MACROVASCULAR AND MICROVASCULAR DISEASE IN DIABETIC FOOT DISEASE

By

DANIELLE LYLIE LOWRY

A thesis submitted to the University of Birmingham for the degree of **DOCTORATE OF MEDICINE**

Institute of Metabolism and Systems Research

College of Medical and Dental Sciences

University of Birmingham

July 2018

ABSTRACT

The objective was to examine how diabetes mellitus (DM) impacts the arterial system of the lower limbs by assessing both macrovascular disease and the microvascular function.

A retrospective cohort study assessed the distribution of disease on digital subtraction angiography in 306 patients, half with DM. The Bollinger score was applied to all infra-inguinal vessels seen. There was a trend towards patients with DM having a higher burden of disease throughout the infra-inguinal arterial tree. When divided by indication for procedure patients without DM had more disease in the pedal vessels.

Secondly, in a prospective study, 24 patients with active foot ulceration were recruited and grouped as having no significant arterial disease (n=14) and those requiring percutaneous angioplasty (PCA, n=10). Laser Doppler fluxmetry (LDF) assessed the microcirculation at regular intervals until healing. Using LDF, the time to maximum flux significantly reduced following PCA, in those that healed (210.5s (72.18-231) to 50.71s (27.38-105.18) p=0.046).

The microcirculation is suggested to improve following PCA; further research is required to explore how changes in the macrocirculation relate to the microcirculation particularly in patients with DM.

ACKNOWLEDGEMENTS

In the course of designing and conducting these studies and the subsequent completion of this thesis, I have had the invaluable support of many people. Firstly, I would like to thank my supervisors Alok Tiwari, Parth Narendran and Mujahid Saeed. Without their support, I would not have known how to make a start and would have likely lost my way during the process.

During the design of the studies, James Hudson has given patient advice and feedback on data management and the statistical design. Jayne Robbie has been a font of information and guidance on the process of gaining ethical approval.

Recruitment and the follow-up of participants would not have been possible without the assistance of the diabetic foot teams at Queen Elizabeth Hospital Birmingham, City and Sandwell Hospitals Birmingham and Russells Hall Hospital Dudley.

Finally, I have to thank my parents who have provided me with calm support during my many episodes of panic.

PUBLICATIONS AND PRESENTATIONS DURING PERIOD OF STUDY

PUBLICATIONS

1. Lowry D, Saeed M, Narendran P, Tiwari A. A review of distribution of atherosclerosis in the lower limb arteries of patients with diabetes mellitus and peripheral vascular disease. *Vasc Endovasc Surg.* 2018; 52(7):535-542
2. Lowry D, Saeed M, Narendran P, Tiwari A. The Difference Between the Healing and the Nonhealing Diabetic Foot Ulcer: A Review of the Role of the Microcirculation. *J Diabetes Sci Technol.* 2017;11(5):914-923.

PRESENTATIONS

Oral

1. Lowry D, Tiwari A. Changes in microcirculation following percutaneous angioplasty in patients with diabetic foot ulcers. British Society of Endovascular Therapy Annual Meeting, South Gloucestershire. 22nd June 2018.
2. Lowry D, Saeed M, Narendran P, Tiwari A. Outcomes following lower-limb angioplasty in diabetes mellitus (DM). West Midlands Surgical Society Autumn Meeting 3rd Dec 2015

Posters

1. Lowry D, Saeed M, Narendran P, Tiwari A. Distribution of infra-popliteal peripheral vascular disease in patients with diabetes mellitus compared to patients without. European Society of Vascular Surgery Annual Meeting. Copenhagen, 28th Sept 2016.
2. Lowry D, Saeed M, Narendran P, Tiwari A. Outcomes following lower-limb angioplasty in diabetes mellitus (DM). Controversies and Updates in Vascular Surgery. Paris, 21st -23rd January 2016
3. Lowry D, Narendran P, Saeed M.A, Hodson J, Tiwari A. Do diabetics have a higher proportion of infra-popliteal disease compared to non-diabetics? A pilot study. International Symposium on the Diabetic Foot. The Hague, 20th-23rd May 2015

TABLE OF CONTENTS

FIGURES

TABLES

ABBREVIATIONS

- ABPI: Ankle brachial pressure index
- AGEs: Advanced Glycation End-products
- A-I: Aorto-Iliac
- ATA: Anterior Tibial Artery
- CM: Capillary Microscopy
- CTA: Computed Tomography Angiography
- DTL: Diabetic Tissue Loss
- DM: Diabetes Mellitus
- DPN: Diabetic Peripheral Neuropathy
- DSA: Digital Subtraction Angiography
- DSPN: Distal Symmetric Polyneuropathy
- eNOS: Endothelial Nitric Oxide Synthase
- ES: Electrical Stimulation
- F-P: Femoro-Popliteal
- HbA1c: Glycated Haemoglobin
- HR: Hazard Ratio
- ICC: Intra-class Correlation
- IDDM: Insulin Dependent Diabetes Mellitus
- IENFD: Intra-epidermal Nerve Fibre Density
- IRS-1: Insulin Receptor Substrate-1
- ITT: Ipswich Touch Test
- IQR: Inter-Quartile Range
- LDF: Laser Doppler Fluxmetry
- LDI: Laser Doppler Imaging
- LDL: Low Density Lipoprotein
- LD-PWA: Laser Doppler – Pulse Wave Amplitude
- LD-SBFV: Laser Doppler – Skin Blood Flow Velocity
- LPA: Lateral Plantar Artery
- MAP-kinase: Mitogen Activated Protein kinase
- MRA: Magnetic Resonance Angiography
- NADPH: Reduced nicotinamide adenine dinucleotide phosphate
- NCS: Nerve Conduction Studies
- NCV: Nerve Conduction Velocity
- NDM: No Diabetes Mellitus
- NF- κB: Nuclear Factor- κB
- NIDDM: Non-insulin Dependent Diabetes Mellitus
- NO: Nitric Oxide
- NPV: Negative Predictive Value
- NSS: Neuropathy Symptom Score
- NTSS-6: Neuropathy Total Symptom Score – 6
- OR: Odds Ratio

- PCA: Percutaneous angioplasty
- PAD: Peripheral Arterial Disease
- PBS: Peripheral Bypass Surgery
- PEA: Peroneal Artery
- PI-3 kinase: Phosphatidylinositol 3-kinase
- PKC: Protein Kinase C
- PORH: Post Occlusive Reactive Hyperaemia
- PPV: Positive Predictive Value
- PTA: Posterior Tibial Artery
- RAGE: Receptor for Advanced Glycation End-products
- ROS: Reactive Oxygen Species
- SD: Standard Deviation
- SE: Standard Error
- SFA: Superficial Femoral Artery
- SPP: Skin Perfusion Pressure
- TASC II: The second Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease
- TBP: Toe Blood Pressure
- TBPI: Toe Blood Pressure Index
- $TcPO_2$: Transcutaneous Oxygen Pressure
- TPT: Tibial-peroneal trunk
- TtM: Time to Maximum flux
- VPT: Vibration Perception Threshold
- VSMC: Vascular Smooth Muscle Cells
- WHO: World Health Organisation

CHAPTER 1: INTRODUCTION

1.1. DIABETES MELLITUS

The World Health Organisation (WHO) describes diabetes mellitus (DM) as "*a condition primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage*.[1]" As well as being characterised by chronic hyperglycaemia DM also involves disturbances of carbohydrate, fat and protein metabolism. These are the results of defects in insulin secretion, insulin action or both[2]. Long term the effects of DM include both macrovascular and microvascular complication, namely retinopathy, nephropathy, neuropathy, ischaemic heart disease, cerebrovascular disease and peripheral vascular disease[1,2].

The current diagnostic criteria for DM based on WHO recommendations are as follows

- Diabetes symptoms plus
    - A random venous plasma glucose concentration ≥11.1 mmol/l or
    - A fasting plasma glucose concentration ≥7.0 mmol/l (whole blood ≥6.1 mmol/l) or
    - Two hour plasma glucose concentration ≥11.1 mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance test.
    - With no symptoms diagnosis should not be based on a single glucose determination but requires confirmatory plasma venous determination. At least one additional glucose test result on another day with a value in the diabetic range is essential, either fasting, from a random sample or from the two hour post glucose load. If the fasting random values are not diagnostic the two hour value should be used.
- A laboratory glycated haemoglobin (HbA1c) ≥48 mmol/l (6.5%)
    - A value of less than 48 mmol/l (6.5%) does not exclude diabetes diagnosed using glucose tests.
    - In patients without symptoms of DM the laboratory venous HbA1c should be repeated. If the second sample is <48 mmol/l (6.5%)the person should be treated as at high risk of DM and the test should be repeated in six months or sooner if symptoms develop.[1,2]

1.2. EPIDEMIOLOGY OF DIABETES MELLITUS

DM is an increasing problem worldwide. Between 1980 and 2014 the prevalence of DM increased from 4.3% to 9.0% in men and from 5.0% to 7.9% in women[3]. This is based on a sophisticated statistical analysis, performed by the NCD Risk Factor Collaborative, that pooled the population-based data for 751 studies, 4.4 million adults, from 146 countries. The definition the NCD Risk Factor Collaborative used for DM was fasting plasma glucose of 7.0 mmol/l or higher or history of diagnosis with diabetes, or use of insulin or oral hypoglycaemic drugs. Their methods accounted for different definitions of DM over time and was able to demonstrate that while some of the increase in prevalence is due to change in population size and age structure and an interaction between change in prevalence and change in population size and age structure there is a significant element of the increase in prevalence that is due to a change in prevalence alone[3].

As of 2014, 422 million adults were living with DM worldwide[3]. In the United Kingdom, there are almost 3.7 million people currently living with DM, with an estimated million people who are as yet undiagnosed[4]. The increase in prevalence includes patients with both type 1 and type 2 DM although 90% of affected patients suffer from type 2[4]. The accepted causes for type 1 DM include genetic factors[5], viral infections and other environmental factors[6,7]. With type 2 DM there are also factors related to genetics and ethnicity[8,9] however the main driving factors for the continued increase in prevalence are thought to be the obesity epidemic, lack of physical activity and increasing age of the population[9,10]. As the prevalence of DM has increased so have the complications related to persistent hyperglycaemia. These complications include diabetic retinopathy, nephropathy and diabetic peripheral neuropathy (DPN) as well as increased cardiovascular risk and risk of

amputation[11-15]. DM is now a leading cause of blindness, renal failure and lower limb amputation[16].

1.3. VASCULAR PATHOPHYSIOLOGY IN DIABETES MELLITUS

The global odds ratio for peripheral arterial disease (PAD) in the presence of DM is 1.68 (95% CI 1.53 – 1.84, p = 0.009)[17]. In the population of the United States of America, aged over 40 years, the prevalence of PAD has been found to be double in those with DM compared to those without (4% [95% CI 2.9 – 5.2] vs 9.5% [5.5 – 13.4])[18]. There is overlap between the risk factors for coronary artery disease (CAD) and PAD, however some risk factors seem to play a larger role in the development of atherosclerosis in one vascular bed over the other. A large prospective cohort study based in Scotland found the strongest predictors for development of PAD were DM (hazard ratio 3.38) and smoking (2.15)[19]. For CAD the largest hazard ratio was still for DM (2.21) followed by gender (1.99) however the spread of risk across the other risk factors considered was much more evenly spread compared to PAD[19]. While there is overlap between the risk factors for PAD and CAD it has been proposed that there are significant differences in how the peripheral vasculature responds to pathological insults in particular in the expression of cell surface receptors and signalling pathways[20,21].

The basic process of development of atherosclerotic plaques involves deposition of low-density-lipoproteins (LDLs) in the vascular sub-endothelial space, increased expression of cell adhesion molecules leading to macrophage migration, increased tissue factor and matrix metalloproteinase expression and smooth muscle cell proliferation with vasovasorium neovascularisation (Figure 1.3-1)[22]. DM predisposes to accelerated atherosclerosis through a combination of insulin resistance, hyperglycaemia and

dyslipidaemia. Through different and interconnected processes these lead to vasoconstriction, inflammation and thrombosis which in turn contribute to atherogenesis[23,24].

Dysfunction of the endothelium may well precede the development of insulin resistance or promote the conversion from a pre-diabetes state to overt DM[25]. The endothelium consists of a single layer of cells that coat the inner surface of all blood vessels. These cells are dynamic and as well as providing semi-permeable barriers have both metabolic and synthetic functions[26]. The autocrine, paracrine and endocrine functions of the endothelium combine to regulate vascular tone, mediate thrombogenicity and reduce inflammation[23,27].

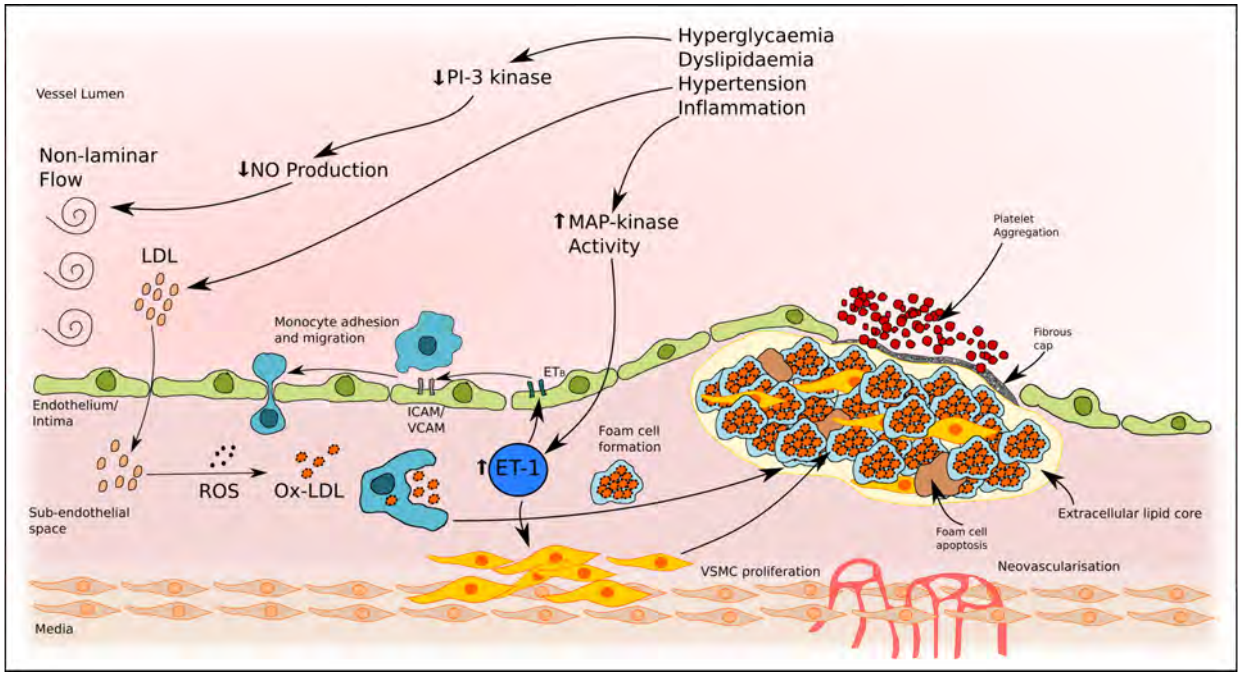

Figure 1.3-1: Development of atherosclerotic plaque in diabetes mellitus
The inter-related combination of hyperglycaemia, dyslipidaemia, hypertension and inflammation lead to endothelial dysfunction. There is reduced production of nitric oxide (NO) and subsequent non-laminar flow. Low-density-lipoproteins (LDLs) are deposited in the sub-endothelial space and interact with reactive oxygen species (ROS) to form oxidised-LDLs (ox-LDL). The combination of down regulation of the insulin regulated phosphatidylinositol 3-kinase (PI-3 kinase) pathway and up regulation of the mitogen-activated protein kinase (MAP-kinase) pathway leads to increased expression of endothelin-1 (ET-1). ET-1 promotes vascular smooth muscle cell (VSMC) proliferation and stimulates the expression of the ET_B receptor on endothelial cells and subsequent increased expression of inter-cellular adhesion molecule and vascular cellular adhesion molecule. Monocytes couple with the adhesion molecules and migrate into the sub-endothelial space. In the sub-endothelial space, they differentiate into macrophages and scavenge the ox-LDL, forming foam cells. The lesions progress sequentially from isolated foam cells to fatty streaks with mainly intracellular lipid accumulation. Extracellular lipids then also start to accumulate and form an extracellular lipid core. Fibroatheromatous lesions subsequently form, containing a combination of lipid, foam cells, VSMC. Cellular apoptosis leads to the formation of a lipid-rich necrotic core. Fibrous tissue forms over the core and may eventually rupture activating platelet aggregation and thrombosis.

In the physiological state insulin binds with insulin receptors on endothelial cells which induce the production of nitric oxide (NO). NO causes vasodilatation, increasing blood flow, which augments the disposal of glucose in skeletal muscle[28]. In more detail when insulin binds to the insulin receptor the transmural ß subunit undergoes auto-phosphorylation with adenosine triphosphate at specific tyrosine sites[29]. The activated receptor itself becomes tyrosine kinase which in turn phosphorylates intracellular substrates like insulin receptor substrate-1 (IRS-1) and Shc proteins[28,29]. There are two main insulin signal transduction pathways (Figure 1.3-2) The phosphatidylinositol 3-kinase (PI-3 kinase) pathway, in vascular endothelium, leads to increased activity in endothelial nitric oxide synthase (eNOS), increased production of NO and vasodilatation[22,28]. The growth factor like pathway is mediated by mitogen-activated protein kinase (MAP-kinase). It initiates a cascade of signalling events that lead to the induction of genes involved in cell proliferation and differentiation[22,28]. In DM the anti-atherogenic PI-3 kinase pathway is down regulated so the protective factors of decreased expression and secretion of vascular adhesion molecule-1 and E-selectin, pro-inflammatory cytokines, tumour necrosis factor-α, monocyte chemoattractant protein-1 and subsequent reduced platelet adhesion and prostacyclin production are lost. This leads to increased platelet aggregation[22]. Conversely the pro-atherogenic MAP-kinase pathway becomes dominant leading to vasoconstriction, increased vascular permeability, vascular smooth muscle cell proliferation and increased production of interleukin-6[22].

Hyperglycaemia in DM contributes to oxidative stress and formation of advanced glycation end-products (AGEs)[25]. Oxidative stress is a condition in which the overproduction of reactive oxygen species (ROS) overwhelms endogenous antioxidant defence mechanisms[30]. AGEs occur as a result of non-enzymatic glycation of proteins and lipids. They

are found deposited in macrophages and vascular smooth muscle cells and cause mechanical dysfunction in vessel walls among other actions[22]. By interacting with the receptor for AGEs (RAGE) AGEs cause a variety of adverse effects including uncoupling of eNOS and its inactivation, increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression, increased protein kinase C (PKC) activity, increased MAP-kinase activity and increased nuclear factor- κB (NF- κB) expression[25]. NADPH oxidase is an enzyme, or family of enzymes, that are the predominant source of the superoxide anion ($O_2\bullet^-$), a ROS, in the vasculature. As well as being activated by RAGE, NADPH oxidase is also activated by angiotensin II, endothelin-1, growth factors, cytokines and mechanical shear stress and stretch[30]. Increased PKC activity and other kinases lead to serine phosphorylation of IRS-1 which means that PI-3 kinase is not activated leading to reduced eNOS activity. PKC activity also leads to increase NF- κB expression which lead to expression of pro-inflammatory and pro-fibrotic genes[22,28]. Increased MAP-kinase activity stimulates the MAP-kinase dependent insulin pathway and promotes secretion of endothelin-1 leading to vasoconstriction and vascular smooth muscle cells (VSMC) proliferation[22,25,28].

Insulin resistance and type 2 diabetes are associated with hypercholesterolaemia there is a relative reduction in high density lipoproteins, which protect against atherosclerosis, and an increase in LDLs and free fatty acids(FFAs)[23]. FFAs impair eNOS and subsequently reduce the production of NO[24,25]. They are also associated with mitochondrial uncoupling and increased expression of NADPH oxidase leading to increased ROS[24,25,30]. The combination of oxidative stress and hyperglycaemia leads to an oxidative modification of LDLs (oxLDL). OxLDLs contribute to eNOS uncoupling are associated with endothelial cell apoptosis[25]. OxLDLs also promote pro-inflammatory cytokines and upregulation of angiotensin-II type 1

receptors leading to vasoconstriction and are easily ingested by macrophages to form foam cells and contribute to atherosclerotic plaque formation[25].

Adipose tissue is itself an active endocrine-paracrine organ[22] and its function is altered in obesity and DM[22,25]. As well as producing inflammatory molecules like tumour necrosis factor-α, interleukins-6,8 and 10, and plasminogen activator inhibitor-1, adipocytes produce adipokines like adiponectin, leptin and angiotensinogen[22]. Adiponectin, unlike the other adipokines, increases sensitivity to insulin and is downregulated in obesity and type II DM and so its anti-inflammatory and anti-thrombotic properties are reduced. It has these effects by decreasing the expression of adhesion molecule, reducing oxLDL uptake, reducing foam cell formation and reducing proliferation of VSMCs[22,25,28,31]. Leptin conversely is upregulated in DM and reduces sensitivity to insulin, stimulates cholesterol accumulation by macropahges and encourages VSMC proliferation[22,25]. There is also increased production of angiotensinogen which is a precursor to angiotensin II. Angiotensin II is a major vasoconstrictor which also enhances foam cell formation, stimulates adhesion molecules and enhances the conversion of NO to ROS via the NADPH oxidase pathway[22].

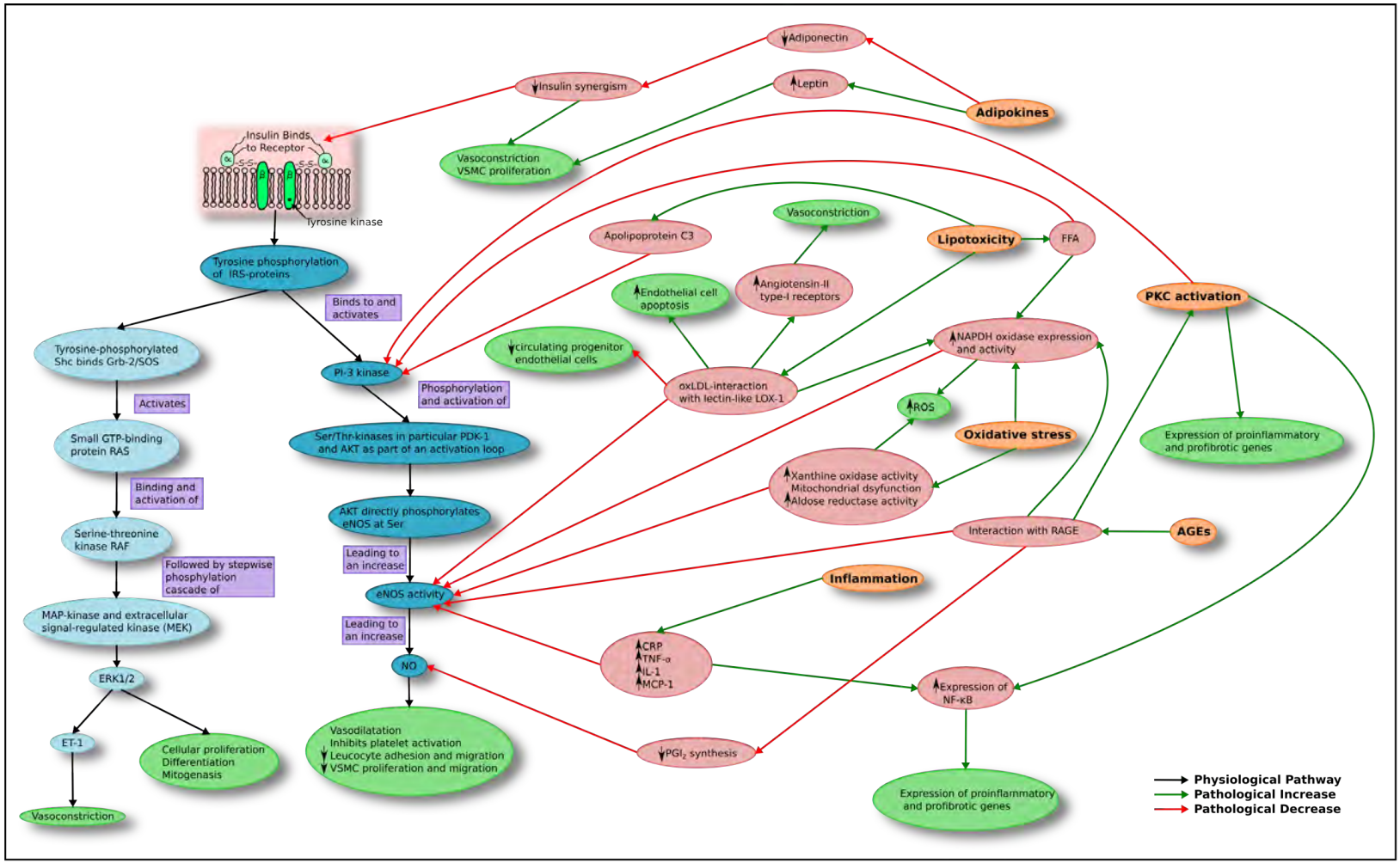

Figure 1.3-2: Endothelial insulin signalling pathways including pathological pathways leading to dysfunction.

Physiologically the dominant pathway is the phosphatidylinositol 3-kinase (PI-3 kinase) pathway (Dark blue) which has vasoprotective effects. In DM the PI-3 kinase pathway is down regulated by the combined results of hyperglycaemia and dyslipidaemia (Orange) making the mitogen-activated protein kinase (MAP-kinase) pathway dominant (Light blue) and up regulating outcomes that lead to endothelial dysfunction.

AGEs, advanced glycation end products; AKT, serine/threonine-specific protein kinase B; CRP, C-reactive protein; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal regulated kinase 1 or 2; ET-1, endothelin–1; FFA, free fatty acids; Grb-2, growth factor receptor-bound protein 2; IL-1, interleukin-1; IRS, insulin receptor substrates; MCP-1, monocyte chemoattractant protein-1; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; NO, nitric oxide; PDK-1, phosphoinositide-dependent protein kinase-1; PGI_2, prostacyclin; RAGE, receptor for advance glycation end products; ROS, reactive oxygen species; Ser, serine; PKC, protein kinase C; SOS, son of sevenless; Thr, threonine; TNF-α, tumour necrosis factor-α; VSMC, vascular smooth muscle cells;

1.4. SKIN CIRCULATION IN DIABETES MELLITUS

The skin is the largest organ of the body, covering the body with a thickness between 0.5mm on the eyelids to 4.0mm on the heels[32]. Two layers form the skin, the epidermis and the dermis. The epidermis is a thin avascular layer of cells. The dermis consists of connective tissue and contains hair follicles, sweat and sebaceous glands and blood vessels[33]. The blood supply for the skin is derived from a combination of, tributaries of the major vessels, musculocutaneous perforators and fasciocutaneous perforators. These tributaries are organised into anastomotic plexi that give off capillary loops into the dermal papillae. Within the deep layers of the dermis, there are arterio-venous anastomoses which can rapidly increase and decrease the blood flow to the skin[34]. In normothermic conditions the blood flow to the skin is approximately 250ml/min, however, this can range from almost zero to 8l/min in extreme conditions[35]. There are many more anastomoses in glabrous skin (non-hairy skin, i.e. palms, soles and lips) than non-glabrous (hairy) skin[36]. Two sets of sympathetic nerves control the cutaneous circulation; sympathetic adrenergic vasoconstrictors and sympathetic cholinergic vasodilators. Within non-glabrous skin, both these systems are active whereas in glabrous skin only sympathetic vasoconstrictors are found. The vasoconstrictors have tonic activity during normothermic conditions and increase their activity during cooling to reduce blood flow to the skin. However, the vasodilators are only active when the body temperature rises, i.e. during exercise[35].

