve damage in your foot and lower leg.
2. It might help you in knowing higher-pressure areas of your feet so you can keep your feet healthy.
3. You will be well informed about the condition of your Achilles tendon (at the back of your ankle).

Medical Records

Responsible members of the NHS Trust research team will look at relevant sections of medical notes and data. All information will be kept confidential.

Reimbursement of travel expenses

We will reimburse your travel expenses to attend the university (we will just need a public transport receipt, details of car mileage etc.).

Who has reviewed the study?

The study protocol has been reviewed extensively by the research team across two universities and three hospital sites. The study has also been reviewed by the central ethics and governance department at the Manchester Metropolitan University and the North West Greater Manchester East NHS Research Ethics Committee.

Storage and Disposal of Study Data

All research data will be held in secure storage at the Manchester Metropolitan University and will only be accessed by the research team. The study sponsor (the Manchester Metropolitan University) and/or any regulatory authorities may also access the data for routine monitoring. All the information we collect from you as part of the tests will be anonymous and will only be identified by a unique study number (not by name).

Manchester Metropolitan University is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Manchester Metropolitan University will keep identifiable information about you for a maximum period of 3 years after the data collection has been finished. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible.

You can find out more about how we use your information by contacting ethics@mmu.ac.uk.

As a University we use personally identifiable information to conduct research to improve health, care and services. As a publicly funded organisation, we have to ensure that it is in the public interest when we use personally identifiable information from people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order

for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible.

Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research.
If you wish to raise a complaint on how we have handled your personal data, you can contact our Data Protection Officer who will investigate the matter. If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO).

Our Data Protection Officer is Mr. David Worrall and you can contact them at D.Worrall@mmu.ac.uk.

Lancashire Teaching Hospitals or Manchester University Foundation Trust, and Manchester Metropolitan University will collect information from you and your medical records for this research study in accordance with our instructions.

Manchester Metropolitan University will use your name, and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Manchester Metropolitan University and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Lancashire Teaching Hospitals or Manchester University Foundation Trust will pass these details to Manchester Metropolitan University along with the information collected from you and/or your medical records. The only people in Manchester Metropolitan University who will have access to information that identifies you will be people who need to contact you to for attendance of the study or dissemination of the results, or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name or contact details.

Manchester Metropolitan University will keep identifiable information about you from this study for 3 years after the study has finished.

Manchester Metropolitan University will collect information about you for this research study from Lancashire Teaching Hospitals or Manchester University Foundation Trust. This information will include your name, contact details and health information, which is regarded as a special category of information. We will use this information to contact you regarding taking part in the study.

Results of the Study
The results of the study will be published as scientific articles and presented at conferences. All the information provided will be anonymized to protect your identity. A written or oral summary of the results will be provided if you would like to be informed of the outcome. In case you wish to have a written summary of the results, please inform the research team while signing the consent form.

What if I have any concerns?

If you have a concern about any aspect of this study you can speak to the research team in the first instance who will do their best to answer your questions (Researcher: Neeraj Sharma: 0161-247 40482; Co-Investigator: Dr. Steven Brown: 0161 247 5952, Chief Investigator: Prof. Neil Reeves: 0161 247 5429). If you remain unhappy and wish to complain formally, you can do this by contacting the Manchester Metropolitan University Research Ethics and Governance team via ethics@mmu.ac.uk. , telephone no. 0161 247 2836

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the Manchester Metropolitan University, but you may have to pay your legal costs.

Indemnity

In the event that something does go wrong, and you are harmed as a result of negligence during the research study, the Manchester Metropolitan University has made indemnity and insurance arrangements. The normal National Health Service complaints mechanism is available to you (if appropriate). For independent advice, you may contact either the Research and Development office on 0161 276 4962 or the Patient Advisory and Liaison Service (PALS) on 0161 276 8686.

Who should I contact if I am interested in taking part?

If you are interested in taking part in this study, or would like further information, please contact:

Neeraj Sharma
Tel: 0161-247 40482
Email: neeraj.sharma@stu.mmu.ac.uk