The historical concept was that patients with DM suffered from occlusion of the microcirculation meaning that macrovascular revascularisation was often considered hopeless. However further investigation using various techniques has not found occlusive

microvascular disease[37] in these patients. Histological examination of the capillaries supplying the skin, muscle and nerves has shown thickening of the basement membrane compared to non-diabetic patients and an increase in porosity[37-42]. The basement membrane is a specialised form of the extracellular matrix. It provides mechanical stability for cells and can also act as a substrate for cellular interactions[43]. The layer contains a combination of the glycoproteins entactins and laminins, heparin sulphate proteoglycans and type IV collagen[43]. Chronic hyperglycaemia leads to the stimulation of the hexosamine and PKC pathways, both of which lead to the overexpression of transforming growth factor-ß (TGF- ß), plasminogen activator-1 and vascular endothelial growth factor (VEGF)[44-46]. These induce the increased synthesis of collagen IV, the degradation of proteoglycans and the unbalanced synthesis of other basement membrane constituents[38,42,44,46]. Changes to the basement membrane appear early in the diabetic disease process, before the development of overt complications[47]. It is thought that thickening of the basement membrane reduces the distensibility of the vessels and provides a barrier to the diffusion of NO[48,49]. These changes contribute to a reduction in the hyperaemic response to trauma (thermal or occlusive) [41,47,49,50] and it is this inability to vasodilate and achieve maximal blood flow that is thought to be a major contributory factor to diabetic foot problems[51].

1.5. THE IMPACT OF DIABETES MELLITUS ON FOOT ULCER DEVELOPMENT AND WOUND HEALING

Foot ulcers in DM can be classified as neuropathic, neuro-ischaemic or ischaemia[15]. It has been suggested that there is a stepwise progression from the development of DM to neuropathy to development of an ulcer which is complicated by ischaemia and/or infection leading to amputation[52].

For a wound to heal there has to be coordination between a complex array of biological and physiological functions. As already discussed in sections 1.3 and 1.4 DM has a significant impact on the function of the macro and microcirculation leading to ischaemia.

Diabetic neuropathies are a group of conditions that affect different parts of the nervous system. As such the symptoms that patients experience can vary significantly[53], they can be focal or diffuse, proximal or distal[54]. The most common types of diabetic neuropathies are chronic sensorimotor distal symmetric polyneuropathy (DSPN) and autonomic neuropathies[53]. Other types of neuropathies include acute sensory neuropathy, focal and multifocal neuropathies including cranial nerve neuropathies and diabetic amyotrophy[53]. I am not going to concentrate on these rarer forms any further here as they have less relevance to the development of foot ulceration.

Like PAD the causes of DPN are multifactorial, glycaemic control, cardiovascular risk factors including dyslipidaemia, inflammation and activation of multiple molecular pathways all contribute[54-57]. Duration of DM is a risk factor for DPN[56] but tight glycaemic control has been shown to slow the progression of DPN[58-60]. Pathways for the effect of hyperglycaemia on peripheral nerves include oxidative stress, polyol shunting, accumulation of AGEs, and activation of PKC[54,55,61]. Persistent hyperglycaemia, as mentioned above (Section 1.3), causes

oxidative stress in many tissues including peripheral nerves[54]. Multiple free radicals have been shown to be associated with DPN and they have been shown to both activate pathological pathways (i.e. polyol pathway) and occur as the result of these pathways[54-56]. The free radicals cause damage to the lipids in myelinated structures and there is associated apoptosis of neurons and Schwann cells[57]. The damage also results in hyperexcitability in afferent nociceptors and central neurons resulting in neuropathic pain[57]. In addition, there is damage to the microvasculature of the neurons leading to endogenous hypoxia and decreased neurological function[57].

Polyol shunting occurs in the presence of hyperglycaemia and associated ROS leading to inhibition of glyceraldehyde 3-phosphate dehydrogenase and subsequent upregulation of the polyol pathway[55]. In normoglycaemic conditions the glycolytic pathway is dominant but becomes saturated in hypergylcaemia[57]. In the polyol pathway aldose reductase uses NADPH to reduce glucose to sorbitol. Sorbitol dehydrogenase then uses oxidised nicotinamide adenine dinucleotide to reduce sorbitol to fructose[55,57]. Sorbitol and fructose are too large to cross cell membranes and so accumulate in nerve cells[55]. This leads to change in osmotic pressure within the cells, efflux of other electrolytes and swelling of the axons[57]. In addition, there is reduction in the osmolytes myoinositol and turin, and inhibition of the Na^+/K^+ ATPase pump with accumulation of intracellular sodium[55,57]. As well as damage to the Schwann cells and a reduction in nerve conduction velocity, these changes cause consumption of NADPH leading to endothelial damage and reduced NO-dependant vasodilatation and increases in MAP-kinase and NF-κB activity[55,57]. AGEs accumulate in peripheral nerves and there is an associated upregulation in RAGE. The AGEs themselves induce tissue damage, including endothelial damage, by causing protein cross linking,

apoptosis of Schwann cells and driving oxidative stress[55,57]. Associated upregulation of RAGE leads to increased NF-κB, ROS and nuclear DNA degradation leading to apoptosis, axonal degradation and nerve atrophy[55,57]. As well as stimulation of the hexosamine and PKC pathways (Sections 1.3 and 1.4), which are also active in peripheral nerves, leading to upregulation of TGF-ß and VEGF and subsequent endothelial fibrosis, there is down regulation of nerve growth factor (NGF)[54-57]. Physiologically NGF stimulates neuronal growth and differentiation and protects nerve cells from apoptosis, particularly small sensory and sympathetic neurons[56,57]. Hyperglycaemia downregulates the production of NGF and prevents it from binding with the tropomyosin receptor kinase A and so there is decreased availability within neuronal cells[57]. Subsequently there is reduction in the p75 neurotrophin receptor and impaired PI-3 kinase and extracellular signal regulated kinase 1 or 2 signalling leading to decreased survival, growth, proliferation and increased apoptosis in small sensory and autonomic nerve fibres[57].

The above pathways combine to contribute to the clinical presentation of DPN. A simple definition of DPN is “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with DM after exclusion of other causes”[62]. Many patients are asymptomatic but careful examination may elicit signs of neuropathy[53]. Symptoms of DSPN include burning, tingling or shooting pains and/or hyperaesthia that affect the feet or hands in a symmetrical way. Over time the symptoms spread proximally in a length dependant fashion[53,63]. On examination there may be loss of vibration, pressure, pain and/or temperature perception and ankle reflexes may be absent[53]. In patients who have associated autonomic dysfunction a clinician may find that the skin of the foot is dry or cracked with distended dorsal veins and that it is a different temperature compared to proximal skin[53].

Loss of sensation leads to loss of protective symptoms in response to trauma of the foot, so a minor injury can easily be neglected[64,65]. Motor and autonomic dysfunction lead to wasting of the intrinsic muscles of the foot causing the characteristic deformities of a high-arched foot with clawing of the toes[65,66]. These deformities alter the biodynamics of the foot resulting in abnormal foot pressures that lead to callus formation. Further repetitive injury in the presence of callus eventually leads to tissue injury and ulceration[67].

Once ulceration has occurred, healing may be interrupted by a combination of chronic inflammation, hyperglycaemia, disruption of collagen synthesis and abnormal action of growth factors[68,69]. Normal healing involves four phases, haemostasis, inflammation, proliferation and remodelling. Patients with DM suffer from chronic low-grade inflammation and may experience a prolonged inflammatory phase following injury; they can also stall in any of the other phases[68,69]. All of the above factors combine to make a diabetic wound very vulnerable to chronic infection. Hypoxia and hyperglycaemia and biomechanical disturbances combine to encourage overgrowth of bacteria and adversely affect the function of neutrophils and macrophages[66]. In any open wound there is the risk of invasion of micro-organisms, if the organisms multiply, within the tissues, at a rate sufficient to cause inflammation then this is defined as infection[70]. These infections can be graded as mild, moderate or severe. The International Working Group on the Diabetic Foot and Infectious Diseases Society of America definitions are shown in Table 1.5-1. Samples taken from mildly infected wounds are more likely to have a single organism isolated whilst severe infections in chronic wounds are more likely to be polymicrobial[70,71]. Potential organisms that cause infection are *Staphylococci, Streptococci, Proteobacters, Pseudomonas aeruginosa and Coliforms*[65,70-72]. The most common growths are *Staphylococcus aureus* and *Escherichia coli,*

chronic and ischaemic wounds are more likely to grow anaerobic organisms[65,71]. In a study, that examined the isolates from the same patient population as the studies presented over the next chapters, mixed anaerobes, *Staphylococcus aureus, Escherichia coli* and coagulase negative *Staphylococcus* where the most common organisms identified. Twenty percent of patients (n=71) grew both gram negative and positive organisms[72]. Most mild to moderate infections will settle with one to two weeks of oral antibiotics. Whereas severe and some moderate infections with require intravenous antibiotics with an appropriate oral switch once there has been a satisfactory response. In cases of osteomyelitis where the bone has not been resected at least 6 weeks of treatment will be required[15].

To optimise the chances of a diabetic foot ulcer healing, all elements contributing to the initiation of the ulcer must be tackled. Glycaemic control should be tightened, blood supply enhanced, infection treated, wounds dressed and pressure areas offloaded[73,74]. For each of these areas there are clinicians with specialist knowledge and skills, e.g. diabetologists, vascular surgeons, microbiologists and podiatrists. By working together as a multi-disciplinary team, in conjunction with the patient, it has been shown to be possible to improve outcomes and reduce rates of major amputation[15,73,75]. Unfortunately despite improvements being made, as mentioned above, amputation rates in patients with DM remain high. This is in part due to the difficulties faced when revascularisation of the lower limb is attempted.

Table 1.5-1: International Working Group on the Diabetic Foot (IWGDF) and Infectious Diseases Society of America (IDSA) classification system for defining the presence and severity of an infection of the foot in a person with diabetes. Adapted from Lipsky *et al*[15]

IWGDF/IDSA Classification	Definition
1 (Uninfected)	No systemic or local symptoms or signs of infection
	Infection At least two of the following items are present: • Local swelling or induration • Erythema >0.5 cm* around the wound • Local tenderness or pain • Local warmth • Purulent discharge - Other causes of an inflammatory response of the skin should be excluded (e.g. trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis and venous stasis)
2 (Mild infection)	- Infection involving only the skin or subcutaneous tissue (without involvement of deeper tissues and without systemic manifestations as described next) - Any erythema present extends <2 cm* around the wound - No systemic signs or symptoms of infection (see the following discussions)
3 (Moderate infection)	- Infection involving structures deeper than skin and subcutaneous tissues (e.g. bone, joint, tendon or muscle) or erythema extending ≥2 cm* from the wound margin - No systemic signs or symptoms of infection (see the following details)
4 (Severe infection)	Any foot infection with the systemic inflammatory response syndrome, as manifested by ≥2 of the following: - Temperature >38 °C or <36 °C - Heart rate >90 beats/min - Respiratory rate >20 breaths/min or PaCO2 <4.3 kPa (32mmHg) - White blood cell count >12 000/mm3 or <4000/mm3, or >10% immature (band) forms

*In any direction, from the rim of the wound. The presence of clinically significant foot ischaemia makes both diagnosis and treatment of infection considerably more difficult.

1.6. VASCULAR SURGERY IN DIABETES MELLITUS

Patients with DM have been found to have higher rates of failed revascularisation compared to patients without DM. This is true of both peripheral bypass surgery (PBS) and percutaneous angioplasty (PCA). In 2008 Söderström *et al.* performed infra-inguinal bypass surgery on 150 limbs with tissue loss. Fifty percent of the participants had DM. Wound healing had occurred in 63% of the patients with DM and 87% of patients without DM at 12 months follow-up[76]. Lee *et al.* compared the restenosis, occlusion and amputation rate in 239 (176 with DM, 63 without) patients undergoing PCA for symptomatic PAD. They found that those with DM had a higher rate of restenosis after 2 years follow-up (54.4% vs 31.5%, p=0.02) and a trend towards occlusion (38.2% vs 26.3%, p=0.21) and major amputation (5.1% vs 1.5%, p=0.46)[77]. The reasons for these high failure rates are poorly understood and the following work aims to investigate this further.

1.6.1. Changes in neuropathy and the microcirculation following revascularisation

Akbari et al.[78] assessed 55 patients with type I and type II DM who required distal arterial bypass. The proportion of patients whose indication was ulceration was not stated, and only 54% made it to the follow-up appointment due to a combination of unsuccessful procedure, death or not attending the appointment. Neuropathy was assessed using peroneal nerve conduction velocity (NCV) and Neuropathy Symptom Score (NSS) and microcirculation using transcutaneous oxygen pressure ($TcPO_2$). A significant improvement in $TcPO_2$ was found in the revascularised leg but not in the non-operated leg. When comparing patients operated on legs to non-operated legs at follow-up (mean 19 months), they found that the operated

leg experienced stabilisation in NCV while the non-operated experienced a significant decrease during the follow-up period.

Arora et al.[51] compared 13 patients with diabetic peripheral neuropathy (DPN) and PAD (DI group) requiring distal arterial bypass to patients with DPN but no PAD (DN group) and patients with DM in the absence of DPN or PAD (D group). Neuropathy was assessed using NSS, Neuropathy Disability Score, vibration perception threshold (VPT) and monofilament. Laser Doppler fluxmetry (LDF) was used to measure hyperaemic response to heat, acetylcholine and sodium nitroprusside and the neurovascular response. Follow-up occurred at 4 to 6 weeks. Following bypass, there was a statistically significant improvement in cutaneous vasodilatory response that brought the DI group up to a similar level to the DN group. The eight patients with tissue loss showed full healing in four patients, signs of healing in two and healing digital amputation sites in the two patients who required minor amputation. There was no significant change in the neurovascular response.

Toursarkissian et al.[79] considered 113 patients with PAD who required distal arterial bypass. Ninety-five of these patients had tissue loss and 88 suffered from DM. There is no report of the healing status post-operatively. Neuropathy was assessed using VPT. After six-months of follow-up no significant difference was found between the pre and post-operative VPTs.

Hinchcliffe et al.[80] conducted a systemic review of the role of revascularisation in diabetic foot ulcers. In 7 studies a healing rate of 60% or more was reported. They suggested that future research should be focused on the indications for and timing of intervention for these patients.

1.7. HYPOTHESIS

Patients with DM have a different distribution of arterial disease compared to patients without DM and this impacts the healing of foot ulcers.

1.8. AIMS

This research project aims to examine both the macrovascular and microvascular blood supply to the leg and foot in patients with DM.

1.9. STUDY DESIGN

Two reviews of the literature and two studies were planned to address this. The first review gathered the current evidence regarding how the distribution disease in the lower limbs differs in those with DM. This was followed by a retrospective cohort study with the aim of adding to the literature in this area. The study compared the digital subtraction angiograms (DSA) of patients with DM to those without using the Bollinger scoring system. For full details see Chapter 2 and Chapter 3.

The second review gathered evidence on how the function of the microcirculation differs between diabetic foot ulcers that heal and those that do not. The research project undertaken following this, recruited patients with active ulceration, with the aim of measuring how the function of the microcirculation changed during the process of healing, see Chapter 4 and Chapter 5.

The final sections of this introductory chapter present the methods of examining for PAD and PND considered for use in the following body of work.

1.10. METHODS OF EXAMINING THE VASCULAR TREE

1.10.1. Macrovascular

Angiography is the gold standard for assessing the patency of the lower limb arteries[81] and various methods of quantifying the degree of disease in each arterial segment have been described. The most commonly used scoring system is the one described by Bollinger in 1981[82-90]. The system semi-quantitatively assesses ten arterial segments (per leg) from the infra-renal aorta down to the proximal 3cm of the anterior tibial artery (ATA) and the proximal 5cm of the posterior tibial artery (PTA) and peroneal artery (PEA) (Figure 1.10-1). Each segment is scored individually using the scoring matrix shown in Table 1.10-1. One of the limitations of the scoring system is that it does not extend beyond the proximal tibial arteries which means the arterial runoff is not adequately assessed. Three studies have extended the Bollinger score to include the distal vessels. Willenberg *et al.* in their assessment of progression of disease in patients with intermittent claudication imply that they are assessing the whole of the tibial arteries although this, and the boundaries used, is not explicitly stated. They found a higher proportion of disease in the ATA and PTA compared to the PEA and that DM was an independent predictor of progression of disease at 2-5 years follow-up[89]. For the Bypass versus Angioplasty in Severe Ischaemia of the Leg (BASIL) trial Bradbury *et al.* defined different arterial segments to those originally described by Bollinger. This included dividing the popliteal artery (PA) into proximal and distal segments (above knee and below knee), counting the tibial-peroneal trunk (TPT) as a discrete segment, assessing the whole length of the ATA, PTA and PEA (divided into proximal and distal segments) and assessing the plantar arch. Inter-observer reliability between two

observers was evaluated after the Bollinger scores had been combined into three segments (whole leg, above knee and below knee) and divided into four scoring groups (<3, 3-5, 6-8, ≥9). The plantar arch was excluded from this assessment due to the high proportion of missing data. They found that in approximately 75% of patients the observers agreed on their Bollinger score group and in less than 1% of patients the discrepancy was greater than one Bollinger score group [91]. When the arterial segments were assessed individually in this cohort of 418 patients with critical limb ischaemia, in whom approximately 40% suffered from DM[91], they found the highest burden of disease was in the distal superficial femoral artery (SFA) and proximal popliteal artery. Below the knee the PTA was most diseased with relative sparing of the PEA[84]. Diehm *et al.* (2008) extended the score down to include the pedal arch to enable comparison of patients with DM and renal failure and included the TPT, ATA, PTA and PEA in between. They found a high atherosclerotic burden in the pedal arch compared to controls in both the DM group and renal failure. The presence of renal failure predisposed to worse disease compared to DM[90].

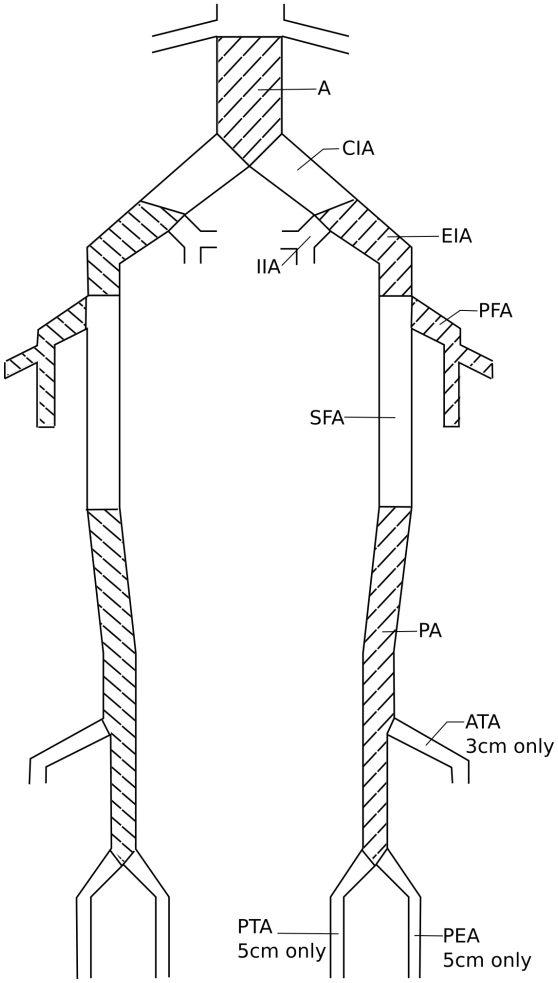

Figure 1.10-1: Arterial segments as described by Bollinger.
A; infrarenal aorta, CIA; common iliac artery, IIA; internal iliac artery, EIA; external iliac artery, PFA; profunda femoris artery, SFA; superficial femoral artery, PA; popliteal artery, ATA; proximal 3cm of anterior tibial artery, PEA; proximal 5cm of peroneal artery, PTA; proximal 5cm of posterior tibial artery.

Table 1.10-1: Bollinger scoring system

Location	Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%
Single	-	4	2	1
Multiple ≤ half	13	5	3	2
Multiple > half	15	6	4	3

Adapted from Bollinger *et al.* 1981. Each arterial segment will have an additive score based on the above scoring matrix. To avoid inadequate scoring the following rules should be obeyed. 1) In the presence of occlusions, plaques or stenosis are not considered. 2) When both categories of stenosis (>50% and ≤50%) are present plaques are not scored. 3) For each type of occlusive lesion only one length category is indicated

1.10.2. Microvascular

The screening tests above and further investigative tests such as duplex ultrasound, computed tomography angiography and magnetic resonance angiography all assess the macrovascular disease burden. The microcirculation (arterioles, capillaries and venules) also has a significant impact on the disease process in patients with DM. The relationship of the microvascular impairment with macrovascular disease is less clear but may be related to the inhibition of rapid refilling of the arterial segments in the presence of arterial stenosis and that in critical ischaemia maximum vasodilatation has already been reached in the resting period[92]. One study has found no difference in microvascular impairment between those with and without PAD[47] but Arora[51] found an improvement in the cutaneous vasodilatory response following peripheral vascular bypass.

1.10.2.1. Capillary microscopy

The skin receives its blood supply from deep and superficial dermal plexi, which are made up of multiple arteriovenous anastomoses. The superficial plexus has capillaries arising from it that provide the nutritional supply to the skin. The deep plexus is predominantly concerned with thermo-regulation[93]. Light microscopy can be used to assess the number and morphology of capillaries in the superficial plexus. Most commonly the nailfold is used. By use of videophotometric capillaroscopy it is possible to non-invasively visualise the size, shape, and the number of nutritional skin capillaries, in addition to this it is possible to measure the velocity of the blood in the capillaries[93-95].

Capillary microscopy (CM) has the advantage over the other non-invasive methods of being the only method that assesses only the nutritional vessels. It has been shown to be

highly reproducible when performed by one observer and when the findings are classified using a staging system it has the ability to discriminate between the severity of ischaemic disease[93]. However, the method can be time-consuming, and as capillary morphology varies between the toes on one foot in a single patient, it is important that there is consistency in the area being measured. Velocity measurements are also heavily influenced by movement[95].

1.10.2.2. Laser Doppler fluxmetry

LDF uses a helium-neon laser to penetrate the superficial layers of the skin[96-98]. The light is scattered by the tissue it encounters, any moving blood cells cause the light to be Doppler shifted. This results in an arbitrary unit, flux, that reflects the concentration and average speed of the cells[97,99]. The laser penetrates the skin to approximately 1.5mm [93] and as such provides information on both the superficial and deep dermal capillary beds with the possibility of also detecting flow in small arterioles and arterio-venous anastomoses. This means unlike CM it does not assess nutritional blood flow only and should be regarded as a total measure of blood flow in the skin [94,95]. Resting flux of the skin can be measured and provocation tests like post occlusive reactive hyperaemia (PORH), skin perfusion pressure (SPP) and thermal challenge can be performed[100-102]. LDF has been used in research to diagnose PAD[103], predict wound healing[104], assess outcomes following revascularisation[105,106] and examine patients with DM[107,108].

1.10.2.3. Transcutaneous measurement of oxygen pressure

$TcPO_2$ looks at the end product of perfusion. A heated probe is attached to the skin and induces localised hyperaemia allowing excess oxygen to diffuse across the skin and be

measured by the probe [95]. $TcPO_2$ has been shown to be able to predict clinical response to revascularisation, the risk of amputation and has the ability to discriminate between severity of disease [109-112]. Various factors affect the accuracy of $TcPO_2$ including arterial and venous blood pressure, epidermal thickness, capillary density, inflammation and oedema [95] and when compared to LDF it has less accuracy [110,112].

1.11. METHODS FOR EXAMINING FOR PERIPHERAL NEUROPATHY IN DIABETES MELLITUS

The American Diabetes Association advises that screening for DPN should be carried out using more than one of pinprick testing, VPT, 10g monofilament and ankle reflexes[53,113].

1.11.1. Pinprick

Pinprick testing assesses the small fibres of the somatic nervous system[114]. A proximal site is compared to a distal site and the result recorded as normal or abnormal[62]. Pinprick testing has been found to be less objective than VPT or monofilament testing[114].

1.11.2. Vibration perception threshold

The ability of a patient to detect vibration is a test of somatic nerve function, specifically large fibre activity[114]. While a tuning fork can be used as a crude screening tool the use of a neurothesiometer or vibratron allows a quantitative assessment of the degree of sensation loss [115]. The neurothesiometer has better accuracy than the vibratron and comparable variability to NCV[116]. VPT is usually measured on the pulp of the great toe, and a value of >25 is associated with a seven-fold increase in the risk of ulceration when compared to patients

with a VPT of <15[117,118]. VPT has a significant correlation with NCV and when diagnosing DPN has a sensitivity of 87%[116,119].

1.11.3. 10g Monofilament

Like VPT monofilament tests the large fibres of the somatic nervous system[114]. The test involves the placement of a monofilament on the skin area to be tested. A negative test is when the patient is unable to detect the presence of the monofilament when the pressure applied is sufficient to cause the monofilament to buckle. The most sensitive and specific areas to test are the plantar aspect of the hallux and the bases of the third and fifth metatarsal heads[120]. A positive result (patient unable to detect the monofilament) is a significant and independent predictor of foot ulceration[121,122].

1.11.4. Ipswich touch test

The Ipswich touch test (ITT) is an alternative to monofilament testing that has the advantage of not requiring any specialist equipment. The test involves touching the tip of the 1st, 3rd and 5th toes lightly for 1 to 2 seconds. The patient's eyes are closed and are asked to respond yes whenever they feel the touch. Two or more insensate areas are classified as neuropathy[123]. The test has been shown to have similar sensitivity and specificity (76% and 90% respectively) to monofilament (81% and 90%) and high reproducibility[123].

1.11.5. Ankle reflex

A reduced or absent ankle reflex is an independent predictor of ulceration[122]. When compared to VPT ankle reflex has been found to have a high sensitivity of more than 90%, but a low specificity of less than 40%[124] and when compared to NCV has a sensitivity of 91%

and specificity of 67%[125]. Ankle reflex is seldom used alone and generally forms part of an assessment that encompasses the signs and symptoms of neuropathy.

1.11.6. Nerve conduction studies

Nerve conduction studies (NCS) are the gold standard for the diagnosis of peripheral neuropathy[114,126]. In the diagnosis of peripheral neuropathy of the lower limb the peroneal and sural nerves are commonly used. This provides a measure of both the motor and sensory deficit. Recording and stimulating electrodes are placed at either end of the nerve and electrical stimulation is passed between them. The velocity, amplitude and latency of the nerve are recorded[126]. In experienced hands NCS have been found to have low variability, good reproducibility and high sensitivity. In combination with symptoms and signs of DPN NCS have been recommended as the investigation of choice in research of DPN[126-129]. The limitations of NCS are the expensive equipment and technical expertise required.

1.11.7. Neurological symptom scores

There are multiple different scores for the symptoms of neuropathy. Many of the scores, for example the Neuropathy Symptom and Change score (also known as the Number Severity and Change score) (NSC), McGill Pain Questionnaire, Neuropathy Impairment Score and Lower Limb Function Test include assessment of sensory, motor and autonomic symptoms and include an element of examination[130]. Due to this they are lengthy questionnaires and time consuming to administer, such as the NSC which has 38 questions[131]. The NSS has been extensively used in clinical trials and has been found to correlate well with NCS and have reasonable sensitivity[132,133] but like those above is lengthy

and was not explicitly developed for evaluating changes in neuropathy after treatment[130]. The Neuropathy Total Symptom Score – 6 (NTSS-6) overcomes some of these shortcomings. It consists of six questions focusing solely on sensory symptoms in the lower limbs. The patient is asked to consider the frequency and intensity of the symptoms they have experienced over the last twenty-four hours. The maximum score is 21.96 and a score of more than six indicates clinically significant symptoms. On testing the score was found to have good internal consistency and results that correlate with NCS [130]. NTSS-6 has been used in clinical trials testing changes in DPN symptoms and been able to demonstrate a difference between treatment groups[134-136].

1.11.8. Intra-epidermal nerve fibre density

Full-thickness punch biopsy of the skin allows for staining with protein gene product (PGP) 9.5 and subsequent immunofluorescence. PGP 9.5 is a pan-axonal and pan-neuroendocrine marker and means the density of nerve fibres per millimetre squared can be measured[137]. A reduction in intra-epidermal nerve fibre density (IENFD) is associated with severity of diabetic neuropathy[138,139]. It has also been shown to be possible to observe axonal regeneration over a period of months using this method[140]. As such IENFD represents an interesting area of current research that would be relevant in our cohort of patients. However, the consensus among the project designers was that it would be ethically unviable to create a new area of skin loss in patients already suffering from the complications of skin loss on a background of DM and or PAD.

CHAPTER 2: THE DISTRIBUTION OF ATHEROSCLEROSIS IN THE LOWER LIMB ARTERIES: A REVIEW OF HOW THE DISTRIBUTION OF DISEASE IS AFFECTED BY DIABETES MELLITUS

2.1. INTRODUCTION

Infra-popliteal disease is associated with critical limb ischaemia which is the final stage in the disease course of peripheral arterial disease (PAD)[141]. The pattern of vascular disease influences the options that are available for revascularisation. Management of distal disease is more challenging than proximal disease, although advances in this area are being made[142-145]. Despite these advances patients with distal disease have a higher risk of amputation and shorter amputation-free survival[146].

The prevalence of diabetes mellitus (DM) is increasing worldwide and is a significant risk factor for PAD[3,16]. Patients with DM have a predisposition towards a higher burden of atherosclerotic disease below the knee compared to patients without DM (NDM). This is considered to have an impact on both the treatment options available and prognosis following revascularisation in patients with DM[147,148].

This hypothesis of a higher burden of disease in the tibial arteries is widely accepted on an anecdotal level within the medical community. Many medical students and surgical trainees will have heard this stated as fact as part of bedside teaching in vascular surgery. This review aimed to summarise the quality of the evidence supporting this hypothesis.

2.2. METHODS

A literature search was performed using the search terms “diabetes mellitus”, “peripheral vascular disease”, ‘distribution of disease”, “angiography”, “computed tomography angiography” and “magnetic resonance angiography”. Synonyms and various combinations were used in the search strategy which involved both Medical Subject Headings (MESH) and keyword searches. Embase and MEDLINE databases were searched including papers published from 1946 to present day and in-process citations (Search terms in Appendix I). References from relevant studies were also scrutinised for potential studies.

Papers were included if arterial imaging of the lower limb was undertaken using digital subtraction angiography (DSA), Computed Tomography Angiography (CTA), or Magnetic Resonance Angiography (MRA). They were excluded if the indication for imaging was not PAD, if there was no separation of patients with and without DM or only patients with DM were included. The final requirement was an anatomical description of the arteries affected by atherosclerotic disease. This description could be given using a scoring system or proportions of arterial segments affected.

2.2.1. Statistical analysis

For papers that included proportions of patients with PAD by arterial segment the number of patients who had disease in each arterial segment was extracted by one author (DL). These papers were included in a forest plot that was produced using Revman 5.3 (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.). Data were summarised as odds ratio (OR) with 95% confidence intervals.

2.3. RESULTS

From the literature search, 151 potential papers were identified and following review of titles, abstracts, full text and references 14 studies were included in the review (Figure 2.3-1). The papers dated from 1964 to 2009 and were all cross-sectional studies apart from one cohort study[90] and two case-control studies[149,150] (Table 2.3-1 and Table 2.3.2). The majority of papers did not state if their analysis was by patient or by limb, in most, it appeared that a single treated limb was included per patient[90,150-155]. Four papers included all treated limbs[156-159], one paper included both legs for all patients[149], one paper analysed by lesion[160] and one paper only used the data from the left leg if there was bilateral imaging as they found the legs to be comparable[83]. How risk factors for PAD were treated varied between papers. Four papers performed some form of multivariate analysis to stratify for risk factors[149,155,159,160], the majority of remaining papers reported proportions of risk factors and comparability between groups however two papers made no mention of risk factors[151,156]. No studies considered type I and type II DM separately. One paper found a significant difference in the proportion of men and women in their cohorts[149] and one paper found significant differences in the proportions of smokers[157]. Most papers had cohorts with a mean age in the mid-sixties although two papers deliberately selected young cohorts[151,152] and two papers had older cohorts[90,158]. The majority of cohorts consisted of approximately 60% men apart from Ozkan *et al.* who had 85.9% men[155]. The proportion of smokers in each group ranged from 13.5% to 83.2% (Table 2.3-3).

All the studies used angiography to visualise the arterial tree and in total 15 different arterial segments were described (Table 2.3-1 and Table 2.3.2). The most commonly used segments that differentiated between proximal and distal disease were aorto-iliac (A-I),

femoro-popliteal (F-P), and tibial. Seven studies also included a category that represented disease at multiple levels. These segments were included in the forest plot along with smaller segments that fitted in the same group. I.e. patients with disease in the popliteal artery could be included in the F-P group but those in a popliteal/tibial group could not be included. The description of what constituted significant disease varied between papers. Of the papers that described proportions of arterial segment involved five only included occlusions[151,154,156-158], two defined a significant stenosis as involving more than 20% of the lumen[152,153], two defined it as more than 50%[149,155] and in one paper the definition was not stated[160].

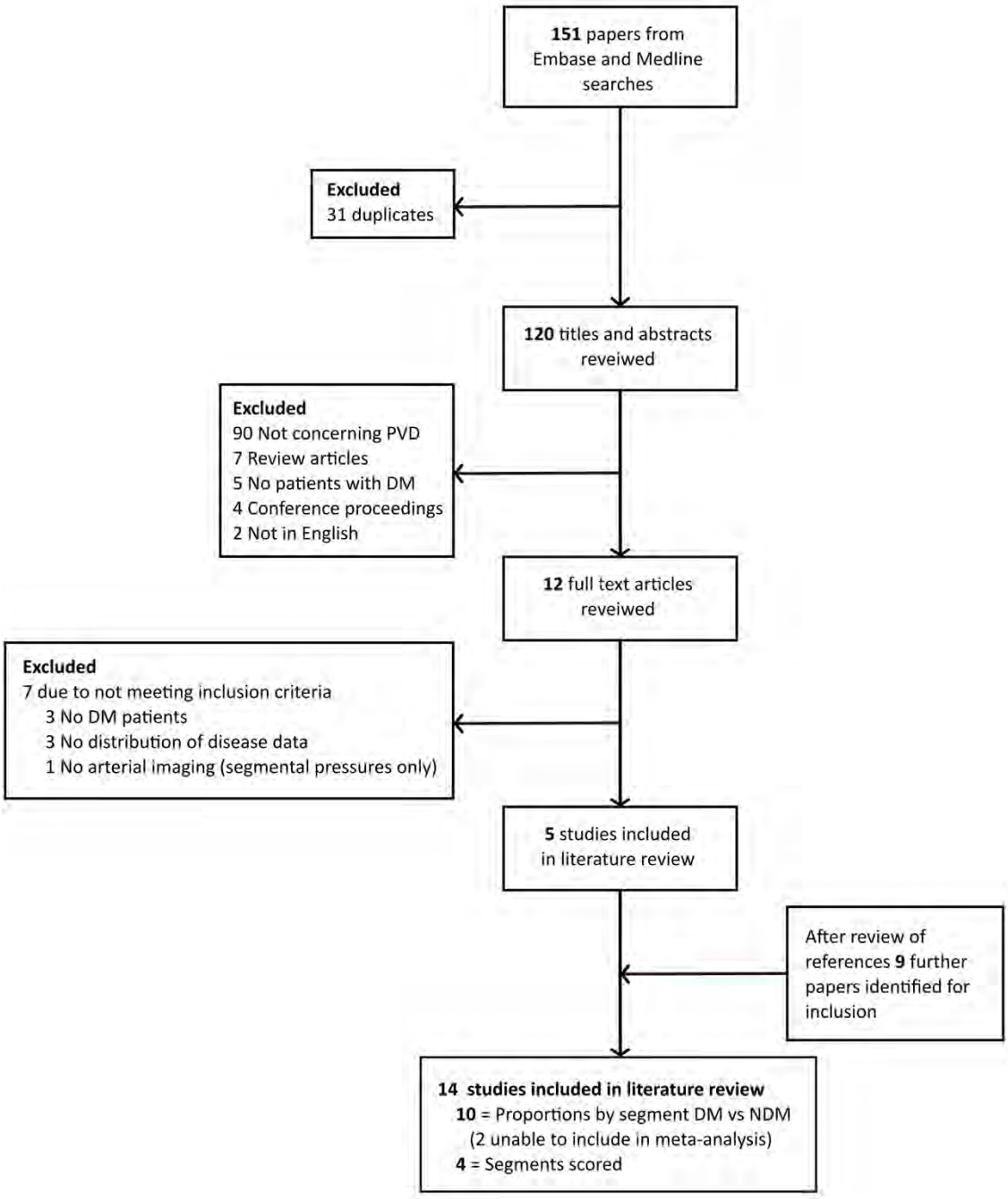

Figure 2.3-1: Distribution of disease review inclusion flow chart

Table 2.3-1: Characteristics of included studies. Studies which presented proportions

Author	Year	Country	Study design	Patients	Groups	Method of describing pattern
De Bakey ME[151]	1964	USA	Cross-sectional study	Patients with occlusive disease of the lower extremities	DM=6 NDM = 41	A-I, F-P, A-I/F-P, PEA/tib, F-P/PEA/tib, A-I/F-P/PEA/tib, ML
Haimovici H[156]	1967	USA	Cross-sectional study	Patients with arteriosclerosis obliterans	DM=91 NDM=98	A-I, F-P, F-P/tib, P-tib, P, tib, ML
Ciavarella A[157]	1993	Italy	Cross-sectional study	Patients with symptomatic PAD	DM=89 NDM=61	A-I, F-P, tib, F-P/tib, ATA, PTA, PEA, DP, Plant, ML
Hansen ME[152]	1995	USA	Cross-sectional study	Patients with symptomatic PAD	DM=22 NDM=37	A-I, F-P/tib, ML
Kroger K[153]	2000	Germany	Cross-sectional study	Patients with PAD	DM=46 NDM=86	A-I, F-P, tib, ML
Haltmayer M[149]	2001	Austria	Case-control study	Patients with symptomatic PAD	DM=41 NDM=65	A-I, F-P, tib,
Lazaris AM[158]	2004	UK	Cross-sectional study	Patients undergoing sub-intimal angioplasty	DM=33 NDM=66	F, F-P, F-P-tib, P-tib, tib, ML
Rueda CA[154]	2008	USA	Cross-sectional study	Patients undergoing infrainguinal revascularisation	DM=262 NDM=168	A-I, F, P-tib, ML
Diehm [N160,a]	2006	Switzerland	Cross-sectional study	Patients undergoing endovascular therapy of lower limb	DM=891 NDM=1768	I, F-P, tib
Ozkan [U155,a]	2009	Turkey	Cross-sectional study	Patients with PAD	DM=261 NDM=365	A-I, F-P, tib, ML

[a]Not included in meta-analysis. A=Aorta, I-Iliacs, F=Femoral, P=Popliteal, tib=Tibials, PEA=Peroneal, ATA=Anterior tibial artery, PTA=Posterior tibial artery, Plant=Plantar vessels, TPT= Tibial- peroneal trunk, ML=Multi-level disease, USA=United States of America

Table 2.3-2: Characteristics of included studies. Studies which presented scores

Author	Year	Country	Study design	Patients	Groups	Method of describing pattern
Menzoian JO[159]	1989	USA	Cross-sectional study	Patients with PAD	DM=115 NDM=119	ATA, PTA, PEA, Plant
Jude EB[83]	2001	UK	Cross-sectional study	Patients undergoing infrainguinal revascularisation	DM=58 NDM=78	Individual vessels I to tib
van der Feen C[150]	2002	Netherlands	Case-control study	Patients with symptomatic PAD	DM=37 NDM=37	I-F, P-tib
Diehm N[90]	2008	Switzerland	Cohort study	Patients undergoing angiography for chronic lower limb ischaemia	DM= 25 NDM=25	Individual vessels TPT to tib, TPT-tib average score, Plant

A=Aorta, I-Iliacs, F=Femoral, P=Popliteal, tib=Tibials, PEA=Peroneal, ATA=Anterior tibial artery, PTA=Posterior tibial artery, Plant=Plantar vessels, TPT= Tibial- peroneal trunk, ML=Multi-level disease

Table 2.3-3: Demographics of studies included in the review.

Author	Age (Mean ± SD)		Gender (♂/♀)		Smoking status (smokers/non-smokers)	
	DM	NDM	DM	NDM	DM	NDM
De Bakey ME[151,a]	16-37[b]		4/2	25/16	2/4	29/12
Haimovici H[156]	-	-	-	-	-	-
Ciavarella A[157]	65 ± 9	64 ± 10	62/27	44/17	45/44	52/9
Hansen ME[152,a]	43.4 ± 5.8		29/30		45/14	
Kroger K[153]	61 ± 13		85/47		-	-
Haltmayer M[149]	66.4 (57.9-74.4)[c]	63.9 (59.5-68.7)[c]	80/26	32/21	48/58	7/45
Lazaris AM[158]	78.5 (42-92[b])		53/46		21/19	47/25
Rueda CA[154]	66 ± 12		302/148		-	-
Diehm N[160]	70 ± 11		1583/1076		1144/1515	
Ozkan U[155]	62 ± 11		538/88		494/132	
Menzoian JO[159]	67 ± 1.2/69.8 ± 1.6[d]	64 ± 1/75.4 ± 1.3[d]	-	-	73/42	98/21
Jude EB[83]	63.83 ± 10.4	65.3 ± 11.11	34/24	47/31	47/11	60/18
van der Feen C[150]	65.5 ± 13.6	65.7 ± 12.7	20/17	20/17	12/25	12/25
Diehm N[90]	74.2 ± 10.3	77.4 ± 9.6	10/15	12/13	11/14	9/16

[a]Deliberately selected young age group, [b]age range, [c]Median (IQR), [d]Smokers/non-smokers, SD= standard deviation

2.3.1. Proportions of arterial segments affected

Ten studies described the proportions of arterial segments affected by PAD and included 1682 patients with DM and 2775 without DM[149,151-158,160]. For two papers, it was not possible to extract sufficient data[155,160] they were not included in the analysis but their results will be discussed.

The resulting forest plot (Figure 2.3-2) demonstrates that those with DM were significantly less likely to have disease in the aorto-iliac segment (OR 0.25 (0.15-0.42), n=DM 466/NDM 458) and significantly more likely to have disease in the tibial segment (OR 1.94 (1.27-2.96), n=DM 306/NDM 417). In the DM group, there is a trend towards relative sparing in the femoro-popliteal segment but this does not reach significance (0.66 (0.33-1.31), n=DM 568/NDM 585). There is also a suggestion that those with DM were more likely to have multilevel disease, again this does not reach significance (1.26 (0.93-1.70), n=DM 549/NDM 557).

The two papers not included in the meta-analysis showed a similar pattern. Diehm *et al.*[160], in a retrospective cohort that examined the risk factors for distribution pattern of lower limb atherosclerosis in 2659 patients (891 with DM), on multivariate logistic regression found that DM had a relative risk ratio of 0.59 (0.49-0.72, p<0.001) for iliac disease compared to 1.68 (1.47-1.92, p<0.001) for tibial disease. Ozkan *et al.*[155] performed a similar analysis in 626 patients with symptomatic PAD 261 of whom had DM. They found, on univariate analysis, the presence of DM was related to odds ratios of 0.56 (p=0.001) for aorto-iliac disease, 1.16 (p=0.39) for femoro-popliteal disease and 2.44 (p=0.001) for tibial disease.

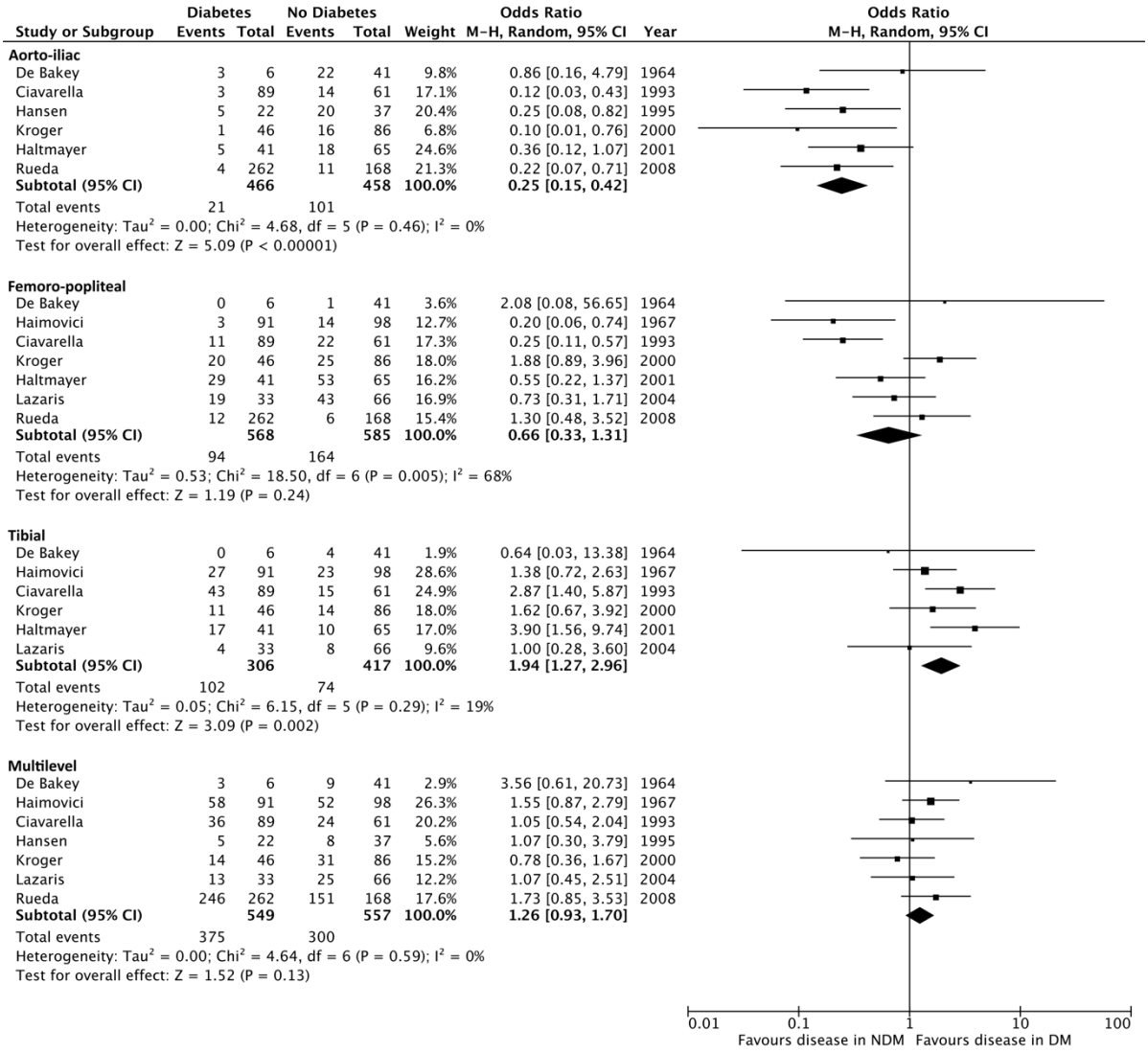

Figure 2.3-2: Forest plot comparing the presence of arterial disease by arterial segment in patients with diabetes compared to patients without.

2.3.2. Scores to describe the distribution of disease

Four papers reported scores by arterial segment[83,90,150,159]. Three of these used the Bollinger score[82] and one[159] a score described by LaMorte *et al.* in 1985[161]. Briefly, Bollinger's score is a semi-quantitative score that considers each arterial segment separately. Each

arterial segment is assessed for the presence of plaques less than 25% of the lumen, stenoses less than 50% of the lumen, stenoses more than 50% of the lumen and occlusions. A higher score is achieved if these lesions are multiple and involve more than half the length of the segment. The minimum score is zero and maximum fifteen (occlusion for more than half the length). The segments and the scoring matrix used for each segment individually are shown in Figure 1.10-1 and Table 1.10-1. LaMorte *et al.*'s score assigns a score of zero to a non-visualised vessel, one to a partially compromised vessel and two to an intact vessel. By applying this score to 227 patients with PAD, Menzoian *et al.* demonstrated significantly lower scores (i.e. more disease) in the posterior tibial artery (PTA) (0.51 vs 1.02, $p<0.05$) and peroneal artery (PEA) (0.9 vs 1.28, $p<0.05$) as well as the sum of the tibial vessels (2.17 vs 3.13, $p<0.05$) in the DM group[159].

Despite all using the Bollinger score it is hard to compare the results for Jude *et al.*, van der Feen *et al.* and Diehm *et al.* due to the different vessels reported. Jude *et al.* used the segments originally described by Bollinger (ten arterial segments (per leg) from the infra-renal aorta down to the proximal 3cm of the anterior tibial artery (ATA) and the proximal 5cm of the PTA and PEA[82]) and reported the median score for each segment. In 136 patients they found those in the DM group (n=58) had a significantly higher score in the profunda femoris (mean score 3 (Inter-quartile range (IQR) 0-5) vs 0 (0-2)), popliteal (7 (3-10) vs 3 (0-4)), ATA (13 (4-15) vs 3 (0-4)), PTA (15 (0-15) vs 4 (0-14)) and PEA (5 (0-5) vs 0 (0-6))[83]. Van der Feen *et al.* also used the original description of the segments but did not report the individual scores for each segment. Instead, the scores were combined to form the "upper leg" (aorta, iliacs, profunda femoris and superficial femoral artery) and "lower leg" (popliteal, ATA, PEA, and PTA). In 37 patients with DM matched for age gender and smoking to 37

patients without DM, there was a higher mean score for the lower leg in the DM group but this was not significant (47.4 vs 37.6, p=0.22). While the scores for the individual segments were not reported, the included bar graphs show that the only segment with a significant difference was the PEA in the right legs ($p<0.05$), those in the DM group had a higher score[150]. Diehm *et al.* only scored the below the knee segments including the plantar vessels and so extended Bollinger's original description. Their patient groups were patients with DM (n=25), patients with renal insufficiency (n=15), patients with both DM and renal insufficiency (n=25) and 25 controls with neither DM or renal insufficiency. They found no significant difference between the groups although those in the DM group and the renal insufficiency group tended towards higher scores.

2.4. DISCUSSION

These results demonstrate that there is a difference in the distribution of atherosclerotic disease in patients with DM compared to those without. The hypothesis that patients with DM have more disease below the knee is supported. Patients with DM are less likely to have disease in the aorto-iliac segment and more likely to have disease in the tibial segment. This is demonstrated in the forest plot, the papers that applied scores, and also the papers that it was not possible to include in the forest plot. In the femoro-popliteal segment, the trend is towards those without DM having more disease, although this does not reach significance. There was a trend towards multi-level disease being more common in patients with DM. Four papers assessed the severity of disease in individual vessels rather than segments[83,90,159] although only Jude *et al.* did for both above and below knee vessels [83]. In

patients with and without DM, the least affected of the tibial vessels was consistently the PEA.

The PEA as a target vessel for revascularisation has been considered to have limitations due to success relying on indirect collateralisation to supply the forefoot[162]. The patency of the PEA has also been demonstrated to be less critical in preventing amputation[163]. The angiosome model holds that the areas supplied by the PEA are the anterior and lateral ankle and plantar heel[164]. However increasingly the PEA has been shown to have multiple collaterals and to commonly supply the pedal arteries and as such has comparable outcomes for both surgical and endovascular revascularisation compared to other distal target vessels[165-168]. Only three papers included in the review considered pedal vessels[90,157,159]. Ciavarella *et al* found that a higher proportion of patients with DM had complete obstruction of the plantar vessels (53% vs 29% p<0.001). There was however no significant difference in the rate of occlusions in the dorsalis pedis artery (43% vs 40%)[157]. Diehm *et al,* using the Bollinger score found that both DM and renal failure were associated with more disease in the pedal vessels when compared to the calf vessels. However, when patency of at least one pedal vessel suitable for bypass was considered, patients with DM where comparable to controls without DM[90]. Menzoian *et al* found that there was less disease of the pedal arch in patients with DM, the difference was not significant[159]. Whether there is sparing or not of the pedal vessels in DM is an inconsistent finding in the literature[169-171], but there are case series that suggest that revascularisation with bypass to pedal vessels is a viable option on patients with DM[172].

DM is known to have an impact on both the presentation of PAD and outcomes following revascularisation[173,174]. The distribution of atherosclerotic disease has also been shown to be

related to outcomes following revascularisation procedures in patients both with and without DM[84]. The pathophysiology behind why patients with DM have increased PAD is complex but thought to be related to a combination of down-regulation of nitric oxide and prostacyclin, upregulation of vasoconstrictors, apoptosis of endothelial cells, activated coagulation, abnormal platelet activation and propensity towards plaque rupture (Section 1.3 for more detail)[175]. There is not any clear evidence why the distal vessels are predominantly affected and while these results support the hypothesis that patients with DM have a more significant disease burden below the knee they provide us with limited information on the degree to which individual vessels or areas of vessels are affected.

A strength of the review is that all the papers used DSA as the imaging modality. DSA remains the gold standard for imaging of the lower limbs and describing the anatomic distribution of stenotic disease[176]. The literature search did include CTA and MRA as imaging modalities but no papers that used these modalities and met the inclusion criteria were found. These modalities could be used to assess distribution of disease keeping in mind their limitations compared to DSA[81,176], however the studies have not been done. In terms of scoring systems very few papers have described systems that employ imaging modalities other than DSA and those that have, have not been validated[177-179]. In the forest plot, there was low heterogeneity between the papers apart from those considering the femoro-popliteal segment (I^2=68%). A significant weakness of the review is the low quality of the papers included. They are all observational studies, predominantly retrospective and so the body of evidence is low to very low quality[180]. An attempt to assess the methodological quality of the papers using the Newcastle-Ottawa scale[181] was made. However, all but three papers were cross-sectional studies making it not possible to apply the scale. There was

consistency in the type of patients selected with the majority of papers including patients with Fontaine II to IV disease. However, one paper only included patients with intermittent claudication[155], two papers excluded those with intermittent claudication[154,158] and three papers did not define the patient group beyond symptomatic PAD[152,156,157]. As described in the results section there was also variance in how significant disease was defined. Within each paper the demographics for each group, when reported, were comparable (Table 2.3-3).

Additional weaknesses include that the papers are all relatively historical (earliest 1964, latest 2009) and the variety in how the arterial segments were described and grouped. This grouping meant some data was not able to be included in the meta-analysis because the segment crossed the knee, weakening the data included. During data collection, it was considered that improvements in the medical management of DM and PAD might have had an impact on the distribution of disease. Evidence from high-quality randomised controlled trials on the importance of tight blood glucose control in relation to the complications of DM was published in the late nineties[59,182]. When studies from before the year 2000 were excluded from the meta-analysis, the trends remained the same although the odds ratio for tibial disease was no longer significant (OR 1.99 (0.94-4.24)). Between 1964 and 2019 there have been considerable improvements in imaging technologies[183]. DSA remains the gold standard for research but in clinical practice duplex ultrasound, CTA and MRA are advised as first line investigations for planning revascularisation[184]. This means that there has been a relative reduction in the number of DSA's performed compared to non-invasive modalities, particularly in Europe[185,186]. Potentially this means that the types of patients included in the

earlier studies will be different to those in the later studies. This change may also go some way to explaining why there are no more recent studies available.

2.5. CONCLUSIONS

Patients with DM are more likely to have atherosclerotic disease in the tibial vessels compared to patients without. The current published evidence supports this hypothesis. There is very limited data on the degree to which individual vessels are affected. Further information on this and a greater understanding of why the distal vessels are more affected are avenues for future research. In the next chapter a study that aims to examine the distal vessels in detail is presented.

CHAPTER 3: METHODOLOGY AND RESULTS FOR DISTRIBUTION OF ARTERIAL DISEASE IN DIABETES MELLITUS

3.1. HYPOTHESIS

Patients with diabetes mellitus (DM) have a higher proportion of infra-popliteal disease compared to patients without DM.

3.2. PRIMARY OUTCOME

Difference between median Bollinger score in each arterial segment in patients with DM compared to patients without DM.

3.3. DESIGN OF STUDY: PILOT STUDY

All patients who had a lower limb angiogram between September 2010 and April 2014 at Queen Elizabeth Hospital Birmingham were identified from the prospective radiology database. From this cohort, all the patients with DM were identified. Each of these patients was age and sex-matched with a patient without DM (NDM) who underwent an angiogram in the same period. The cohort for the pilot study consisted of 216 patients randomly selected from all matched patients combined, due to this there were uneven numbers of patients between the two study cohorts.

The first angiogram performed within the study period with images saved on the hospitals imaging system (IMPAX) was assessed. Each arterial segment, as described by Bollinger, was scored for all arteries imaged from the infra-renal aorta down to the proximal

anterior tibial (ATA), posterior tibial (PTA) and peroneal arteries (PEA). A limitation of the Bollinger score, as it was originally described[82], is that the described segments only extend 3cm into the ATA and 5cm into the PTA and PEA. Due to this the decision was made to include segments that encompassed the whole of the crural vessels and major pedal vessels. This aimed to collect a fuller picture of the extent of distal disease than would be possible with the original segments. The same scoring matrix was applied to the whole of the ATA, PTA and PEA divided into thirds and the dorsalis pedis, medial and lateral plantar arteries (LPA) (Figure 3.3-1). The decision to divide the vessels into thirds was based on advice from consultant radiologists and the endovascular surgeons in our department who routinely describe the vessels in these terms. For the pilot study, both legs were scored if there were available images.

3.3.1. Statistics

Parametric data (age) is reported as mean (± standard deviation (SD)) and the groups compared using unpaired T-test. Non-parametric data (Bollinger score) is reported as median (inter-quartile range) and the groups compared using Mann-Whitney U test. Categorical data (ethnic group, smoking status, hypertension, hypercholesterolaemia and renal function) was compared between groups using Fisher's exact test. A p-value of less than 0.05 was considered to be statistically significant.

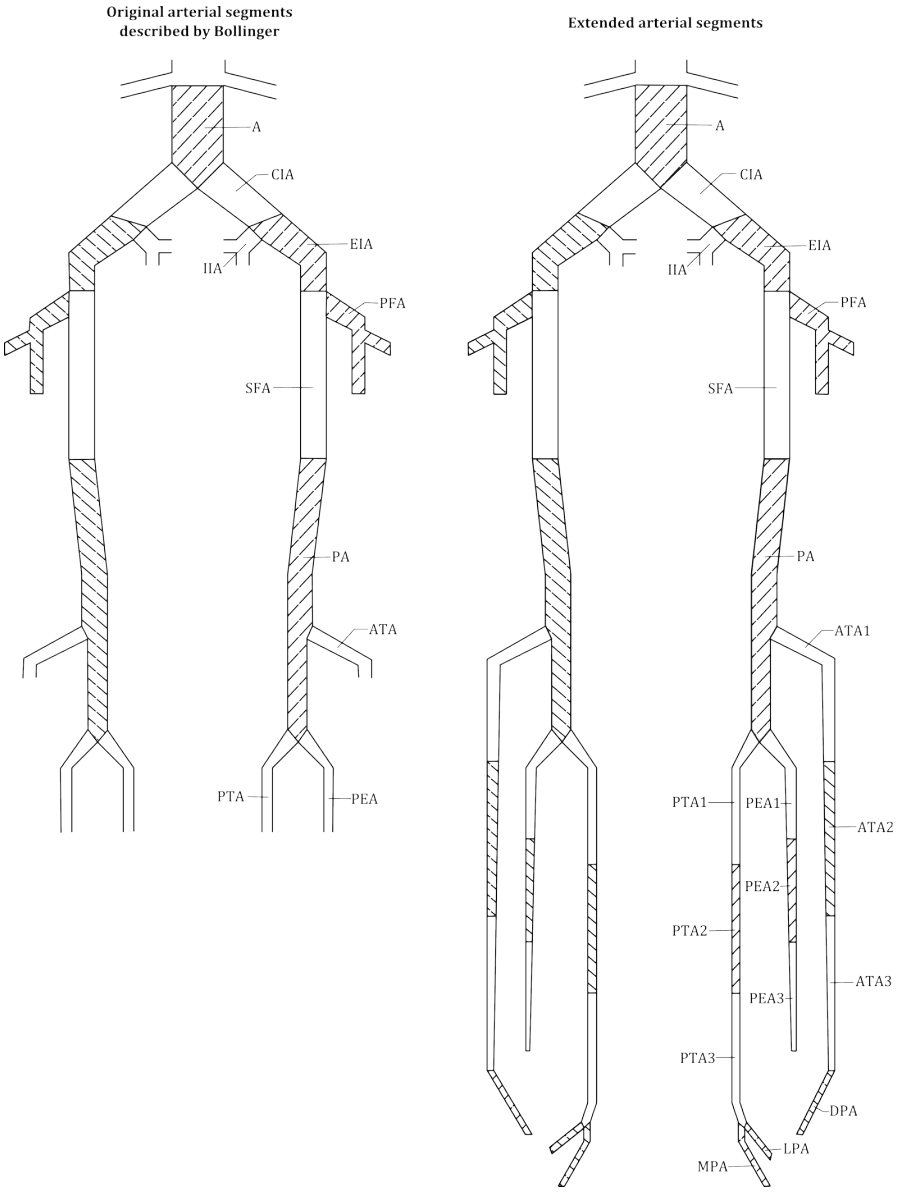

Figure 3.3-1: Schematic representation of arterial segments described by Bollinger compared to the arterial segments examined in the pilot study.

A; infrarenal aorta, CIA; common iliac artery, IIA; internal iliac artery, EIA; external iliac artery, PFA; profunda femoris artery, SFA; superficial femoral artery, PA; popliteal artery, ATA; proximal 3cm of anterior tibial artery, ATA1; proximal 3rd of anterior tibial artery, ATA2 middle 3rd of anterior tibial artery, ATA3; distal 3rd of anterior tibial artery, DPA; dorsalis pedis artery, PEA; proximal 5cm of peroneal artery, PEA1; proximal 3rd of peroneal artery, PEA2; middle 3rd of peroneal artery, PEA3 distal 3rd of peroneal artery, PTA; proximal 5cm of posterior tibial artery, PTA1; proximal 3rd of posterior tibial artery, PTA2; middle 3rd of posterior tibial artery, PTA3; distal 3rd of posterior tibial artery, MPA; medial plantar artery, LPA; lateral plantar artery**.**

3.4. PILOT STUDY RESULTS

There were 119 patients in the DM group and 97 in the NDM group. The mean age of the DM group was 70 years (SD ±11) and NDM group 69 years (SD±13, p=0.51). Seventy-four percent of NDM patients and 71% of DM were males (p=0.65). Significantly more patients without DM had bilateral angiograms than patients with DM (26.8% vs 9.2%, p <0.01). Due to this difference and the potential to skew the data the decision, in consultation with statisticians, was made to only report and include in the analysis the results for the left leg in those who had bilateral imaging. In the diabetes group, there were significantly higher proportions of patients in the Asian and Black ethnic groups and also more patients with hypertension. The other demographic groups, smoking status, hypercholesterolaemia and renal function, were comparable (Table 3.4-1).

Table 3.4-1: Demographics for pilot study groups

		Percentage (n)		
		No Diabetes (n=97)	Diabetes (n=119)	p-value
Mean age (SD)*		69 (12.8)	70 (11.20)	0.51
Sex**	**Male**	74.2 (72)	70.6 (84)	0.65
	Female	25.8 (25)	29.4 (35)	
Ethnic group**	**White**	90.7 (88)	73.9 (88)	**<0.05**
	Asian	2.1 (2)	13.4 (16)	
	Black	1.0 (1)	6.7 (8)	
	Other	6.2 (6)	5.9 (7)	
Smoking**	**Never smoked**	10.3 (10)	19.3 (23)	0.19
	Ex-smoker	48.5 (47)	50.4 (60)	
	Still smoking	29.9 (29)	21.0 (25)	
	Unknown	11.3 (11)	9.2 (11)	
Hypertension**	**Yes**	48.5 (47)	73.9 (88)	**<0.05**
	No	40.2 (39)	17.6 (21)	
	Unknown	11.3 (11)	8.4 (10)	
Hypercholesterolaemia**	**Yes**	40.2 (39)	38.7 (46)	0.51
	No	29.9 (29)	24.4 (29)	
	Unknown	29.9 (29)	37.0 (44)	
Renal function**	**Normal**	93.8 (91)	81.5 (97)	0.09
	Creatinine >150 umol/L	3.1 (3)	10.1 (12)	
	Dialysis	2.1 (2)	4.2 (5)	
	Functioning transplant	1.0 (1)	2.5 (3)	

*Unpaired T-test, **Fisher exact test, SD = standard deviation

The external iliac artery was the only artery to have a significantly higher score in the NDM group (3 (Inter-quartile range (IQR) 0-7)) than the DM group (2 (IQR 0-3) $p<0.05$). The DM group had significantly higher scores in all segments of the PTA. The proximal, middle and distal thirds of the ATA in the DM group all had a higher median score than the NDM group; however, this difference did not reach statistical significance (Table 3.4-2).

Table 3.4-2: Pilot study: comparing the median Bollinger score of patients with diabetes to patients without diabetes by arterial segment.

Arterial segment	Median Bollinger score (IQR)		p-value*
	No Diabetes (n=97)	Diabetes (n=119)	
Aorta	3 (1-3)	3 (3-3)	0.497
Common iliac	3 (2-7)	3 (1-3)	0.192
Internal iliac	3 (0-7)	3 (0-7)	0.994
External iliac	3 (0-7)	2 (0-3)	**<0.05**
Superficial femoral	8 (3-13)	7 (3-13)	0.411
Profunda femoris	0 (0-3)	0 (0-3)	0.889
Popliteal	5 (2-13)	6 (3-10)	0.369
Anterior tibial (proximal 3cm)	2 (0-8)	3 (0-7)	0.182
Anterior tibial (proximal 3rd)†	3 (0-13)	5 (2-13)	0.109
Anterior tibial (middle 3rd)†	3 (0-13)	4 (0-15)	0.112
Anterior tibial (distal 3rd)†	3 (0-15)	7 (0-15)	0.417
Dorsalis pedis†	13 (0-15)	13 (0-15)	0.573
Peroneal (proximal 5cm)	3 (0-7)	3 (0-10)	0.386
Peroneal (proximal 3rd)†	3 (0-7)	3 (0-13)	0.246
Peroneal (middle 3rd)†	3 (0-7)	3 (0-13)	0.675
Peroneal (distal 3rd)†	3 (0-13)	3 (0-15)	0.992
Posterior tibial (proximal 5cm)	3 (0-13)	6 (0-15)	**<0.05**
Posterior tibial (proximal 3rd)†	3 (0-13)	8 (2-15)	**<0.05**
Posterior tibial (middle 3rd)†	3 (0-15)	10 (0-15)	**<0.05**
Posterior tibial (distal 3rd)†	3 (0-15)	13 (0-15)	**<0.05**
Medial plantar†	15 (0-15)	15 (0-15)	0.463
Lateral plantar†	15 (0-15)	15 (0-15)	0.978

* Mann-Whitney U Test

† Not originally included in Bollinger score

There was a high proportion of missing data throughout the dataset used in the pilot study (Table 3.4-3). This was due to absent or incomplete imaging of arterial segments with eighteen patients having only data for the supra-inguinal vessels. The proportion of missing data points ranges from 15.5% (popliteal and ATA) to 75.3% (aorta) in the DM group and 3.4% (ATA) to 89.2% (aorta) in the NDM group. The mean proportion missing for those without DM was 30.6% (SD 19.3%) compared to 23.0% (32.4%, p=0.16).

Table 3.4-3: Proportion of patients with missing data by arterial segment

	No Diabetes (n-97)		Diabetes (n=119)		Difference in percentage missing
	Missing	%	Missing	%	
Aorta	107	89.9	73	75.3	14.66
Common iliac	97	81.5	60	61.9	19.66
Internal iliac	96	80.7	60	61.9	18.82
External iliac	95	79.8	60	61.9	17.98
Superficial femoral	5	4.2	16	16.5	17.98
Profunda femoris	15	12.6	20	20.6	12.29
Popliteal	4	3.4	15	15.5	8.01
Anterior tibial	4	3.4	15	15.5	12.10
Anterior tibial 1	4	3.4	17	17.5	12.10
Anterior tibial 2	5	4.2	19	19.6	14.16
Anterior tibial 3	6	5.0	22	22.7	15.39
Dorsalis pedis	16	13.4	29	29.9	17.64
Peroneal	4	3.4	16	16.5	16.45
Peroneal 1	4	3.4	17	17.5	13.13
Peroneal 2	5	4.2	19	19.6	14.16
Peroneal 3	6	5.0	22	22.7	15.39
Posterior tibial	4	3.4	16	16.5	17.64
Posterior tibial 1	4	3.4	17	17.5	13.13
Posterior tibial 2	5	4.2	19	19.6	14.16
Posterior tibial 3	6	5.0	22	22.7	15.39
Medial plantar	21	17.6	34	35.1	17.64
Lateral plantar	21	17.6	34	35.1	17.40
Paired T-test p=0.016					

3.4.1. Inter-observer reliability

Five clinicians, with experience of reading lower limb angiograms, independently scored twenty-five randomly selected angiograms from the dataset. The scorers included the author of this project, a vascular surgery consultant, a radiology senior trainee and two surgical research registrars with vascular backgrounds. Intra-class correlation (ICC) and Cohen's kappa coefficient were calculated to assess the level of agreement. ICC was also calculated for those patients that were scored by Scorer 1 for both the pilot study and the matched cohorts.

3.4.1.1. Inter-class correlation

Bland-Altman plots were calculated for each scorer pair. These were calculated for each arterial segment separately and all segments combined. There was no pattern that demonstrated any significant disagreement by arterial segment between scorers and so only the combined data is presented here. Examination of the Bland-Altman plots (Figure 3.4-1) showed that scorer 2 had significantly different results compared to all four other scorers (Table 3.4-4). Due to this ICC was calculated both with and without Scorer 2's results.

Table 3.4-4: Inter-observer reliability: Mean difference and 95% limits of agreement for combined arterial segments by scorer pairs.

Scorer pairs (n)	Mean Difference (SD)	p-value*	95% Limits of Agreement	
			Lower	Upper
1 vs 2 (427)	-1.76 (4.44)	**<0.001**	6.95	-10.47
1 vs 3 (421)	-0.40 (5.04)	0.105	9.49	-10.29
1 vs 4 (446)	-0.45 (4.55)	**0.037**	8.46	-9.37
1 vs 5 (442)	-0.74 (4.25)	**<0.001**	7.58	-9.07
2 vs 3 (405)	1.45 (4.10	**<0.001**	9.49	-6.58
2 vs 4 (424)	1.38 (3.85)	**<0.001**	8.93	-6.16
2 vs 5 (425)	1.04 (3.24)	**<0.001**	7.38	-5.31
3 vs 4 (420)	-0.13 (4.00)	0.519	7.72	-7.97
3 vs 5 (418)	-0.46 (3.74)	0.013	6.88	-7.80
4 vs 5 (438)	-0.32 (3.07)	**0.032**	5.70	-6.33

*One sample T-test (test value = 0)

A two-way random effects model with measures of absolute agreement was used. For all the scorers the ICC coefficient was 0.74(95% CI 0.71-0.78) and with scorer 2 removed 0.76(0.72-0.79). The result with all scorers suggests moderate agreement and with scorer 2 excluded there was good agreement. There was good internal consistency with Cronbach's alpha 0.94 for all scorers and 0.93 with scorer 2 excluded.

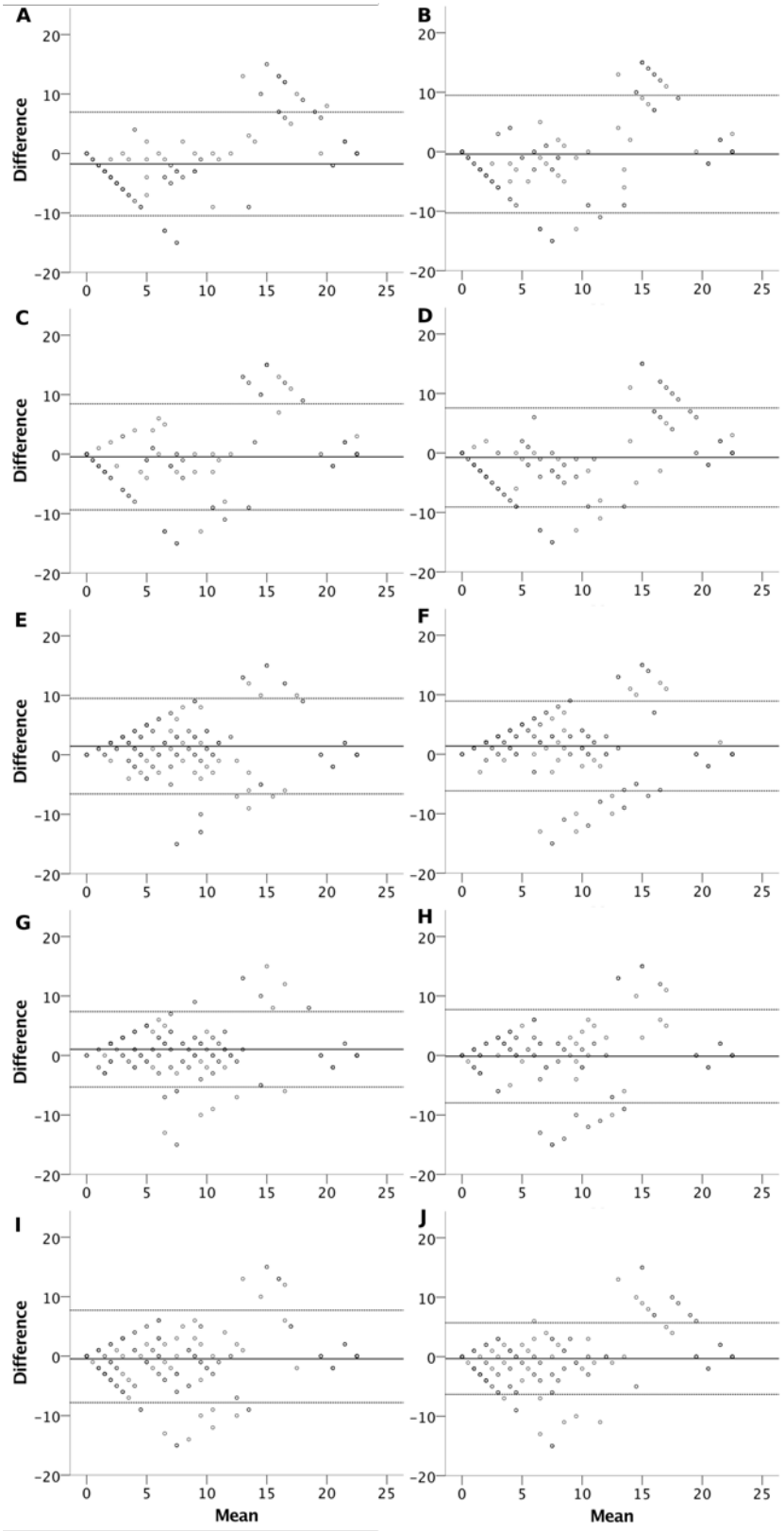

Figure 3.4-1: Bland-Altman plots for all arterial segments by scorer pairs.
A: Scorer 1 vs Scorer 2. B: Scorer 1 vs Scorer 3. C: Scorer 1 vs Scorer 4. D: Scorer 1 vs Scorer 5. E: Scorer 2 vs Scorer 3. F: Scorer 2 vs Scorer 4 G: Scorer 2 vs Scorer 5 H: Scorer 3 vs Scorer 4 I: Scorer 3 vs Scorer 5 J: Scorer 4 vs Scorer 5

3.4.1.2. Cohen's kappa coefficient

The Bollinger scores were categorised as 1=<3, 2=3-5, 3=6-8, 4=≥9. The kappa values ranged from 0.26 (Standard Error (SE) 0.026) to 0.54 (0.030) demonstrating a fair to moderate correlation. As before scorer 2 had the lowest levels of agreement (Table 3.4-5).

Table 3.4-5: Cohen's kappa coefficient by scorer pair.

Scorer pairs	Kappa Value (SE)	p-value
1 vs 2	0.26 (0.026)	**<0.001**
1 vs 3	0.39 (0.033)	**<0.001**
1 vs 4	0.54 (0.032)	**<0.001**
1 vs 5	0.48 (0.031)	**<0.001**
2 vs 3	0.36 (0.031)	**<0.001**
2 vs 4	0.35 (0.028)	**<0.001**
2 vs 5	0.39 (0.031)	**<0.001**
3 vs 4	0.53 (0.032)	**<0.001**
3 vs 5	0.47 (0.032)	**<0.001**
4 vs 5	0.54 (0.030)	**<0.001**

3.4.2. Intra-observer reliability

Sixty-six patients were scored by Scorer 1 as part of both the pilot study and matched cohorts, twenty patients without DM and 46 with DM. This represents 30.5% of the pilot cohort and 21.5% of the matched cohorts. Due to the difference in how the popliteal artery and tibial-peroneal trunk (TPT) were treated during the pilot and the matched analyses it was not possible to include them in this analysis.

3.4.2.1. Intra-class correlation

The Bland-Altman plot was similar to those obtained in the inter-observer analysis, the mean of the difference was 0.34 (SD3.77, p=0.007) and limits of agreement -7.05-7.74 (Figure 3.4-2). The ICC was 0.81 (95% CI 0.80-0.83) and Cronbach's alpha 0.90 reflecting good correlation and internal consistency.

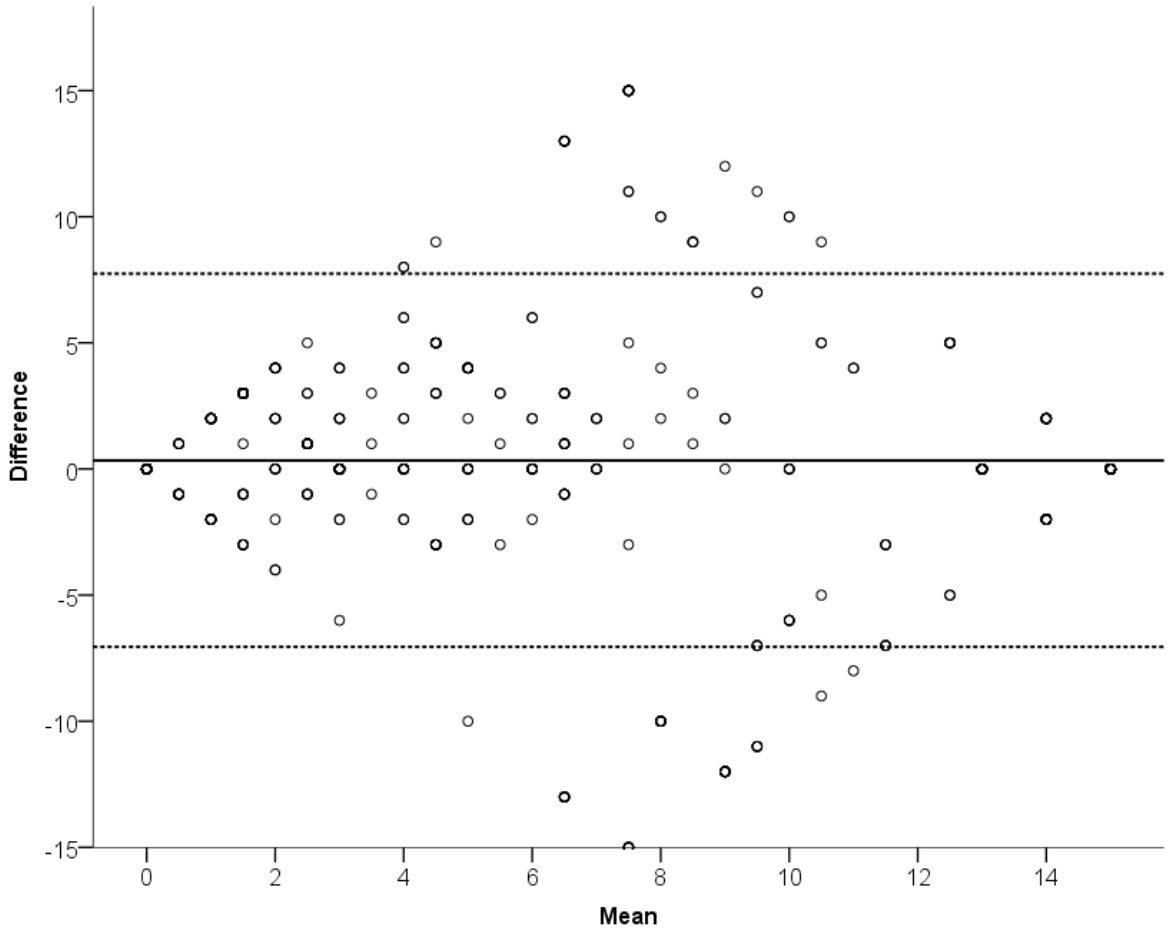

Figure 3.4-2: Bland-Altman plot for all arterial segments in intra-observer reliability.

3.5. CONCLUSIONS FROM PILOT STUDY AND PLAN FOR FULL STUDY

The results from the pilot study show that patients with DM had a higher severity of disease in the PTA; there was also a non-significant trend towards worse disease in the ATA. The PEA appeared to be relatively spared in the DM group. Within the demographics, there were potential sources of bias in the results with a significantly higher proportion of patients with hypertension and from Asian backgrounds in the DM group. During the scoring, it was noted that the popliteal segment as described by Bollinger (from the distal end of the adductor canal until bifurcation into PTA and PEA) regularly had a different pattern of disease in the TPT, i.e. distal to the ATA branch, compared to rest of the segment. There was a high proportion of missing data on individual arterial segments particularly in the aorto-iliac segments, and this proportion seemed to be higher in the DM group.

The inter-rater reliability results were mixed and in particular there was poorer reliability between the trainees (0.69 (0.65-0.74)). An attempt was made to recruit more consultant level raters to improve the validation but unfortunately it was not possible to achieve this. Of note, the best agreement was between the consultant surgeon and the scorer who went on to interpret the angiograms for the rest of the project (ICC 0.87 (0.84-0.89) and Cohen's kappa coefficient 0.54 (0.030)).

Due to these findings and following advice from a statistician, the following adjustments were made to the protocol for the full study.

- All patients with aorto-iliac imaging only were excluded.
- To improve matching between the two cohorts SPSS (IBM Version 22) was used to match for age (±5 years), sex, ethnicity, smoking, hypertension, hypercholesterolaemia and renal impairment. Matching was performed on a one-to-one basis, and only exact matches were initially included.
- Only infra-inguinal arterial segments were scored.

- The originally described popliteal segment was divided into the popliteal artery (PA) and TPT (Figure 3.6-1).
- The crural vessels were assessed both in thirds as per Figure 3.6-1 and as a complete vessel.
- If bilateral images were available, only the left leg was scored.
- Outcome data for all included patients were collected from each patient's electronic medical record.

3.6. SECONDARY OUTCOMES

Due to the above changes, new secondary outcomes were decided on:

- Difference in short to medium term outcomes between cohorts, particularly; amputation-free survival, further revascularisation (open or endovascular), minor amputation, major amputation, all-cause mortality.

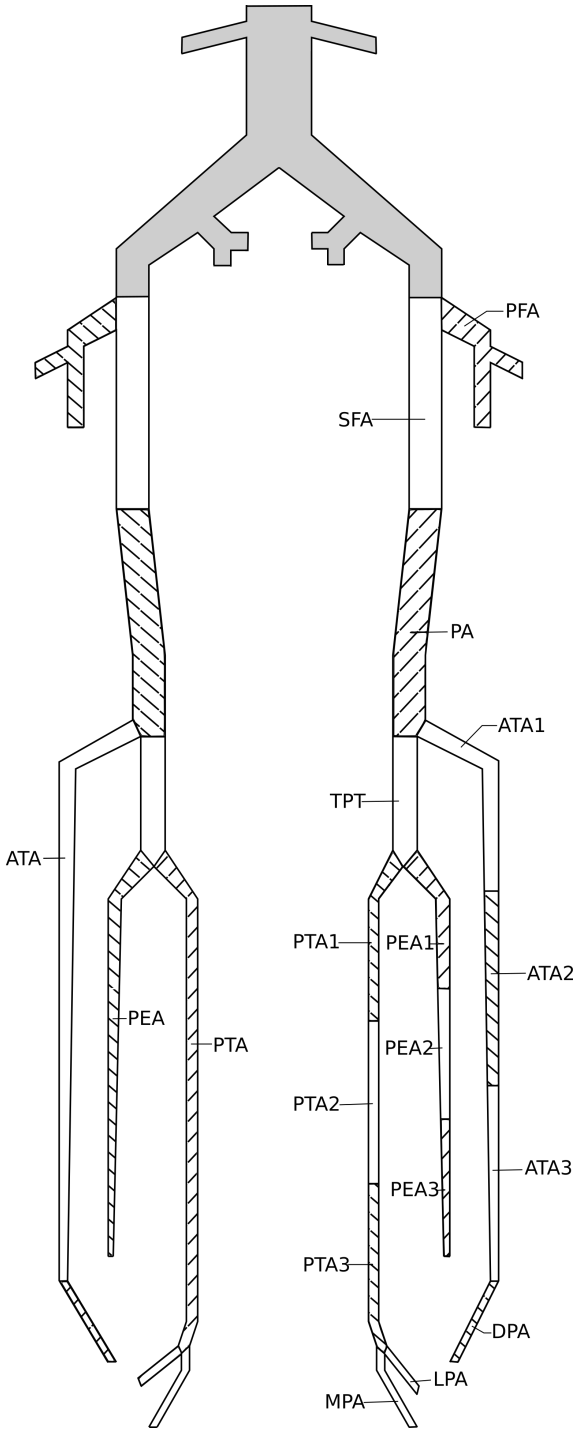

Figure 3.6-1: Schematic representation of arterial segments used in full study.
PFA; profunda femoris artery, SFA; superficial femoral artery, PA; popliteal artery, TPT; tibial-peroneal trunk, ATA; anterior tibial artery, ATA1; proximal 3rd of anterior tibial artery, ATA2 middle 3rd of anterior tibial artery, ATA3; distal 3rd of anterior tibial artery, DPA; dorsalis pedis artery, PEA; peroneal artery, PEA1; proximal 3rd of peroneal artery, PEA2; middle 3rd of peroneal artery, PEA3 distal 3rd of peroneal artery, PTA; posterior tibial artery, PTA1; proximal 3rd of posterior tibial artery, PTA2; middle 3rd of posterior tibial artery, PTA3; distal 3rd of posterior tibial artery, MPA; medial plantar artery, LPA; lateral plantar artery.

3.7. STATISTICAL ANALYSIS FOR THE FULL STUDY

All angiograms were assessed by one observer (author) who also carried out the statistical analysis. During assessment of the angiograms the observer was unaware which study group the patient belonged too (only identifying information on the datasheet, patient number and date of angiogram).

As our cohorts are matched, paired statistical analysis was used where possible. Normality of continuous data (age, length of follow-up) was tested using Shapiro-Wilk's test and Q-Q plots[187]. Parametric data were reported as mean (± SD) and compared using paired T-tests. Non-parametric data were reported as median (IQR) and compared using Wilcoxon signed rank test. Categorical data were compared using Fisher's exact test or Chi-squared test.

The Bollinger score runs from zero to 15. It is only possible to score integers, and it is not possible to score eleven or twelve, as such the scores are non-parametric data and were reported using median (IQR) and appropriate non-parametric tests. Each arterial segment was analysed separately.

3.8. RESULTS

3.8.1. Demographics

3.8.1.1. Raw data

Within the raw data, once duplicates and those with aorto-iliac imaging only were removed, there were 355 patients with DM and 631 without. In this raw data, the mean age was 69.4 (SD 12.8), 639 of patients were male (64.8%) and 347 female (35.2%). Seventy-two percent of the procedures were performed electively, and 90% of patients were of a white ethnic origin, 4.2% were of a black ethnic origin, and 4.5% were of an Asian ethnic origin.

3.8.1.2. Tests of Normality

Tests of normality were performed on the matched cohorts. For age at intervention the Shapiro-Wilk statistic was 0.993 ($p=0.141$) meaning the null hypothesis that the data is normally distributed was accepted. The corresponding statistic for length of follow-up was 0.973 ($P<0.001$) meaning the null hypothesis was rejected. The Q-Q plots demonstrated for age at intervention a very close relationship between the observed and expected values whereas this relationship was weaker for length of follow-up (Figure 3.8-1). For these reasons, age at intervention was treated as parametric data and length of follow-up as non-parametric.

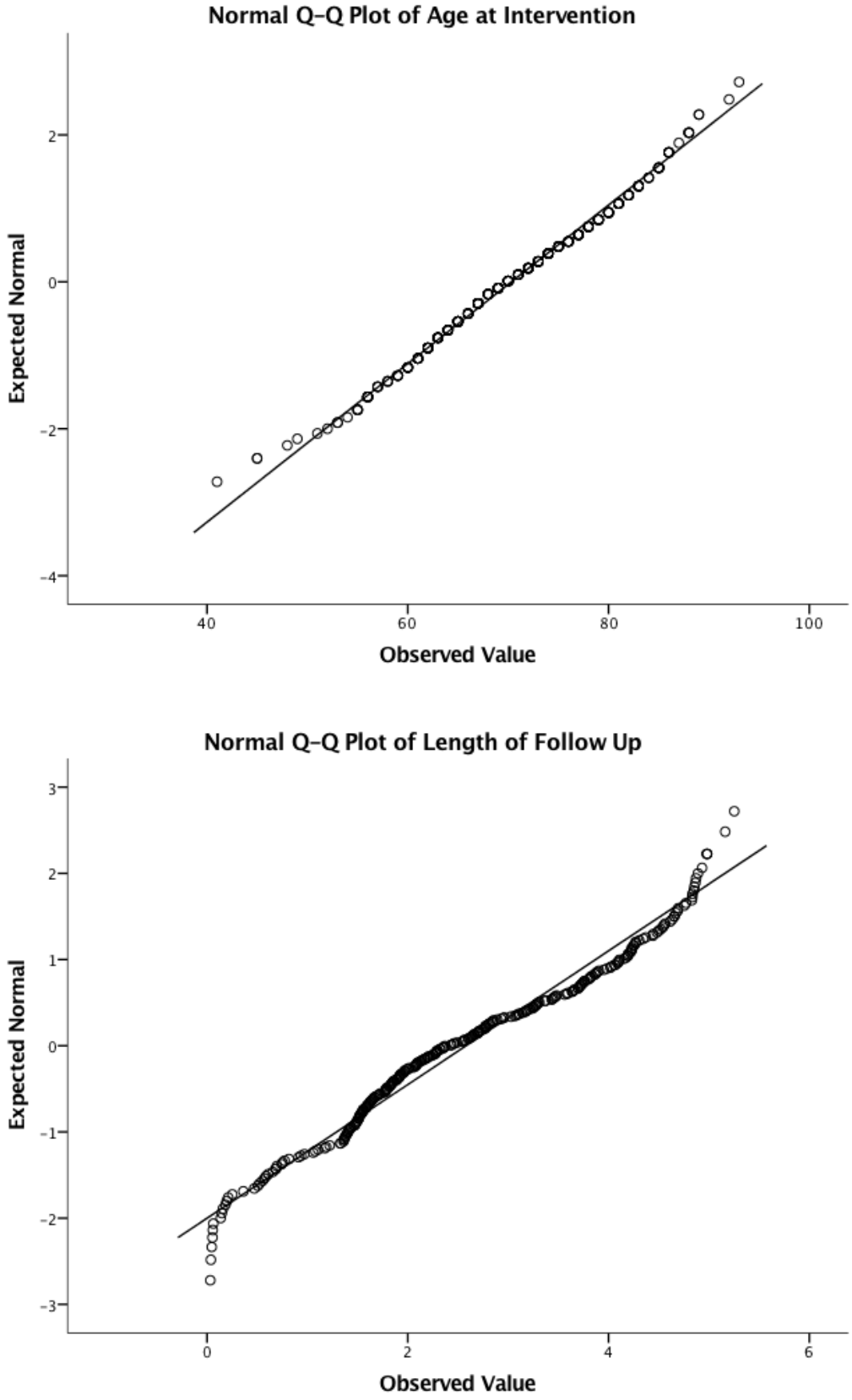

Figure 3.8-1: Q-Q plots for age at intervention and length of follow-up

3.8.1.3. Matched Cohorts

Three hundred and ten patients were identified as being matched by the criteria set out above (Section 3.5), 155 per cohort. During the process of collecting the data for the Bollinger score in the DM cohort, two duplicate entries were identified. This necessitated the removal of the matching pairs from the NDM group and reduced the cohort sizes to 153 patients. Eight other patients were manually replaced with the next nearest match due to the following reasons. Five patients had iliac images only saved, two patients underwent their angiogram to plan for plastic surgery rather than for peripheral arterial disease (PAD), and one patient's angiogram failed with no images stored.

The resulting cohorts were significantly matched for age, gender, ethnicity, smoking status, hypertension, hypercholesterolaemia and renal function (Table 3.8-1). They were also closely matched for length of follow-up, with the median being just under 2.5 years for both cohorts. There were, however, differences between the cohorts in indication and timing of the procedure. Those with DM were more likely to present with critical ischaemia (54.9% v 32%), and those without DM more like to present with claudication (60.1% v 37.9%, $p<0.001$). Those with DM were also more likely to require the procedure as an emergency (29.4% v 10.5%, $p<0.001$).

Table 3.8-1: Demographics for matched cohorts

		Cohort (%)		p-value
		No Diabetes (n=153)	**Diabetes (n=153)**	
Mean age (SD)*		70.3 (9.4)	70.3 (9.2)	0.937
Median LoF (IQR)**		2.3 (1.24-3.34)	2.4 (1.38-3.78)	0.292
Sex‡	**Male**	72.5	71.9	1.00
	Female	27.5	28.1	
Ethnic group‡	**White**	97.4	97.4	1.00
	Asian	1.3	1.3	
	Black	1.3	1.3	
Smoking‡	**Never smoked**	11.1	11.1	1.00
	Ex-smoker	67.3	68.0	
	Still smoking	21.6	20.9	
Hypertension‡	**Yes**	16.3	16.3	1.00
	No	83.7	83.7	
Hypercholesterolaemia‡	**Yes**	38.6	39.2	1.00
	No	61.4	60.8	
Renal function‡	**Normal**	96.7	96.7	1.00
	Renal Failure	3.3	3.3	
Indication‡	**Asymptomatic**	7.8	7.2	**<0.001**
	Claudication	60.1	37.9	
	Critical ischaemia	32.0	54.9	
Timing of procedure‡	**Elective**	89.5	70.6	**<0.001**
	Emergency	10.5	29.4	

*Paired T-test, **Wilcoxon signed rank test, ‡Chi-squared test, LoF: Length of Follow-up, SD=standard deviation

3.8.2. Bollinger score results

Due to the significant difference in indication for procedure by group the results are presented separately for each indication. The only segments in which there was difference where the lateral and medial plantar arteries in the patients with critical ischaemia (Tables 3.8-2 to 3.8-4).

Table 3.8-2: Results of Bollinger scores for asymptomatic patients

	Median Bollinger score (IQR)		p-value*
	No Diabetes (n=12)	Diabetes (n=11)	
Superficial femoral	15 (12-15)	15 (13-15)	0.880
Profunda femoris	2 (0-2)	0 (0-2)	0.606
Popliteal	10 (3-14)	13 (4-13)	0.674
TP Trunk	3 (0-4)	3 (0-7)	0.740
Anterior tibial	13 (2-15)	3 (0-15)	0.652
Peroneal	0 (0-3)	0 (0-13)	0.847
Posterior tibial	2 (0-15)	15 (3-15)	0.116
Dorsalis pedis	14 (2-15)	0 (0-13)	0.161
Lateral plantar	0 (0-15)	15 (1-15)	0.397
Medial plantar	0 (0-15)	15 (1-15)	0.281

*Mann-Whitney U Test

Table 3.8-3: Results of Bollinger scores for patients with claudication

	Median Bollinger score (IQR)		p-value*
	No Diabetes (n=91)	Diabetes (n=58)	
Superficial femoral	9 (6-13)	8 (6-13)	0.871
Profunda femoris	0 (0-0)	0 (0-0)	0.225
Popliteal	3 (0-13)	3 (0-5)	0.339
TP Trunk	0 (0-2)	0 (0-3)	0.763
Anterior tibial	0 (0-15)	13 (2-15)	0.121
Peroneal	2 (0-13)	2 (0-13)	0.745
Posterior tibial	0 (0-13)	0 (0-13)	0.516
Dorsalis pedis	4 (0-15)	15 (0-15)	0.125
Lateral plantar	15 (0-15)	13 (0-15)	0.092
Medial plantar	3 (0-15)	0 (0-15)	0.508

*Mann-Whitney U Test

Table 3.8-4: Results of Bollinger scores for patients with critical ischaemia

	Median Bollinger score (IQR)		p-value*
	No Diabetes (n=49)	Diabetes (n=84)	
Superficial femoral	7 (4-13)	7 (3-13)	0.653
Profunda femoris	0 (0-2)	0 (0-3)	0.505
Popliteal	3 (3-9)	5 (3-9)	0.526
TP Trunk	3 (0-8)	3 (0-7)	0.795
Anterior tibial	13 (1-15)	13 (3-15)	0.377
Peroneal	4 (0-13)	3 (1-15)	0.621
Posterior tibial	13 (4-15)	15 (2-15)	0.404
Dorsalis pedis	4 (0-15)	13 (0-15)	0.643
Lateral plantar	15 (15-15)	15 (0-15)	**0.015**
Medial plantar	15 (4-15)	13 (0-15)	**0.006**

*Mann-Whitney U Test

When the crural vessels were divided into thirds there was no significant difference between the groups with the exception of the distal segment of the PTA in the asymptomatic group (NDM, n=11, 0 (0-13) vs DM, n=11, 13 (3-15), p=0.023).

Overall those with DM had a higher burden of disease throughout the infra-inguinal arterial tree (median total Bollinger score 88 vs 42) this difference was not significant (p=0.061).

Within the matched cohorts, compared to the pilot study, there was less missing data by arterial segments (Table 3.8-5). The mean proportion of missing data in the DM cohort was 9.73% (SD 11.60%) compared to 10.85% (11.23%) this was not a significant difference (p=0.127).

Table 3.8-5: Proportion of missing data by arterial segment in matched cohorts

	No diabetes (n=153)		Diabetes (n=153)		Difference in % missing
	Missing	%	Missing	%	
Superficial femoral	2	1.31	0	0.00	1.31
Profunda femoris	12	7.84	17	11.11	3.27
Popliteal	2	1.31	2	1.31	0.00
TP Trunk	2	1.31	2	1.31	0.00
Anterior tibial	10	6.54	4	2.61	3.93
Dorsalis pedis	34	22.22	32	20.92	1.31
Peroneal	9	5.88	4	2.61	3.27
Posterior tibial	7	4.58	2	1.31	3.27
Medial plantar	44	28.76	43	28.10	0.65
Lateral plantar	44	28.76	43	28.10	0.65
Paired T-test p=0.127					

3.8.3. Outcomes

The median length of follow-up was 2.3 years (IQR 1.24-3.34) in the NDM group and 2.4 years (1.38-3.78, p=0.292) in the DM group (Table 3.8-1). The estimated 1-year risk of event was consistently lower in the DM group for minor and major amputation, mortality, amputation-free survival and further revascularisation (Table 3.8-6). Examination of the Kaplan-Meier curves shows that this difference is significant for amputation and mortality but not for revascularisation (Figure 3.8-2).

Table 3.8-6: Estimated 1-year risk of event for outcomes

	Estimated 1-Year Risk of Event (SE)		p-value*
	No Diabetes	Diabetes	
Minor Amputation	98.0 (1.1)	91.3 (2.3)	**0.011**
Major Amputation	98.6 (1.0)	91.9 (2.2)	**0.009**
Mortality	91.5 (2.3)	87.6 (2.7)	**0.002**
Amputation-Free Survival	90.2 (2.4)	81.0 (3.2)	**0.001**
Revascularisation	72.5 (3.7)	71.9 (3.7)	0.681
*Log-rank test, SE=Standard error			

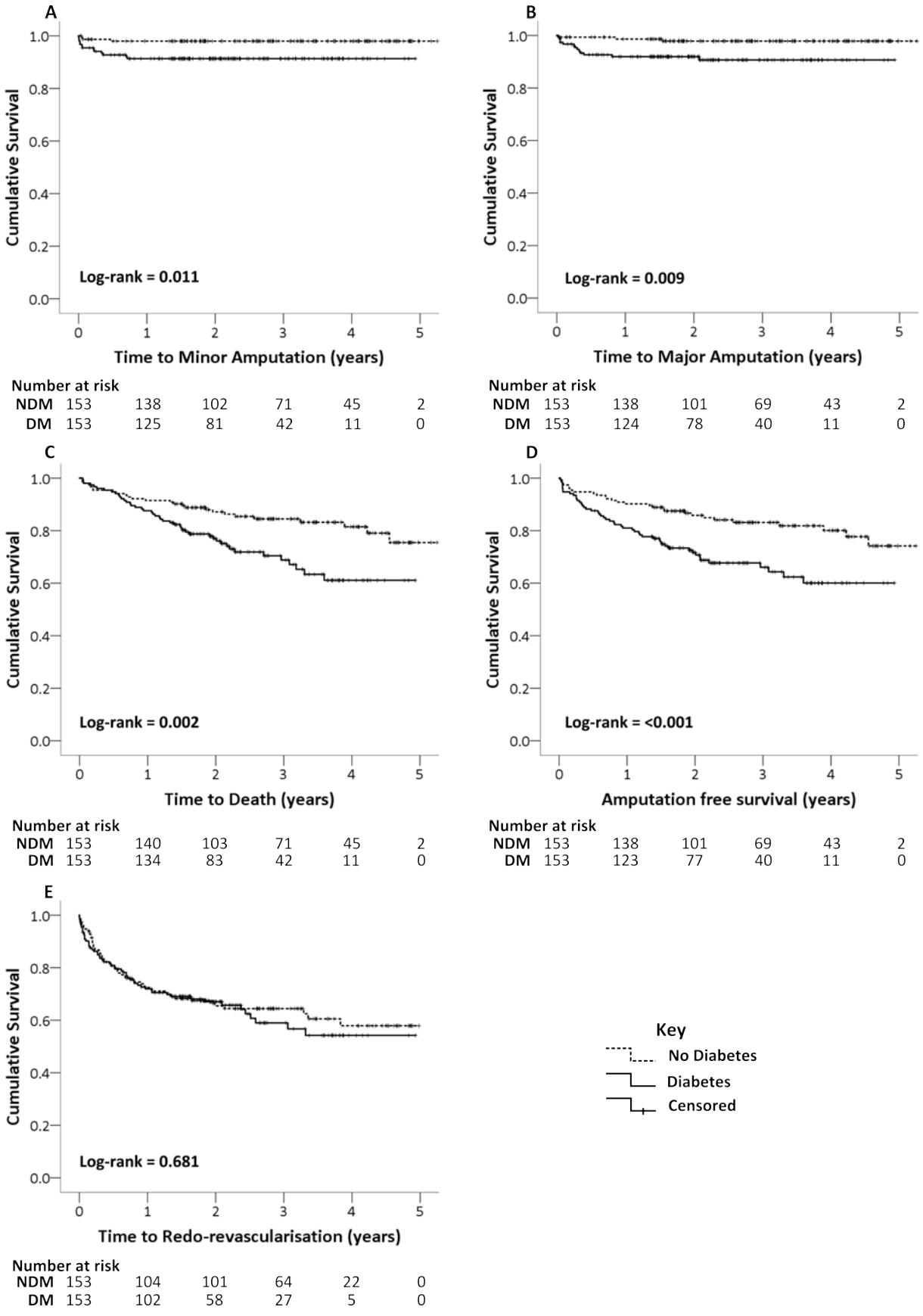

Figure 3.8-2: Kaplan-Meier curves for outcomes.

CHAPTER 4: ASSESSMENT OF THE MICROCIRCULATION IN DIABETIC FOOT DISEASE

4.1. INTRODUCTION

As already discussed in Chapter 1, patients with diabetes mellitus (DM) have a high risk of foot ulceration and subsequent amputation. DM is known to have a significant effect on the microvasculature, causing dysfunction of the arterioles and capillaries supplying the retina, kidneys and peripheral nerves[188]. Histological examination of capillaries has shown thickening of the basement membrane compared to non-diabetic patients[37,40,41]. Different methods to quantifiably examine the microcirculation and its function have been developed; these include capillary microscopy (CM), transcutaneous oxygen pressure ($TcPO_2$) and laser Doppler fluxmetry (LDF). For the purposes of this thesis the microcirculation is defined as arterioles, capillaries and venules and includes any vessel that measures less than 200-150µm[189].

The aim of this paper is to examine the common non-invasive tests that claim to assess the microcirculation of the foot. This includes a review of the current evidence within the literature on the relationship between the microcirculation in the ulcerated diabetic foot and wound healing. Specifically, the ability to predict healing, how the results for those with DM compare to those without and how the results vary when repeated measurements are taken.

4.2. CAPILLARY MICROSCOPY

CM, at its simplest, is the examination of capillaries in living skin through a microscope[190]. Capillaries in the skin were first observed using a microscope as early as 1879[191]. The technique was formalised and details published in the literature in the 1940s and 50s[190,192,193]. The basic requirements are a binocular microscope and a powerful light[192]. As the technique has developed it has become possible to record both static and dynamic images. Videocapillaroscopy allows dynamic assessment of the microcirculation and sequential magnification of areas of interest[194]. As part of the technique paraffin oil is applied to the skin to be examined to aid visualisation through the outer layers of the epidermis and minimise reflection[93,193]. Classically the nailfold of digits is examined as in this area the vessels run parallel to the skin making it possible to assess capillary morphology[195] and red blood cell velocity[189]. In other areas of the skin the capillaries are perpendicular to the skin and capillary density can be assessed but not morphology[189,196]. Parameters that can be quantitatively assessed include capillary density, capillary diameter and red blood cell velocity (at rest and as part of post occlusive reactive hyperaemia (PORH)) [95,197-199].

As light only penetrates a few micrometres into the epidermis the nutritional skin circulation only is visulised[198,200]. Of the tests used to assess the microcirculation this is the only test that only assesses the nutritional flow of the skin[95].

CM has been used, most commonly, in the investigation of dermatological disorders and rheumatoid conditions among other disease processes both systemic and localised[190,194,201-207]. In the context of DM and peripheral arterial disease (PAD) there is less evidence. Patients with DM have been found to have dilated capillaries and delayed hyperaemia following occlusion[95]. In ischaemic ulcers there is a paucity of capillaries in areas with no evidence of

granulation, areas of granulation had significantly higher capillary density and areas of normal skin surrounding the ulcer had a higher density again[208]. There is lower capillary density in ischaemic ulcers compared to venous[200] but on dependency those with severe ischaemia show an increase in skin perfusion which does not occur in patients with less severe disease[198]. There has also been shown to be lower capillary blood velocity in patients with PND compared to those without[209].

By staging the changes in the morphology of capillaries it has been shown that it is possible to discriminate between patients with critical limb ischaemia and intermittent claudication[93]. There is good sensitivity and specificity when discriminating between the stages of morphological changes[195,196]. There is also good inter and intra-observer reliability of for capillary density[93,195] and measurement of capillary width[195].

There are limitations to the use of CM, particularly, interpretation of images requires significant experience[192]. The morphological appearances of the capillaries vary between patients and areas of skin on the same patient in the normal population and so care must be taken that the same area is being assessed[95,194]. It can also be difficult to fully immobilise the area of interest as small movements will impact the area being examined and can interfere with velocity measurments[95]. Within the literature there is no consensus on definitions or standardised approach to the evaluation of images[194,195]. Papers that report consistent approaches tend to have originated from the same research groups. In addition CM can be challenging on pigmented skin[193].

Overall may be useful in assessing nutritional blood flow as opposed to total skin flow but not widely available[210] and cumbersome to use[95].

4.3. TRANSCUTANEOUS MEASURMENT OF OXYGEN PRESSURE

The technique for measuring the partial pressure of oxygen ($TcPO_2$) non-invasively at the surface of the skin was developed in the 1970s[211,212]. It was initially used in the critical care environment particularly for the monitoring of oxygenation in pre-term infants[211,212].

The probes consist of a polarographic oxygen sensor which is incorporated into a heating element[211,212]. The heating element contributes to the measurement in three ways. Firstly it causes vasodilatation of the surface capillaries and increasing local blood flow. Secondly it causes the shift of the haemoglobin disassociation curve to the right causing additional oxygen to be released into the plasma. Thirdly it enhances the diffusion of oxygen through the stratum corneum[211,212]. These all contribute to raising the partial pressure of oxygen at the skin from the normal resting value of almost zero to a level similar to that of the arterial blood[212]. The temperature that the skin is heated too is between 37-45^{o}C[213]. The resulting figure reflects approximate oxygenation of tissues[214]. The correlation between arterial oxygen pressure and $TcPO_2$ is strong in neonates[213]. In adults the diffusion gradient between the capillaries and the surface of the skin is greater due to thickness of the skin, increased adiposity and pathological factors like oedema[215]. This means the measured $TcPO_2$ may not reflect the arterial partial pressure of oxygen as closely as in neonates, but it has still been shown to be useful for a range applications[215,216].

Researchers started using $TcPO_2$ to investigate to the relationship of $TcPO_2$ and PAD in the early eighties and also the differences between patients with and without DM[213,217-219]. Areas of particular interest, where $TcPO_2$ has been shown to be useful, are in determining levels of amputation and healing potential in wounds[220,221].

Whilst $TcPO_2$ has been shown to be reduced in both patients with DM and patients with PAD and to improve following revascularisation[216,222] the relationship is not a simple one[223-225]. Ueno *et al* demonstrated that resting $TcPO_2$ was influenced by arterial stenosis or occlusions in an angiosomal distribution i.e. $TcPO_2$ of the dorsum of the foot was influenced by the status of the proximal vessels and the anterior tibial artery and the $TcPO_2$ of the calf was influenced by the proximal vessels and the posterior tibial arteries[226]. A recent study performed in Greece has supported that $TcPO_2$ is low in patients with DM and patients with diabetic peripheral neuropathy but these results were independent of the presence of PAD[224]. Other studies have also demonstrated that there is not a linear relationship between arterial supply and oxygenation of distal tissues in patients with DM[223-225]. As these are cross-sectional studies they were unable to comment on the pathophysiology behind the results.

In the lower limbs $TcPO_2$ has been shown to have variable reproducibility[93,227]. However good sensitivity and specificity for the detection of PAD[226], for the healing of diabetic foot ulcers[228] and the prediction of major amputation has been shown[229]. Current guidelines include $TcPO_2$ of <30mmHg as a predictor of poor outcome in patients with diabetic foot ulcers and critical limb ischamia[74,184].

Limitations of the technique include that it takes a relatively long time to perform compared the likes of toe blood pressure and the results are highly sensitive to surrounding conditions[214].

4.4. LASER DOPPLER FLUXMETRY

The laser Doppler technique for examining blood flow in the microcirculation was developed by Stern and Lappe in the early 1970s[97,230]. It was developed as a method to non-invasively monitor the microcirculation and as a replacement for techniques that used radioisotopes to assess blood flow[96].

The LDF probe delivers a laser via an optical fibre to the tissue under investigation. This light is scattered by the tissue encountered, and if that tissue is a moving red blood cell (RBCs) the light is Doppler shifted. The scattered light is then detected within the same probe and processed to form a laser Doppler signal. This signal provides information on the concentration of RBCs and their average speed this is termed flux and expressed as arbitrary perfusion units[97,99] (Figure 4.4-1).

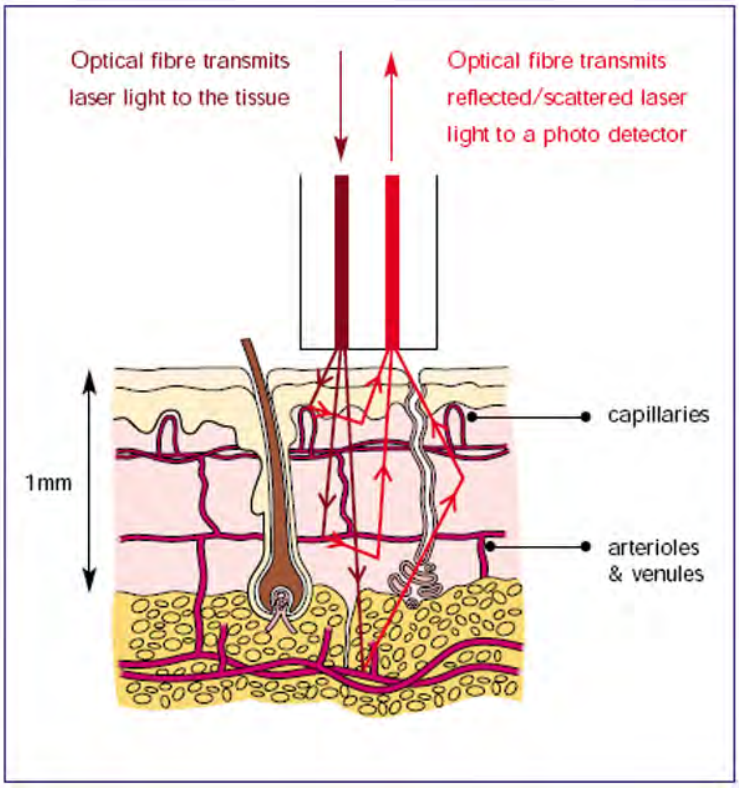

Figure 4.4-1: Schematic representation of Laser Doppler Fluxmetry. Courtesy of Moor Instruments.

The laser penetrates the skin to approximately 1.5mm[93] and as such provides information on both the superficial and deep dermal capillary beds with the possibility of also detecting flow in small arterioles and arterio-venous anastomoses. This means unlike CM it does not assess nutritional blood flow only and should be regarded as a total measure of blood flow in the skin[94,95].

Resting flux has been found to have low clinical value due to its low sensitivity and high variability[231,232]. Reactivity tests have been shown to be more reproducible; these include thermal challenge, iontophoresis, post occlusive reactive hyperaemia (PORH) and skin perfusion pressure (SPP). A thermal challenge is generally delivered using localised heating to the same area where the probe is placed (ideally using an integrated probe) and monitoring the response over a period of time[100-102]. There has been shown to be a decrease in response in people with DM compared to controls[41,50,102].

Iontophoresis involves the delivery of a low current through a chamber containing a vasoactive solution. The current causes ionisation of the substance towards the skin resulting in vasodilatation[102]. The probe is either placed within the chamber or in close proximity to it. The substances generally used are acetylcholine and sodium nitroprusside which assess endothelium-dependent and endothelium-independent vasodilatation respectively[101]. Decreased vasodilatation in response to both acetylcholine and sodium nitroprusside has been demonstrated in patients with diabetic neuropathic ischaemia[102,233], but there is heterogeneity in methods available leading too poor to fair reproducibility[101].

PORH is probably the most commonly used of the microvascular reactivity tests. The test involves occluding the blood supply to the limb being investigated using a blood pressure cuff. The occlusion is maintained for anything between 1 and 5 minutes[101] and then rapidly

deflated. Reperfusion occurs producing a characteristic trace involving hyperaemia followed by a gradual return to the resting flux. It is possible to measure various parameters on the curve which are based on magnitude and temporal relationships[234]. The parameters that have been found to have the best reproducibility and discrimination are time to maximum flux, time to half recovery, time to resting flux, maximum flux (difference between maximum amplitude and biological zero) and ratios of maximum flux/resting flux and maximum flux/time to maximum flux[231,234] (Figure 4.4-2).

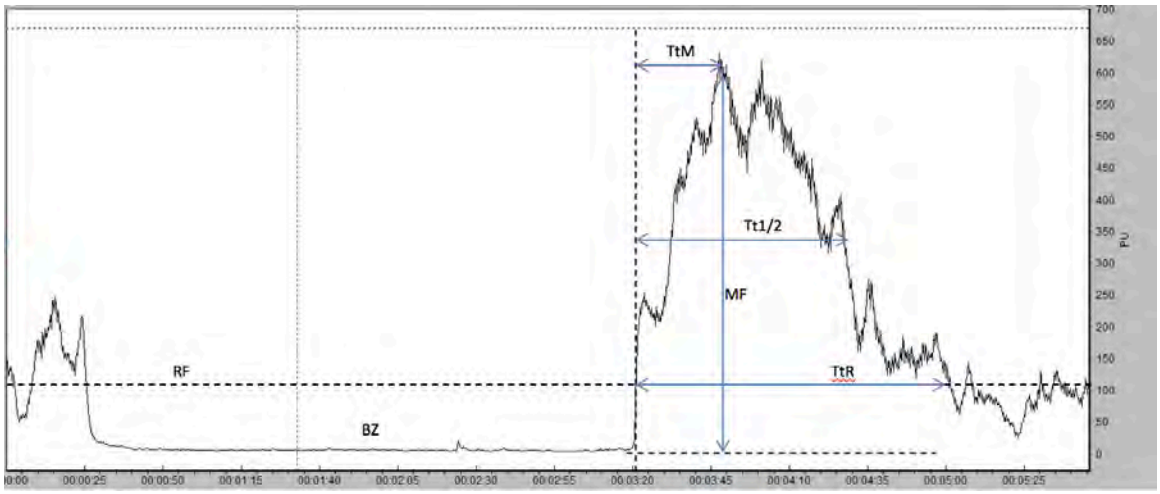

Figure 4.4-2: PORH trace of a healthy subject with parameters marked on.
RF resting flux, BZ biological zero, TtM time to maximum flux, MF maximum flux, Tt1/2 time to half recovery, TtR time to recovery.

As well as in healthy patients PORH has been found to be highly reproducible in patients with rest pain or disabling claudication[93]. Diabetic patients have demonstrated severely impaired PORH[47,49]. Within the literature, there is no evidence linking improvement in PORH to clinical improvement.

SPP is a measure of the pressure required to restore microcirculation to the skin. It is similar to toe blood pressure measurements in that it is not affected by calcification of the vessels but has the advantage over toe blood pressure of being able to be carried out when

the hallux has been amputated or is gangrenous. Also, measurement can be taken adjacent to ulcers to better reflect the disease status in that area[235,236]. Using LDF, SPP is measured using a modified low-profile probe that is placed underneath a blood pressure cuff. The cuff is inflated to a supra-systolic pressure and then slowly deflated. The SPP is taken to be the first of the two measurements that show an increase in flux[236]. SPP has a strong correlation to both ankle and toe pressure and $TcP0_2$[112,236] and has high sensitivity and specificity for wound healing in ischaemic ulcers and diagnosis of critical limb ischaemia[112,235].

LDF has been shown to be responsive to room temperature, posture, respiratory pattern and emotional stimulation of subject[97,100,210]. The spatial positioning of probes has also been shown to impact on the variability of results. This is thought to be due to differences in the density of capillaries[100].

4.5. THE DIFFERENCE BETWEEN THE HEALING AND THE NON-HEALING DIABETIC FOOT ULCER. A REVIEW OF THE ROLE OF THE MICROCIRCULATION

4.5.1. Methods

A search of the Medline, EMBASE and Web of Science databases was performed. The search strategy consisted of the Medical Subject Headings (MESH) “microcirculation”, “wound healing”, “diabetic foot”, “skin ulcer”, “laser Doppler flowmetry”, “blood gas monitoring, transcutaneous”, “microscopic angioscopy”, “xenon radioisotopes”. In addition, a keyword search was performed. The terms used can be found Appendix II. Non-English language and non-human studies were excluded, the date range for the search was 1946 to

February 2015. Final inclusion in the review was dependent on meeting the criteria set out in Table 4.5-1; no limits were applied to length of follow-up or the number of patients included. The original intention was to perform a meta-analysis; however, there were insufficient numbers of high-quality studies to be able to continue this plan, and so a more descriptive approach was taken to reporting the data.

Table 4.5-1: Review Inclusion criteria

Inclusion criteria

• English language article
• At least one method of assessing the microcirculation
• Patients with active tissue loss
• Wound healing as an outcome measure
• Results from patients with diabetes to be analysed separately from patients without diabetes in one of the following three formats.
  o Patients with diabetes compared to patients without diabetes
  o Patients with diabetes who healed compared to patients with diabetes who did not heal
  o Repeated measurements from the same patient during the period of active diabetic ulceration being investigated

4.6. RESULTS

Two-hundred and eighty-seven articles were identified after searching the databases. Full text was obtained for all abstracts that met the inclusion criteria and all relevant data was extracted. After this assessment and review of references, nineteen studies were included in the final review (Figure 4.6-1) [225,228,237-253]. The date of publication ranged from 1985 to 2014, two studies were randomised control trials, there were three pseudo-randomised control trials, and the rest were observational studies (Table 4.6-1). Not all studies included all the comparisons considered below and some studies used more than one method to assess the microcirculation.

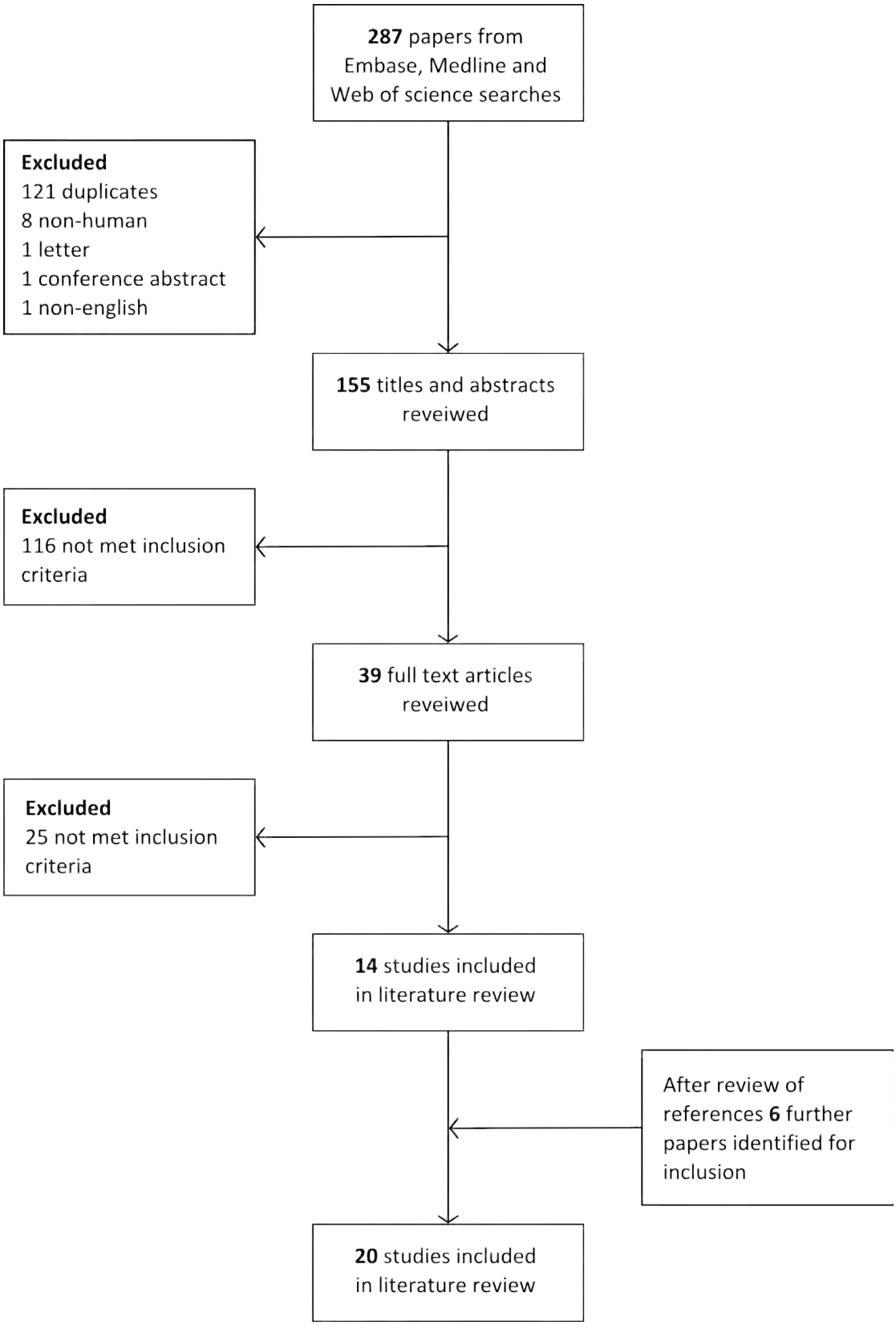

Figure 4.6-1: Flow diagram illustrating study identification process for microcirculation review

Table 4.6-1: Characteristics of included studies

Author	Year	Country	Type of study	Microcirculation method	Number of subjects	
					DM	Non-DM
Faris, I.[237]	1985	Australia	Cross-sectional	SPP using isotope washout	64	-
Karanfilian, R.[238]	1986	USA	Cohort	1)LDF 2)$TcPO_2$	34	22
Pecoraro, R.E.[225]	1991	USA	Cross-sectional	1) $TcPO_2$ 2) $TcPCO_2$	46	-
Jorneskog, G.[239]	1993	Sweden	Cross-sectional	1)LDF-PORH 2)Capillary microscopy	10	-
Padberg, F.T[240]	1996	USA	Case-control	$TcPO_2$	129	97
Kalani, M.[228]	1999	Sweden	Cross-sectional	1) $TcPO_2$ 2)TBP using LDF	50	-
Koblik, T.[241]	2001	Poland	Double-blind RCT	LDF, PORH and resting flow	18	-
Zimny, S.[242]	2002	Germany	Cross-sectional	$TcPO_2$	31	-
Fife, C.E.[243]	2002	USA	Cross-sectional (retrospective)	$TcPO_2$	1144	-
Newton, D.J.[244]	2002	UK	Cross-sectional	LDI	5	
Kalani, M.[245]	2002	Sweden	Pseudo-RCT	1) $TcPO_2$ + $TcPCO_2$ during O2 inhalation 2)TBP using LDF	38	-
Klingel, R.[246]	2003	Germany	Cross-sectional	1) $TcPO_2$ 2)LDF 3)Capillary microscopy	8	-
Petrofsky, J.S.[247]	2007	USA	Pseudo-RCT	LDI	29	-
Lawson, D.[248]	2007	USA	Pseudo-RCT	LDI	10	10
Ichioka, S.[249]	2009	Japan	Case-control	$TcPO_2$	31	22
Petrofsky, J.S.[250]	2010	USA	RCT	LDI	20	-
Yang, C.[251]	2013	China	Cross-sectional	$TcPO_2$	61	-
Wang, A.[252]	2014	China	Cross-sectional	$TcPO_2$	194	-
Yotsu, R.R.[253]	2014	Japan	Cohort	1)SPP using LDF 2) $TcPO_2$	73	-

Abbreviations: RCT – randomised control study, SBF – skin blood flow, SPP – skin perfusion pressure, SVR – skin vascular resistance, LDF – laser Doppler fluxmetry, $TcPO_2$ – transcutaneous oxygen pressure, $TcPCO_2$ – transcutaneous carbon dioxide pressure, PORH – post occlusive reactive hyperaemia, TBP – toe blood pressure, LDI – laser Doppler imaging.

4.6.1. Using the microcirculation to predict healing

Twelve studies out of nineteen compared the microcirculation in patients with diabetes who healed to those who did not heal[225,228,237,238,243-246,249,251-253]. Ten of these studies employed $TcPO_2$[225,228,238,243,245,246,249,251-253], five used LDF[228,238,245,246,253], one used laser Doppler imaging (LDI)[244] and one used isotope washout to measure SPP[237]. These were all observational studies apart from one that randomised the first 14 of its participants but not the final 24[245]. For seven of the studies, the participants received only standard therapy[225,228,237,238,251,252]. Two studies examined the effects of HBO therapy, Kalani *et al.* (2002) had two cohorts, one of which received standard therapy and the other which received HBO. The healed and unhealed groups in this study are made up of participants from either cohort[245]. Fife *et al.* performed a retrospective study of 1144 patients who received HBO therapy[243]. Klingel *et al.* reported the results of a very small pilot study (8 patients) all of whom received rheopheresis[246]. Two studies treated their participants with dermal replacement therapy; Ichioka *et al.* bone marrow impregnated collagen, Newton *et al.,* collagen containing glycosaminoglycans)[244,249]. Five studies only investigated patients with both diabetes and ischaemia[228,238,243,245,246], three studies excluded those with ischaemia[225,244,249], in one study it was unclear[251], and three included a mix of patients[237,252,253]. Only Yotsu *et al.* divided the patients into groups depending on their aetiology (neuropathic, ischemic and neuro-ischemic)[253].

4.6.1.1. Transcutaneous oxygen pressure

Nine studies used $TcPO_2$ to predict wound healing[225,228,238,245,246,249,251-253], the results are summarised in Table 4.6-2. Five studies found that those with a higher $TcPO_2$ had a

statistically significant higher chance of healing, with results ranging from 30±4mmHg to 61.11±21.16mmHg [228,238,246,251,252]. Kalani *et al.* 2002 and Yotsu failed to find a significant difference between the two groups[245,253]. Pecoraro *et al.* found a significant difference between those who had early healing and those who did not (56.3±2.72mmHg vs 26.9±8.26mmHg, p=0.003) however was unable to demonstrate that the difference had persisted in those that healed overall (53.67±2.99mmHg vs 37.57±11.02mmHg, p=0.126)[225].

4.6.1.2. Skin perfusion pressure

Two papers used SPP to compare the healed and unhealed groups[237,253]. Faris *et al.* in 1985 used an isotope washout method on 64 patients with diabetes and foot ulceration or gangrene. Those who healed had a mean SPP of 59 ± 16mmHg compared to those who did not heal whose mean SPP was 35 ± 11 ($p<0.001$)[237]. Yotsu *et al.* in 2014 employed LDF instead of isotope washout to measure SPP on diabetic ulcers divided into the groups described above. They found that neuropathic ulcers had a higher SPP than both ischemic and neuro-ischemic ulcers, 65 ± 13.6mmHg, 27 ± 14.1mmHg and 34 ± 23.2mmHg respectively ($p<0.001$). However, there was no significant difference between the healed and unhealed ulcers in each group (Table 4.6-3)[253].

Table 4.6-2: $TcPO_2$ results for patients who healed compared to patients who did not heal.

Author	Patient type	Measurement/ groups	$TcPO_2$ (mmHg)		p-value
			Healed (n)	Unhealed (n)	
Klingel[246]	T2DM non-healing ischaemic ulcers	Mean change in $TcPO_2$ week 0-12. Improved and deteriorated groups	13.23±9.57 (4)	-2.3 ± 6.65 (2)	<0.05 *
Kalani (2002)[245]	DM non-healing ischaemic ulcers. No reconstruction options	Basal $TcPO_2$, dorsum of foot. All patients	26 ± 10 (23)	24 ± 10 (9)	ns
Karanfilian[238]	DM ischaemic ulcers	Dorsum of foot. All diabetic patients	30 ± 4 (16)	7 ± 2.5 (18)	<0.05
Yang[251]	DM with ulcer	Dorsum of foot. Group 1 (ulcers healed with intact skin),	32 ± 10 (36)	15 ± 12 (17)	<0.001
		Group 3 (Ulcers that did not heal or deteriorated including requiring amputation)			
Ichioka[249]	DM with ulcer	Peri-wound $TcPO_2$. Diabetic subgroup (combination of treatment and conventional therapy group)	34.5 ±19.2 (32)	26.4 ±16.7 (10)	Not stated
Yotsu†[253]	T2DM ulcer >14 days	Multiple measures from 2 areas on foot, lowest value recorded. Contralateral foot used if extensive ulceration. Ischaemic group	38, 12-40 (9)	30, 3-45 (11)	ns
		Neuro-ischaemic group	38, 22-51 (9)	17, 16-32 (5)	ns
		Neuropathic group	48, 40-56 (34)	44, 43-50 (5)	ns

Kalani (1999)[228]	DM ulcer >2months, 32 ischaemia with no reconstruction options	Dorsum of foot. Healed with intact skin compared to impaired ulcer healing	50 ± 20 (20)	13 ± 14 (13)	<0.001
Pecoraro[225]	DM with ulcer	Peri-wound $TcPO_2$ overall healing	53.67±2.99 (39)	37.6±11.0 (7)	ns
		Peri-wound $TcPO_2$ early healing	56.3 ± 2.72 (38)	26.9 ± 8.26 (8)	0.003
Wang[252]	DM with ulcer requiring hospitalisation	Site of measurement not stated. Healing and non-healing groups	61.1±21.2 (162)	46.5 ± 18.1 (20)	<0.01

*Wilcoxon test for matched pairs for significance of change between weeks 0-12 for each group separately. All values mean ± SD apart from †median and inter-quartile range (IQR) reported

Table 4.6-3: Skin perfusion pressure results for patients who healed compared to patients who did not heal[253].

Group	SPP (mmHg)		p-value
	Healed (n)	Unhealed (n)	
Neuropathic	67, 57-75 (34)	65, 40-69 (5)	0.192
Ischemic	37, 17-43 (9)	20, 15-37 (11)	0.341
Neuro-ischemic	38, 22-51 (9)	17, 16-32 (5)	0.141
All values median, IQR			

4.6.1.3. Laser Doppler

Karanfilian *et al.* was the only paper to use laser Doppler fluxmetry to compare between healed and unhealed patients. They demonstrated significantly higher skin blood flow velocity (LD-SBFV), and pulse wave amplitude (LD-PWA) results between those who healed and those who did not in both their study groups (Table 4.6-4)[238].

Table 4.6-4: $TcPO_2$ and LDF results in patients with diabetes compared to patients without diabetes[238].

	Diabetes (Mean ± SE)		No diabetes (Mean ± SE)	
	Healed (16)	Unhealed (18)	Healed (15)	Unhealed (7)
$TcPO_2$ (mmHg)	30 ± 4.0	7 ± 2.5*	42 ± 3.5	2 ± 1.6*
LD-SBFV (mV)	98 ± 13.0	50 ± 8.0*	88 ± 15.0	37 ± 2.0*
LD-PWA (mV)	14 ± 3.0	4 ± 0.5*	8 ± 1.4	2 ± 0.3*
* Significant difference between healed and unhealed groups ($p<0.05$), SE=standard error				

4.6.1.4. Prediction of healing

Three studies reported the accuracy of cut-off values for healing[228,237,243]. Faris and Duncan found an SPP of less than 40mmHg was an indicator of poor healing (sensitivity of 97%, specificity 80%, positive predictive value (PPV) 87% and negative predictive value (NPV) 95%)[237]. Kalani *et al.* (1999) used a cut-off of 25mmHg for $TcPO_2$ and 30mmHg for toe blood pressure (TBP) using LDF. For $TcPO_2$ the sensitivity was 85%, specificity 92%, PPV 79% and NPV 94%. For TBP the sensitivity was 15%, specificity 97%, PPV 67% and NPV 77%[228]. Fife *et al.* tested multiple potential cut-offs for sea level $TcPO_2$ as a predictor of failure of hyperbaric therapy. They found that 25mmHg was the best cut-off with a 2.5 times greater chance of success. However, the accuracy was still relatively poor with sensitivity of 67%, specificity 50%, PPV 35% and NPV 79%[243].

4.6.2. Diabetes compared to no diabetes

Two out of nineteen studies compared subjects both with DM and without DM[238,240]. Both of these papers used $TcPO_2$ to make their comparisons, in addition, Karanfilian *et al.* employed LDF[238]

Padberg *et al.* reported the predictive accuracy, using a probability approach, for healing of $TcPO_2$ in critically ischaemic wounds. 204 wounds were stratified depending on the presence of DM, dialysis-dependent chronic renal failure or neither disease. Probability of healing curves for each group were plotted and compared using multiple logistic regression. When the estimated probability of healing was 50% $TcPO_2$ in DM patients had a predictive accuracy, sensitivity and specificity of 81%, for chronic renal failure (all but two patients also

had DM) these figures were 77%, 73% and 82% respectively, and for neither disease, 84%, 86% and 82%[240].

Only one study identified compared the mean results of microcirculatory tests for patients with diabetes and those without[238]. The patients were all men with ulceration to the foot (34 with diabetes, 22 without). One-off measurements of $TcPO_2$ and LDF (LD-SBFV and LD-PWA) and follow-up of at least 30 days was performed. The results are presented in Table 4.6-4. Patients without diabetes who did not heal had a lower $TcPO_2$, LD-SBFV and LD-PWA than patients with diabetes who did not heal. In the healed groups for the patients without diabetes, the $TcPO_2$ was higher than the patients with diabetes. However, the LD-SBFV and LD-PWA were lower in the group without diabetes. The authors have not reported whether these differences are statistically significant[238].

4.6.3. Multiple measurements during the observation period

Eight out of nineteen studies reported the results of more than one measurement on the same group of patients[239,241,244,246-250]. One study detected no change, and two noted a decrease in reading, a further two noted an increase and three noted a pattern of increasing then decreasing. Jorneskog *et al.* used LDF and CM to examine ten patients with diabetes who received low molecular weight heparin for eight weeks. Measurements of the microcirculation (PORH, the structural appearance of capillaries in the forefoot and toes) were undertaken 1-2 weeks before receiving heparin, after 4-7 weeks of treatment and two weeks after treatment was stopped. They found that there was no significant change in any of the laser Doppler parameters during or after treatment. It was however noted that six patients who had improved healing also had an improvement in their capillary stage, three

others also improved clinically, but one had no change in their capillaries, one initially improved but then deteriorated again and in one it was not possible to determine their capillary stage. One patient deteriorated both clinically and on microscopic examination[239].

Petrofsky *et al.* published on electronic stimulation (ES) for diabetic foot ulcers in both 2007 and 2010[247,250]. In 2007 the study groups were ten patients who received global heating and ES, nine who received local heating and ES and ten patients who received conventional therapy only. The measure of the microcirculation was blood flow using LDI (measured in arbitrary unit flux). The control group did not undergo LDI measurement, only wound area was measured. In 2010 the aim of the study was to examine the role that heating had compared to ES and heating. Ten patients received local heating only and a further ten local heating and ES. The treatment period for both studies was four weeks. In both studies, the blood flow around and in the ulcer had decreased by the end of the study. In the 2007 study, the mean blood flow at baseline was reported for 1cm from the ulcer (182.3±26.1 increasing to 245.0±28.5 with ES) and the edge of the ulcer (223.4±34.1 increasing to 301.0±29.3 with ES). The result for the centre of the ulcer is reported as being similar and is illustrated in a graph, but the actual values are not stated. At four weeks only the values for the centre of the ulcer are stated (228±36.2 increasing to 256.7±46.3 with ES). The change in blood flow before and during ES at baseline and four weeks is displayed in Table 4.6-5; there was a significant reduction in the increase at four weeks (<0.01). The results for the local heating group are illustrated in a graph and stated as being similar but of a smaller magnitude to the global group, but the actual mean values are not quoted[247]. In 2010 Petrofsky found that the mean resting blood flow from all three areas and both groups had reduced by 54.5±22.3% after four weeks[250].

Table 4.6-5: Change in blood flow associated with electrical stimulation at baseline and four weeks (global heating group only)[247].

Position of measurement	Flux±SD		p-value
	Baseline (10)	Week four (10)	
1cm from ulcer	63.5±11.9	18.3±10.8	
Edge of ulcer	77.6±11.6	48.7±9.6	<0.01
Centre of ulcer	33.6±3.1	28.4±15.8	

Lawson *et al.* as described above also investigated the effect of electrical stimulation on wound healing. They measured blood flow at the centre and outside of the ulcer using LDI at baseline, two weeks and four weeks. When looking at the outside of the ulcer, the pre-stimulation results for the DM group showed a larger increase in the blood flow than for the no diabetes mellitus group (DM, 0-2 weeks 35%, 0-4 weeks 21%; Non-DM, 0-2 weeks 0%, 0-4 weeks 18%). However, at the centre of the wound the patients without DM (NDM) had a greater increase (DM, 0-2 weeks 8%, 0-4 weeks 5%; NDM, 0-2 weeks 22%, 0-4 weeks 38%). The statistical significance of these results is not reported[248].

Koblik *et al.* performed an RCT comparing optimisation of insulin therapy and injection of an antithrombotic drug (sulodexide) with optimisation of insulin therapy and placebo injections for ten weeks. Measurements were taken at baseline and eight weeks using LDF. The parameters measured were resting flux (RF), peak hyperaemic flow (pLDF), time to peak hyperaemic flow and hyperaemia duration after an occlusion of thirty seconds. These measures were repeated following a sixty-second occlusion once the readings had stabilised. In the placebo group (6 patients) there was no significant change in the RF at eight weeks (Baseline: mean flux 11.6± standard error of mean 1.3. Eight weeks 12.3±1.1. p = ns). The pLDF for both the thirty (51.7±15.2 to 147.0±16.2, p<0.01) and sixty second occlusion (110.5±13.0 to 164.8 ±15.4, p<0.01) significantly increased at eight weeks[241].

The results from two studies with small numbers are presented in graphical form in Figure 4.6-2. Newton *et al.'s* seven ulcers all healed or showed improvement at eight weeks. Four measurements using LDI were performed at baseline, two, five and eight weeks. Four patients had an increase in blood flow over the first few weeks followed by a decrease to below baseline at eight weeks. One increased throughout the measurement period. One decreased at two weeks, increased at weeks five and eight but did not return to baseline level. One decreased throughout (Figure 4.6-2a). Those that had healed at eight weeks, two increased then decreased, one increased throughout and the other decreased throughout[244]. Of Klingel's eight patients who received rheopheresis five underwent $TcPO_2$ at baseline, twelve and twenty-four weeks and three underwent $TcPO_2$ at baseline and twelve weeks (due to minor amputation in one patient and major amputation in two). Of the four patients who showed an improvement in their ulcer two had an increase in blood flow followed by a decrease, the other two increased throughout. In the patients whose ulcers were unchanged one increased $TcPO_2$ at twelve weeks, the other increased at both twelve and twenty-four weeks. Of the two patients who deteriorated one had a small increase at twelve weeks, and the other had a small decrease (Figure 4.6-2b)[246].

Ichioka *et al.* in their DM subgroup showed, in graphical form, a trend of increasing $TcPO_2$ in the healed group and a decrease at four days in the unhealed group. The mean $TcPO_2$ at four and 14 days are not reported; however, logistic regression analysis showed the results at these time point contributed significantly to the prediction of outcome ($p<0.001$ and 0.002 respectively)[249].

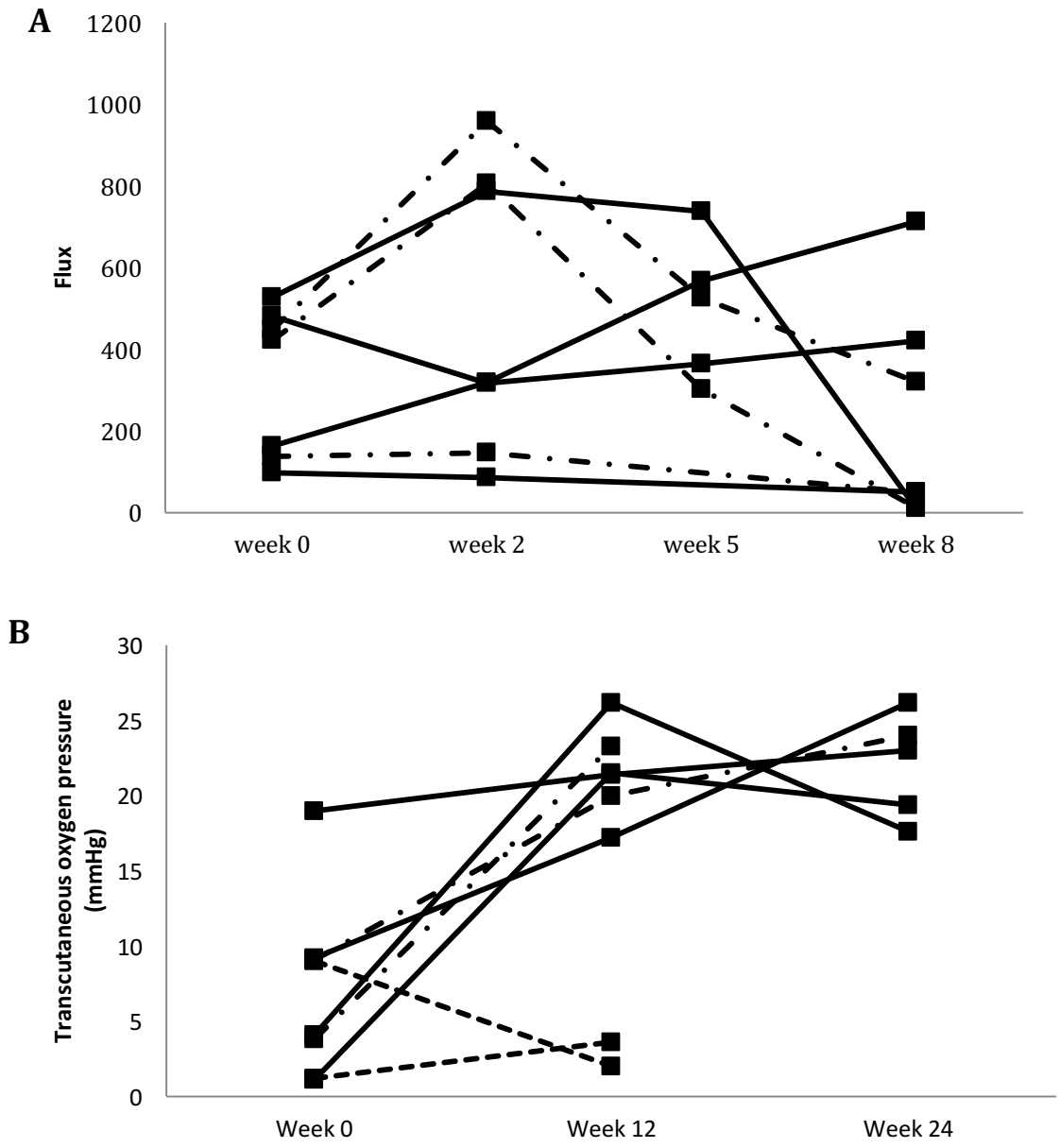

Figure 4.6-2: Trends during healing for LDI and $TcPO_2$.
A: Adapted from Newton et al.[244] Solid line, ulcers healed Alternating dashed line ulcers improved. B: Adapted from Klingel et al.[246] Small dashed line ulcers deteriorated.

4.7. DISCUSSION

Within this group of studies, the most commonly used method to assess the microcirculation was $TcPO_2$ (n=12), followed by LDF (n=7), LDI (n=4), CM (n=2) and isotope washout (n=1). These proportions are probably representative of the current state of clinical usage of these methods with $TcPO_2$ and LDF being the most common.

Within this group of studies, a variety of methods for examining the microcirculation have been used. Some of these methods have now fallen out of favour as technology has developed less invasive methods. This includes Xe clearance and SPP using isotope washout. LDF, $TcPO_2$ and CM remain in regular use. LDF is relatively underrepresented in this cohort, which is surprising considering that its utility in evaluating patients with critical limb ischaemia is well-documented[93,101,254]. One reason for this may be the relative age of many of the studies included (only three since 2000 and going back as far as 1978). $TcPO_2$ was the most commonly used method in this review, which fits with its presence in the literature on critical limb ischaemia and diabetic foot disease as a whole.

There is disagreement on how to carry out each of the methods of assessing the microcirculation, including positioning of the probes and in the case of $TcPO_2$ the skin temperature that recordings were made at. Probes were most commonly positioned on the dorsum of the foot[225,228,238,242,245,251,252], but they are also positioned peri-wound[225,243,246,249], and in one case it was not stated[240]. A possible explanation for Yotsu *et al.* not detecting a significant difference is their method of measurement[253]. Multiple measurements were taken in two areas of the foot and the lowest result recorded. Of particular note, the contra-lateral foot was used if there was extensive ulceration, this may well have skewed their

results. $TcPO_2$ was most commonly measured at 44°C[225,228,243,245,246,249] but also at 45°C [238,240] or not stated[242,251-253]

Due to the variety of countries and inclusion/exclusion criteria, the cohorts differed across the studies. For example, Yang[251] and Lawson[248] excluded patients with evidence of osteomyelitis whereas most of the other studies did not.

Due to the larger number of studies, $TcPO_2$ best demonstrates that if the microcirculation is functioning poorly then outcomes are likely to be worse. Most studies demonstrated a significantly higher $TcPO_2$ in those patients who healed. What is less clear is the threshold at which healing occurs. The $TcPO_2$ thresholds quoted for a successful outcome in this review range from 10mmHg to 34mmHg. Karanfilian quotes sensitivity of 100% and specificity of 88% for healing if the $TcPO_2$ is >10mmHg[238]. Pecoraro found that a $TcPO_2$ of <20mmHg was associated with a 39 fold increased risk of early healing failure[225]. Both Kalani and Yang used the threshold of <25mmHg and quoted sensitivities and specificities of 85% Vs 92% and 88.6% Vs 82.4% respectively[228,251]. This threshold, when looking at the collated results in the healed and unhealed groups in Table 4.6-2, appears to hold true when considering the healed groups, all the mean results are above 25mmHg. However, it is worth observing that the mean $TcPO_2$ is also higher than 25mmHg in six of the unhealed groups[225,249,252,253]. The current consensus among experts is that patients with an SPP ≥40mmHg, TBP ≥45mmHg or $TcPO_2$ ≥25mmHg are more likely to heal than their counterparts with poorer perfusion and that a TBP <30mmHg or $TcPO_2$ <25mmHg is an indication for urgent vascular imaging[15,255]. This is based on a recent review examining the utility of prognostic markers in diabetic foot disease in which the authors faced similar difficulties to us in identifying studies of sufficient quality to draw conclusions from[255]. Eventually, eleven studies involving 5890 patients were

included however there was still significant heterogeneity and difference in the measures used. Their conclusions were based predominantly on three papers of acceptable rather than high-quality (Quality in Prognosis Studies Tool)[228,237,256].

Only one study in this current review truly compared the results of testing the microcirculation in patients with DM and those without[238]. Karanfilian found that the DM patients who healed had a lower $TcPO_2$ than NDM patients who healed. Conversely, the LDF results were higher in the DM healed group. In the unhealed groups, the opposite is true. The accuracy of $TcPO_2$ for predicting healing is shown to be reasonable in those with DM, slightly poorer than those without DM but better than those with CRF. It is not possible to draw relevant conclusions from this paper as it is a historical cohort of low quality with a small number of participants. The pattern seen may be due to cohort selection as the NDM cohort had significant peripheral arterial disease (PAD) whereas the DM cohort was made up of a mix of patients with diabetic foot disease, with and without PAD[238].

The results from the repeated measures suggest that there is a change in the microcirculation during healing but the true trend and how it relates to healing has not yet been identified.

As the heart, arteries, microcirculation and veins link into a circuit it is tempting to consider that there is a direct relationship between the flow of blood as it leaves the heart and how it flows through the microcirculation. However, anybody who has studied the basics in fluid biodynamics will be aware that how fluids, like blood, behave is different on the macro and microscopic scale[257]. This is before the complex control systems related to maintaining homeostasis of blood pressure and tissue perfusion become involved[258]. A patient with diseased macrocirculation is likely to have reduced overall blood flow through

the microcirculation although the degree of collateralisation will have an impact on this to[210,226]. Patients with stenosis or occlusions in anatomically appropriate vessels have been shown to have reduced $TcPO_2$ at the skin[226]. Due to this it is not possible to claim that any of the tests discussed above assess the microcirculation in isolation. However in patients with DM reduced $TcPO_2$ has been demonstrated irrespective of the presence of PAD or DPN[224], highlighting that the test is examining something other than just the macrocirculation. Reactivity tests like PORH may be less impacted by the macrocirculation as the activation of the NO pathway is a local effect of the rapid return of blood flow following occlusion, and impairment of the hyperaemic response is known to precede the development of clinically apparent microvascular and atherosclerotic disease[259]. There is lack of consensus in this area though[260].

4.8. CONCLUSIONS

Due to the heterogeneity of the cohorts and the data presented it is not possible to draw any firm conclusions from a review of the current literature. The influence of DM and associated neuropathy is not clear, and neither is the degree of improvement required to achieve healing. Studies that examine a clearly defined cohort both with and without DM are required. Accurate quantitative assessment of microcirculation will greatly aid predicting feet at risk, of predicting wound healing with and without surgery, and for identifying those at greatest risk of amputation. A study was designed to examine how the function of the microcirculation changed through the process of wound healing, particularly how the function changed following percutaneous angioplasty.

CHAPTER 5: WHAT IS THE RELATIONSHIP OF REVASCULARISATION AND IMPROVEMENT IN MICROCIRCULATION TO WOUND HEALING AND PERIPHERAL NEUROPATHY IN DIABETIC FOOT DISEASE? AN OBSERVATIONAL COHORT STUDY.

5.1. INTRODUCTION

As demonstrated in the literature review above (Chapter 4) there is a need for further evidence relating to the relationship between the microcirculation and wound healing in patients with DM. The following chapter describes work that was carried out with the aim of addressing this question. Due to problems with recruitment it was not possible to complete the planned study and the results presented represent a truncated version. They should be viewed as pilot data that could be used to guide the direction of future research.

5.2. HYPOTHESIS

Improving the microcirculation of the foot in patients with diabetic foot disease improves wound healing and degree of peripheral neuropathy.

5.3. PLAN FOR COHORT STUDY

The original plan for the cohort study was to recruit three groups of patients all with active pedal tissue loss. The groups would have comprised a group with diabetes but no significant peripheral arterial disease (PAD), the diabetic tissue loss group (DTL). Two groups with significant PAD which required a revascularisation procedure, those who had been

listed for a percutaneous angioplasty (PCA) and those that had been listed for peripheral bypass surgery (PBS). The initial comparison would have been between these three groups. The second comparison would have been between the patients with and without diabetes mellitus (DM) in the PCA and PBS groups (Figure 5.3-1).

5.3.1. Main questions to be addressed

To address the hypothesis, the following questions were asked.

- Which of PCA or PBS provides a greater improvement in the microcirculation?
- What is the level of improvement in the microcirculation at the time of wound healing in patients with DM and PAD?
- Is there any improvement in the neurological status of patients with neuro-ischaemic ulceration who have undergone revascularisation?

The microcirculation was to be assessed using laser Dopper fluxmetry (LDF), toe blood pressure (TBP), and skin perfusion pressure (SPP). Neuropathy was assessed using a combination of vibration perception threshold (VPT), monofilament detection, Ipswich touch test (ITT) and neuropathy total symptom score-6 (NTSS-6).

5.3.2. Sample size calculation

Data previously obtained at our institution[261] found the following values for the mean (Standard Deviation) times (in seconds) to maximum post-occlusive reactive hyperaemia in patients who underwent PBS or PCA:

PBS Patients (N=29): Preoperative = 100 (22); Postoperative = 59 (38)

PCA Patients (N=9): Preoperative = 69 (27); Postoperative = 55 (30)

The average improvement was 41 seconds (100-59) in the PBS group and 14 seconds (69-55) in the PCA groups. Hence, the difference in the improvement between the two groups was 27 seconds (41-14).

The pooled standard deviation from this data is 30. If the pre and post-operative measurements on the same patient are assumed to be independent, the standard deviation of the change will be 30 times the square root of 2, i.e. 42

The study was powered based on a t-test, assuming a standard deviation of 42. For a detectable difference of 27, a sample size of 40 patients per group (i.e. 80 total) would be sufficient for 80% power at 5% alpha.

There is no equivalent data available with which it would be possible to calculate an appropriate sample size for the group with DM and no PAD (DTL group). For this reason, they have not been included in the primary outcome. A sample size of 20 will be able to detect a difference of 33 seconds between the initial measurement and the point of healing. This was agreed to be reasonable as a consensus within the research group.

Due to the demographics of patients being investigated previous experience suggests that a high dropout rate (up to 20%) should be expected. This increases the size of our study groups to 48 for both the bypass and angioplasty groups and 24 for the DTL group. This brings our total sample size to 120 participants.

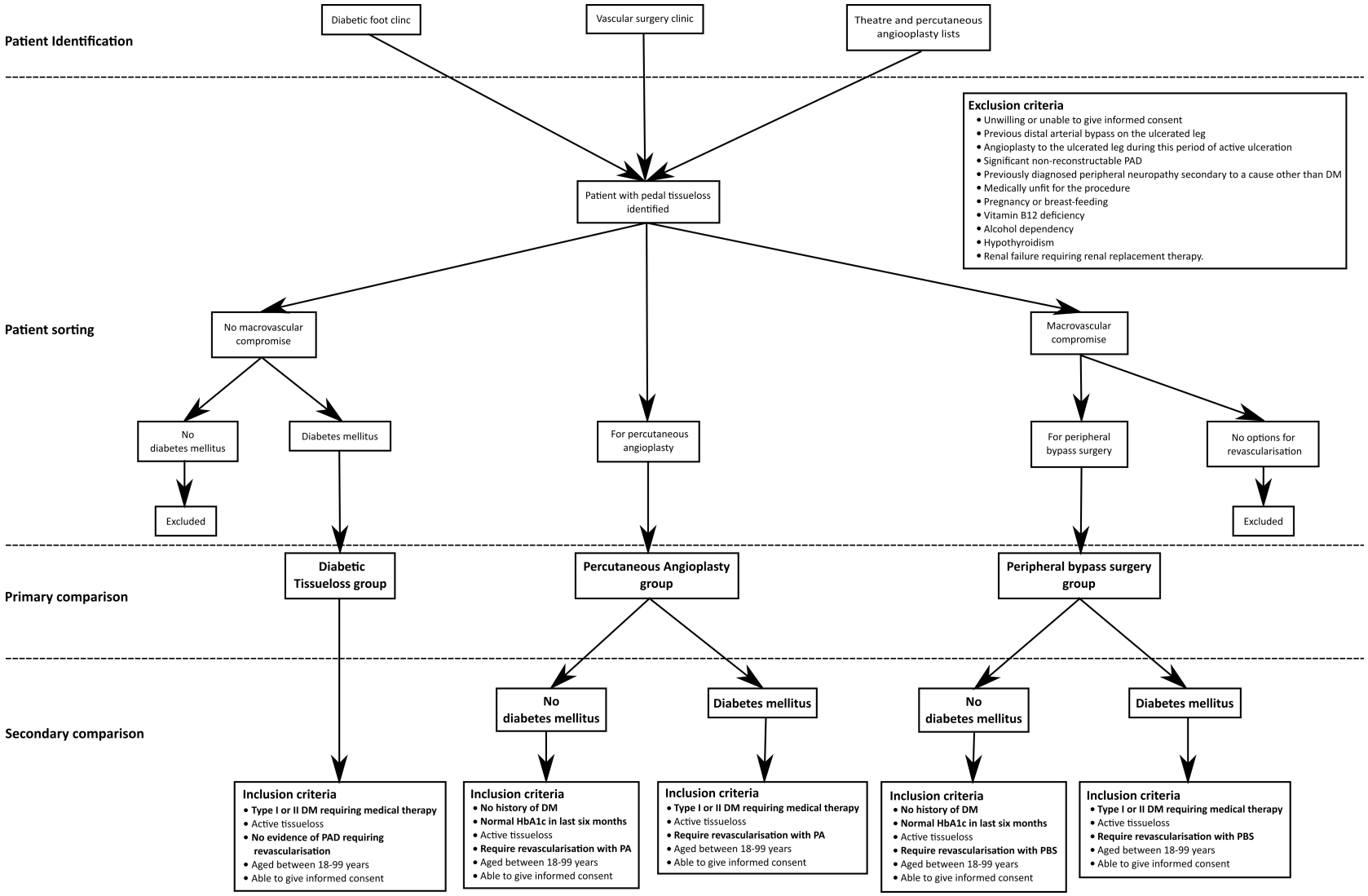

Figure 5.3-1: Inclusion flow chart for planned cohort study

5.4. RECRUITMENT ISSUES

Recruitment began based on the protocol set out in Appendix III. Recruitment levels were low throughout the study period, reasons for this include issues with the identification of potential participants and issues with the sites. At the beginning of the study, at all sites, the purpose of the study and the type of patient being recruited was presented at relevant team meetings (vascular surgery and diabetes and endocrinology). In areas where clinics took place, posters were placed with a list of inclusion criteria and contact details for the study team. Despite this, no participants were identified unless the lead recruiter (DLL) was present in the clinic. In clinics which were specific diabetic foot clinics a relatively high proportion of patients could be considered as potential participants. The majority of these would be eligible for the DTL group rather than the PCA or PBS groups. Potential members of the PCA and PBS groups were more likely to be identified from vascular surgery clinics. Within an individual clinic of fifteen to thirty patients, there may only be one, or less, patients potentially eligible for the study. It was not possible for DLL to be present at all vascular clinics across all sites particularly after the follow-up of recruited patients had started.

In an effort to improve identification of PCA patients, after a review of recruitment at six months, an amendment to the study protocol was applied for. This requested permission to review clinic lists, angiography and theatre lists for potential participants. Any patient who appeared to meet the inclusion criteria was sent a letter of invitation and then contacted by phone if they responded favourably. This was only possible at the UHB site as DLL was not a

regular member of staff at the other sites and so would not normally have access to the appropriate procedure lists.

This did improve recruitment of PCA patients slightly, but PBS patients remained challenging. The main reason behind this was due to bed pressures. Ideally, patients being admitted for major surgery would be admitted on the evening before surgery. Pressures on beds during the period of recruitment meant that predominantly patients were being admitted to an admissions lounge on the morning of surgery. There was no space within the admissions lounge for the study procedures to be undertaken, in addition, the patients were usually first on the list, and so there was not the time to organise an alternative room.

5.4.1. Amendments

5.4.1.1. Amendment 1: August 2014

Amendment 1 was made in response to an internal review of the protocol performed by the University of Birmingham as part of the application process for an MSc by Research Clinical and Experimental Medicine. The changes to the protocol are as follows:

1. Rewording of the principle research question to "What is the level of improvement in the microcirculation at the time of wound healing in patients with diabetes mellitus and peripheral arterial disease?" from "What is the level of improvement in microcirculation required to achieve wound healing in patients with diabetes mellitus and peripheral arterial disease?"
2. Clarification of the timelines for assessment of the patients.
3. Rewritten description of how 10g monofilament assessment will be performed.
4. Sample size recalculated to include a dropout rate of 20%.

5. Proposed statistical analysis clarified.
6. Proposed handling of missing data clarified.

5.4.1.2. Amendment 2: October 2014

Amendment 2 requested permission to perform a glycated haemoglobin (HbA1c) on all patients who were believed not to have diabetes but had not had a HbA1c in the last six months.

If a patient was found with an HBA1c of >6.5% (diagnostic of diabetes as per WHO guidance 2011) and assuming they were stable and well in themselves then the researchers would provide basic counselling in the clinic and then refer them to their GP for further management and investigation of their diabetes. If there was any indication the patient was acutely unwell, the researchers would organise admission to hospital for further management. In both cases, these patients would be excluded from the study as they would not yet meet the inclusion criteria of being on medication for their diabetes.

5.4.1.3. Amendment 3: August 2015

Amendment 3 requested permission to review clinic, theatre and angioplasty lists for potential participants. This changed the wording on how patients would be identified to that now found in section 5.6.5.

5.5. RECRUITMENT ACHIEVED

Recruitment started on the 1st January 2015. As the recruitment period went on it became clear that it would not be possible to recruit the 120 planned patients in the available time period. As of February 2016, twenty-six patients had been recruited, fourteen in the DTL group, ten in the PCA group and two in the PBS group (Figure 5.5-1). The decision was made to stop recruitment and analyse the data that had been collected up until this point. The hope was to use the data for new power calculations and to redesign the study. In essence treating this data as a pilot study. As the PBS group only included two patients, and one of those was the only patient without DM, they were not included in the data analysis.

The following sections set out the outcome measures of the study, data collection protocol and data analysis that was carried out.

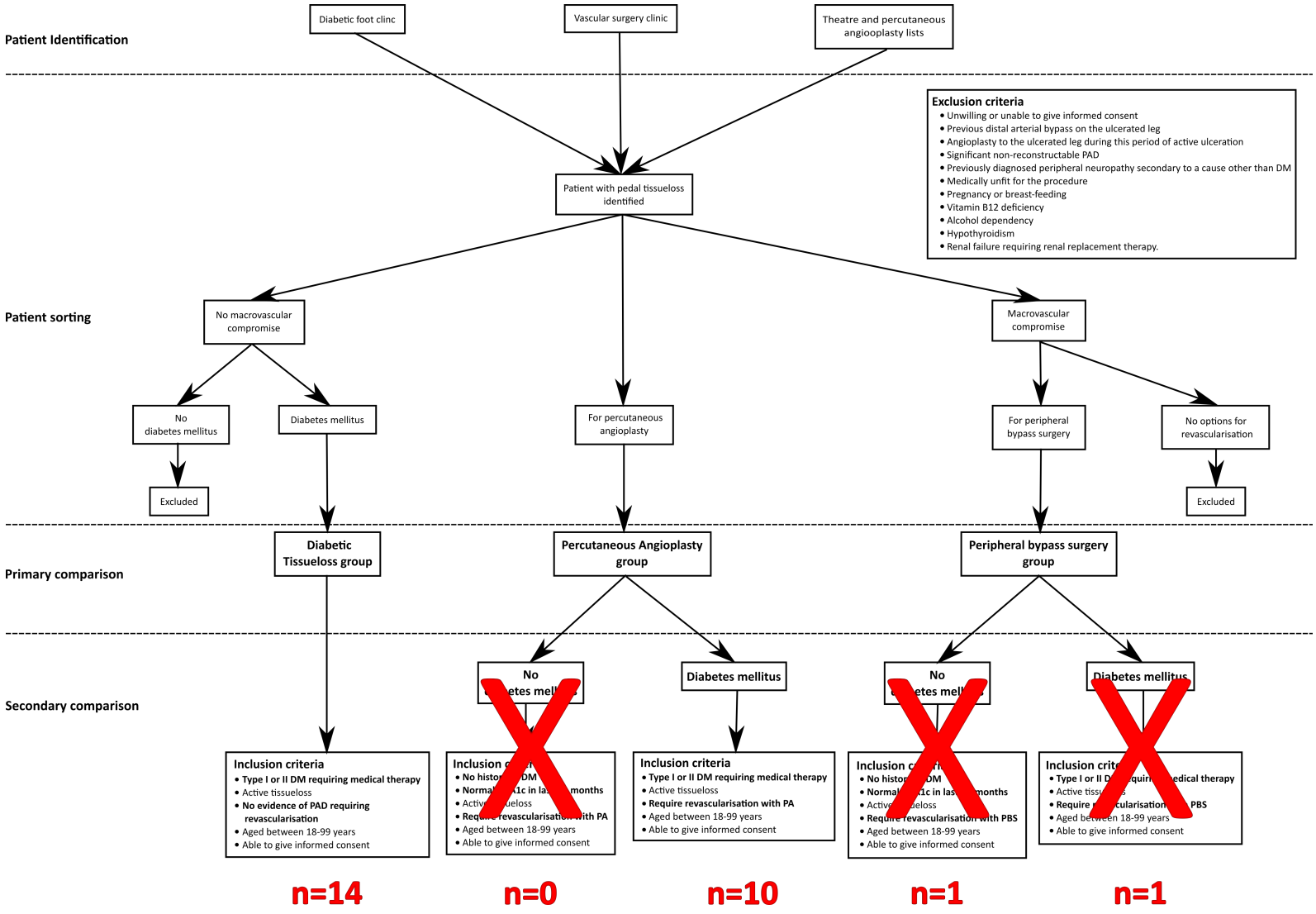

Figure 5.5-1: Inclusion flow chart for pilot study

5.6. DESIGN OF PILOT STUDY

5.6.1. Outcome Measures

5.6.1.1. Primary outcome

- Evidence of difference in the level of change in the time to maximum flux between, before, and after PCA.

5.6.1.2. Secondary outcomes

- Evidence of difference in the level of change in the time to maximum flux between when ulceration active and when ulceration healed in patients with DM and no significant PAD.
- Time to wound healing
- Time to major amputation
- The change in skin perfusion pressure (SPP) at the end of the study
- The change in toe blood pressure (TBP) at the end of the study
- The change in vibration perception threshold (VPT) at the end of the study
- The change in monofilament detection at the end of the study
- The change in Ipswich touch test (ITT) at the end of the study
- The change in neuropathy total symptom score - 6 (NTSS-6) at the end of the study

End of the study is defined as either complete wound healing, major amputation of the study limb or time for the conduct of the study elapsing (1st June 2016).

5.6.2. Study groups (Figure 5.5-1)

5.6.2.1. Diabetic tissue loss without peripheral arterial disease (DTL)

Patients with type I or II DM and active tissue loss without clinically significant PAD. These patients had no additional treatment other than the standard wound care and follow-up in the local diabetic foot clinic.

5.6.2.2. Percutaneous angioplasty (PCA)

Patients with active tissue loss and PAD confirmed clinically and on duplex ultrasound, computed tomography angiography or Magnetic Resonance Angiograpy who on assessment by their named consultant require PCA.

5.6.3. Inclusion criteria

5.6.3.1. For participants requiring percutaneous angioplasty

- Type I or II DM requiring medical therapy
- Active tissue loss
- Require endovascular revascularisation
- Aged between 18 and 99 years
- Able to give informed consent

5.6.3.2. For participants with diabetes mellitus and no peripheral arterial disease

- As the first group, apart from no evidence of PAD requiring revascularisation. As assessed by a vascular surgery consultant.

5.6.4. Exclusion criteria

- Unwilling or unable to give informed consent
- Previous distal arterial bypass on the ulcerated leg.
- Angioplasty to the ulcerated leg during this period of active ulceration.
- Significant non-reconstructable PAD.
- Previously diagnosed peripheral neuropathy secondary to a cause other than DM.
- Medically unfit for the procedure.
- Pregnancy or breast-feeding
- Vitamin B_{12} deficiency.
- Alcohol dependency.
- Hypothyroidism.
- Renal failure requiring renal replacement therapy.

5.6.5. Patient identification

Potential patients were identified predominantly from diabetic foot clinics, vascular clinics and inpatient admissions at the study sites. The patients were identified by the investigators and other doctors on the vascular and diabetes teams. As it was not possible for a member of the study team to be present in person in all relevant clinics review of referral documentation to clinics, theatre lists and angioplasty lists was conducted. This was only performed at the University Hospitals Birmingham site due to restrictions in distributing the relevant lists beyond those who would normally be in receipt of them due to their clinical responsibilities. If a potential patient was identified from these lists a patient invitation letter (Appendix IV) and a patient information sheet (Appendix V) was posted to their home address. The patient invitation letter contained a reply slip and stamped addressed envelope which allowed the patient to express their desire or otherwise to be included in the study. If a reply slip was not received, then, after 1-2 weeks the letter was followed-up with a phone call. If the patient expressed an interest at their next clinic appointment or on the day of

procedure the patient was met by a member of the team to answer any questions and confirm their continued desire to participate. If appropriate, consent was taken, and the first assessment carried out at this time.

5.6.6. Consent

All participants were verbally invited to take part in the study by the clinical team providing their care. They were given written information with regards to the purpose and design of the study (Appendix V). They were then invited to participate and, if agreed, consented using a standardised consent form (Appendix VI). This was performed and undertaken by a member of the study team.

Consent to participate included consent to the use of data obtained during their participation in the final analysis. Participants were free to leave the study with only a verbal request, though their non-identifiable data remained within the study unless a written request was made to the contrary. This was made clear at the time of consent.

5.6.7. Assessment

5.6.7.1. Timing of assessments

For the PCA group, the initial assessment was carried out within the 24 hours before their procedure. For the DTL group, the initial assessment was carried out following informed consent being gained. If possible, this was during the same attendance.

Post-procedure assessment for the PCA group occurred at initial follow-up clinic appointment one month following their procedure. Subsequent re-examination occurred

monthly (within a week) at the hospital appointment that fell nearest this time. This included vascular, diabetes and podiatry appointments. Reassessment continued until the ulcer was decided to have clinically healed, the limb was amputated, or the end of the study was reached.

The DTL group had repeat assessments monthly until the ulcer was decided to have clinically healed, the limb was amputated, or the end of the study was reached.

5.6.7.2. Procedure for data collection

The patient was asked to arrive for their appointment having not eaten for the last 2 hours and not had any caffeinated drinks since the proceeding night. They were assessed in a temperature controlled clinic room (22^0C +/- 2^0C) or if for inpatients this was not possible the temperature was recorded. The patient was positioned on a couch in a semi-recumbent position so they could acclimatise to the temperature of the room. During the first fifteen minutes, the NTSS-6 questionnaire was completed, and monofilament, ITT and VPT testing was carried out on both feet. The characteristics of the ulcer/s were also described. During the second fifteen minutes, the patient was asked to relax, and the LDF probes were attached. The protocol for each measurement was as follows.

10g Monofilament

Three areas on each foot was tested. These were the plantar aspects of the hallux, and base of the third and fifth metatarsals[18,120]. A positive or negative response for each area was recorded. Abnormal was defined as an inability to detect the monofilament in one area.

If it was not possible to test any of the areas due to amputation or ulceration, this was recorded.

Ipswich Touch Test

The tips of the 1st, 3rd and 5th toes of each foot were tested in the order described by Diabetes UK in their leaflet "How to do the Touch the Toes Test"[262]. A positive or negative response for each area was recorded. If it was not possible to test any of the areas due to amputation or ulceration, this was recorded.

Vibration Perception Threshold

VPT was assessed on the pulp of the hallux or next dominant toe if amputation had occurred in the past or the toe was necrotic. A neurothesiometer was used. The first measurement was taken increasing from zero to maximum and the level at which vibration was detected recorded. The second measurement was taken from maximum to zero and the level at which vibration disappears was recorded. An average of these two readings was taken. If no vibration was detected, then 50V was recorded.

Ulcer description

The ulcer/s were described in the same way as used by the Eurodiale study [263]. Area of the ulcer was determined by multiplying the largest diameter by the second largest diameter perpendicular to the first. Depth was described as superficial or deep: a superficial ulcer is a full-thickness lesion of the skin not extending through the subcutis, and a deep ulcer is a lesion of the skin extending through the subcutis. An infection was diagnosed if two or more of the following signs are present: frank purulence, local warmth, erythema, lymphangitis, oedema, pain, fever and foul smell. The anatomical location of the ulcer/s was also described. The ulcer was described as healed when complete epithelialisation had occurred.

Laser Doppler Fluxmetry

The patient's feet were placed on a pillow to stabilise them and a laser Doppler probe placed on the pulp of the hallux or remaining dominant toe and dorsal surface of the foot between the 2nd and 3rd metatarsals. A cuff was placed around the patient's ankle. The LDF was then turned on and baseline flux measured for one minute, the cuff was then inflated for three minutes followed by rapid deflation and monitoring of the response for five minutes.

A toe cuff was placed around the great toe or remaining dominant toe to replace the cuff around the ankle. Toe blood pressure was then measured.

Following this, the low-profile probe was placed adjacent to the ulcerated tissue and gently secured with clingfilm, which also protected the ulcerated area. A cuff was placed over around the foot. SPP was measured with slow deflation of the cuff.

Probes were then transferred to the other leg and the process repeated. This whole process took approximately thirty minutes.

The data obtained was processed using the Moor VMS-PC™ software.

Demographics

Demographics that were collected at first appointment are listed below.

- Age
- Sex
- Type of DM
- Duration of DM
- Current medications
- Recent blood results including glycosylated haemoglobin, cholesterol, thyroid stimulating hormone, free T_4 and vitamin B_{12}.

5.6.8. Data Analysis Plan

Initially, the continuous variables were tested for normality The only variables found to meet the assumptions of normality were systolic blood pressure, diastolic blood pressure and room temperature. Consequently, these variables where analysed using parametric tests. For the other variables comparison between groups was made using independent samples non-parametric tests like Kruskal-Wallis and Mann-Whitney U tests. Related variables, i.e. visits, were compared using the related samples Freidman's test and Wilcoxon signed rank test. Categorical data was examined using Chi-squared test or Fisher's exact test as appropriate. Related categorical data was examined using McNemar's test.

Demographics were analysed by group. The differences between characteristics of the ulcers and the patients (days since the last appointment, time of assessment, hours since last ate, hours since caffeine, ulcer area, neuropathy total symptom score – 6, vibration perception threshold, room temperature, systolic blood pressure and diastolic blood pressure) were examined by group and by visit. As only three patients had more than three visits only the data for the first three visits were analysed. The comparisons for the LDF data were by group, by visit, patients who healed compared to those who did not and the study leg compared to the non-study leg.

5.6.8.1. Data collection and management

Data was recorded directly onto a standard data collection proforma, and the LDF data was recorded onto an encrypted computer. The analysis also took place on this computer.

5.6.8.2. Data storage

All identifiable patient data was collected and stored on an encrypted computer and kept locked within secured premises within the vascular department. This will be maintained as per data protection and GCP guidelines then stored for the required five years before destruction.

5.6.8.3. Source data

Source data was kept within the source data file that was, in turn, kept locked within a secure office locked and secure within the Vascular Department. It is accessible only by those signatories within the research group and by the sponsors as requested.

5.6.9. Ethical Considerations

Local Research Ethics Committee approval was sought from the South Birmingham Research Ethics Committee. Approval was granted on 26th June 2014. Approval was also sought from the Research and Development departments of the three study sites, University Hospitals Birmingham NHS Foundation Trust, Dudley Group NHS Foundation Trust and Sandwell and West Birmingham Hospitals NHS Trust. These were approved on 24th July 2014, 18th July 2014 and 30th September 2014 respectively.

All research was conducted in line with the World Medical Association Declaration of Helsinki. All members of the research group received Good Clinical Practice Training as per the National Institute for Health Research, and the research was conducted in line with the same principles. Funding to purchase the equipment (the laser Doppler equipment) for the

study was sourced from the Vascular surgery research fund held by the Queen Elizabeth Hospital Birmingham Charity and not from commercial sources.

5.7. RESULTS

5.7.1. Numbers

In total twenty-four patients were recruited. Fourteen patients in the DTL group and ten in the PCA group. One patient in the PCA group consented to be included in the study but was taken for their procedure before any assessments could be performed, so no data from them is included in the study.

5.7.2. Demographics (Table 5.7-1)

The patients in the PCA group were significantly older than the DTL group. There was no significant difference in duration of DM or ulcer. The site of the ulcer was more likely to be on the plantar surface of the foot in the DTL group and the heel in the PCA group. Those in the PCA group had a significantly lower estimated glomerular filtration rate than the DTL group. There were no other areas of significant difference within the collected demographics. It is worth noting the small number of patients with information available for total cholesterol, thyroid stimulating hormone, free T_4, and vitamin B_{12}.

Table 5.7-1: Demographics for study groups

	Study Group				
	Diabetic Tissue Loss		Percutaneous Angioplasty		p-value*
	n	Median (IQR)	n	Median (IQR)	
Age (years)	14	52 (43-60)	9	76 (75-78)	**<0.001**
Male (%)	14	71.4	9	66.7	1.00††
Type II DM (%)	14	78.6	9	100	0.253††
Glycated Haemoglobin (mmol/mol)	9	75 (52-84)	3	52 (49-53)	0.209
Duration of DM (years)	14	15 (9-20)	8	18 (11-24)	0.525
Duration of Ulcer (months)	14	2 (1-13)	9	5 (3-7)	0.336
Ulcer Site (%)	14		9		
Hallux		21.4		22.2	
Non-dominant toe		0.0		11.1	
MTPJ		28.6		22.2	**0.030††**
Dorsum		0.0		11.1	
Plantar		50.0		0.0	
Heel		0.0		33.3	
Study Leg (% Right)	14	42.9	9	88.9	**0.040††**
Number of Comorbidities	14	1 (1-3)	9	2 (1-3)	0.688
Hypertension (%)	14	57.1	9	77.8	0.400††
Estimated Glomerular Filtration Rate	14	87 (61-90)	8	55 (43-71)	**0.024**
Total Cholesterol (mg/dL)	7	4.9 (4.8-5.3)	4	4.3 (3.4-4.9)	0.109
Thyroid Stimulating Hormone (mU/L)	7	2.1 (0.9-3.5)	3	3.5 (2.6-4.1)	0.183

Free T4 (pmol/L)	4	15.4 (14.6-17.6)	3	19.3 (14.0-22.6)	1.000
Vitamin B_{12} (pg/ml)	3	467 (145-554)	0	-	-
Number of Medications	13	7 (2-9)	9	8 (7-9)	0.357
Cardioactive Drugs (%)	14	46.2	9	44.4	1.000††
Alcohol Units per week	14	2 (1-6)	9	1 (0-7)	0.439
Smoking Status (%)	14		9		
Never Smoked		78.6		44.4	0.225††
Ex-smoker		14.3		44.4	
Current Smoker		7.1		11.1	

*Independent Samples Mann-Whitney U Test, †Chi-squared Test, ††Fisher's Exact Test, MTPJ: Metatarsal Phalangeal Joint

5.7.3. Characteristics of ulcers and patients

5.7.3.1. Days since the last appointment

The median time between appointments was 34 days (Inter-quartile range (IQR) 28-50) for patients in the DTL group and 45 days (35-70) in the PCA group (p= 0.030) (Table 5.7-6). There was no significant difference in the time between appointments for all patients or by group (Table 5.7-7).

5.7.3.2. Time of assessment

The median time of assessment was 11:30 (09:50-13:09) in the DTL group and 11:00 (10:07-12:15, p=0.382) in the PCA group (Table 5.7-6). This difference was not significant, and neither was there a significant difference between visits (Table 5.7-7).

5.7.3.3. Time since eating

The median time since eating was 2.75hrs (1.50-3.25) in the DTL group and 2.38hrs (1.75-3.00, p=0.594) in the PCA group (Table 5.7-6). These values remained similar and non-significant by visit (Table 5.7-7).

5.7.3.4. Time since caffeine

The time since consuming caffeine was similar to the time since eating (DTL 3.00hrs (2.25-5.00) vs PCA 2.50hrs (1.63-3.50), p=0.084) both by group and by visit (Table 5.7-6 and Table 5.7-7).

5.7.3.5. Ulcer area

Between groups over all visits, there was no significant difference in ulcer area (Table 5.7-6). When looked at by visit in both groups there was a decrease in size at the second visit but an increase at the third visit. In the PCA group, the increase is to larger than the baseline value (Table 5.7-7).

5.7.3.6. Depth of ulcer

At baseline sixteen patients (69.6%) had a deep ulcer. There was no significant difference in the depth of the ulcer by the second visit (Table 5.7-2). By the last visit, there was a significant difference in the DTL group (Table 5.7-3) with a high proportion of patients changing from a deep ulcer to a superficial ulcer.

Table 5.7-2: Depth of ulcer at baseline compared to depth of ulcer at second visit

			Depth of ulcer at second visit		p-value*
			Superficial	**Deep**	
Depth of ulcer at baseline	**Diabetic Tissue Loss**	**Superficial**	2	1	0.219
		Deep	5	3	
	Percutaneous Angioplasty	**Superficial**	2	1	1.000
		Deep	2	3	

*McNemar Test

Table 5.7-3: Depth of ulcer at baseline compared to depth of ulcer at last visit

			Depth of ulcer at last visit		p-value*
			Superficial	**Deep**	
Depth of ulcer at baseline	**Diabetic Tissue Loss**	**Superficial**	2	1	**0.039**
		Deep	8	0	
	Percutaneous Angioplasty	**Superficial**	3	0	0.125
		Deep	4	1	

*McNemar Test

5.7.3.7. Infection

Nine patients (39.1%) had evidence of infection at baseline and four (21.1%) at the second visit. Only one of these at the second visit was a new infection (Table 5.7-4). There was also no significant difference at the last visit (Table 5.7-5).

Table 5.7-4: Presence of infection at baseline compared to presence of infection at second visit

			Presence of Infection at Second Visit		p-value*
			No	**Yes**	
Presence of Infection at baseline	**Diabetic Tissue Loss**	**No**	7	1	1.000
		Yes	2	1	
	Percutaneous Angioplasty	**No**	4	0	0.500
		Yes	2	2	

*McNemar Test

Table 5.7-5: Presence of infection at baseline compared to presence of infection at last visit

			Presence of Infection at Last Visit		p-value*
			No	**Yes**	
Presence of Infection at baseline	**Diabetic Tissue Loss**	**No**	7	1	0.625
		Yes	3	0	
	Percutaneous Angioplasty	**No**	4	0	0.250
		Yes	3	1	

*McNemar Test

5.7.3.8. Room temperature

The mean room temperature was 23.06±0.95^{o}C in the DTL group and 22.59±1.51^{o}C in the PCA group. This difference was not significant (p=0.117) neither was there a significant difference by visit (Table 5.7-7).

5.7.3.9. Blood pressure

There was no significant difference in systolic or diastolic blood pressure by group or by visit (Table 5.7-6 and Table 5.7-7).

Table 5.7-6: Ulcer characteristics by group

	Group				p-value **
	Diabetic Tissue Loss		Percutaneous angioplasty		
	n	Median (IQR)	n	Median (IQR)	
Days since last appointment*	27	34 (28-50)	19	45 (35-70)	**0.030**
Time of assessment	41	11:30 (9:50-13:09)	28	11:00 (10:07-12:15)	0.382
Hours since last ate	41	2.75 (1.50-3.25)	28	2.38 (1.75-3.00)	0.594
Hours since caffeine	41	3.00 (2.25-5.00)	28	2.50 (1.63-3.50)	0.084
Ulcer area (mm^2)	41	182 (50-450)	28	180 (19-985)	0.888
Room temperature† (^0C)	41	23.06±0.95	28	22.59±1.51	0.117††
Systolic blood pressure† (mmHg)	41	138.15±20.63	28	139.00±17.89	0.859††
Diastolic blood pressure† (mmHg)	41	76.63±11.04	28	73.25±10.96	0.214††

*Visit 1 excluded **Independent-Samples Mann-Whitney U Test †Mean ††One-way ANOVA

Table 5.7-7: Ulcer characteristics by visit

		Visit						p-value*
		1		2		3		
		n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	
Days since last appointment	**DTL**	-	-	11	31 (28-60)	7	35 (30-42)	0.075
	PCA	-	-	9	60 (35-91)	4	38.5 (35-47)	0.893
Time of assessment	**DTL**	14	11:55 (11:10-13:09)	11	10:45 (9:50-12:00)	7	11:30 (9:50-14:45)	0.368
	PCA	9	11:45 (11:00-13:00)	9	11:30 (10:35-11:45)	4	10:07 (9:40-11:22)	0.504
Hours since ate	**DTL**	14	2.75 (1.500-3.5)	11	3.00 (2.25-4.00)	7	2.00 (1.00-3.00)	0.618
	PCA	9	2.50 (2.00-3.00)	9	2.75 (2.00-3.25)	4	2.13 (1.50-3.13)	0.623
Hours since caffeine	**DTL**	14	2.88 (2.00-4.25)	11	3.00 (2.25-5.50)	7	6.00 (3.00-12.00)	0.392
	PCA	9	2.50 (1.50-3.00)	9	3.25 (2.00-3.50)	4	2.13 (1.50-7.25)	0.392
Ulcer area (mm^2)	**DTL**	14	342.5 (80-782)	11	90 (10-400)	7	125 (0-260)	**0.002**
	PCA	9	270 (180-1750)	9	150 (0-1200)	4	458 (195.5-1090.5)	0.050
Room temperature (^0C)**	**DTL**	14	23.29±1.18	11	22.91±0.91	7	23.14±0.66	0.939†
	PCA	9	22.87±0.99	9	22.900±1.74	4	22.43±2.22	0.943†
Systolic BP (mmHg)**	**DTL**	14	136.86±24.33	11	136.82±15.14	7	130.57±23.63	0.385†
	PCA	9	139.78±19.62	9	146.78±10.22	4	129.75±23.10	0.659†
Diastolic BP (mmHg)**	**DTL**	14	76.57±11.66	11	77.73±13.02	7	75.00±9.71	0.385†
	PCA	9	73.33±11.80	9	78.11±10.07	4	69.25±14.93	0.481†

*Related-Samples Friedman's Two-Way Analysis of Variance by Ranks, **mean±SD, †Two-way repeated measures ANOVA

5.7.4. Post Occlusive Reactive Hyperaemia

The variables calculated for post occlusive reactive hyperaemia (PORH) are shown in Figure 4.4-2. For clarity, the only variable included in the following figures is the time to maximum flux (TtM). The full data set is presented in Appendix VII.

Patients in the PCA group had a significantly longer TtM than the DTL group at baseline on both the study toe (220.8s (200.2-288.78) vs 13.4s (3.68-73.85), p=0.002) and study dorsum (220.28s (93.65-279.8) vs 8.05s (3.45-17.55), p<0.001). At the last visit for the study toe, there was no longer a significant difference between the groups however the difference remained on the study dorsum. There were no significant differences between the groups in the non-study leg (Table 5.7-8).

When the baseline results were compared to the last results (Table 5.7-9 and Figure 5.7-1 to Figure 5.7-4) there was a significant increase in the TtM in the DTL group for all patients (13.4s (3.68-73.85) vs 27.08s (8.5-154.38), p=0.021) and those that healed (13.4s (6.33-73.85) vs 64.43 (22.5-114.2), p=0.028). This was true for the study toe but only for all patients combined on the study dorsum. In the PCA group, there was a decrease, this only reached significance in the study toe healed group (210.5 (72.18-231) vs 50.71 (105.18-105.18), p=0.046).

There was no significant difference between the baseline results and the second visit results (Table 5.7-10 and Figure 5.7-5 to Figure 5.7-8) apart from on the study dorsum in the DTL unhealed group where there was a significant increase in TtM (11.2s (3.83-21.35) vs 20.38s (9.78-209.2), p=0.043).

There was no significant difference between the patients who had healed by their last visit and those who did not (Table 5.7-11 and Figure 5.7-9 to Figure 5.7-12) apart from in one instance. In the DTL group on the study dorsum at the second visit, those in the unhealed group had a significantly longer TtM (3.09s (2.15-5.28) vs 20.38 (9.78-209.2), p=0.030).

When the study leg was compared to the non-study leg, both on the toe and the dorsum, there was a, non-significant, longer TtM in the non-study leg in the DTL group at baseline (13.4s (3.68-73.85) vs 38.98s (12.68-141.05), p=0.158 and 8.05s (3.45-17.55) vs 15.4s (3.58-116.9), p=0.300). In the PCA group, the TtM was shorter in the non-study leg. On the toe this was significant at baseline (220.8s (200.2-288.78) vs 54.8s (13.95-127.43), p=0.028) but not at the last visit (54.5s (34.73-158) vs 35.33s (15.85-146.280, p=0.612) (Table 5.7-12 and Table 5.7-13).

Table 5.7-8: Post occlusive reactive hyperaemia Time to Maximum Flux (s) by group

		Diabetic Tissue Loss Median (IQR) (n=14)	Percutaneous Angioplasty Median (IQR) (n=9)	p-value*
Study toe	**Baseline**	13.40 (3.68-73.85)	220.80 (200.20-288.78)	**0.002**
	Last	27.08 (8.50-154.38)	54.50 (34.73-158.00)	0.442
	% Change	94.47 (18.87-328.06)	-42.14 (-80.90--2.17)	**0.001**
Study Dorsum	**Baseline**	8.05 (3.45-17.55)	220.28 (93.65-279.80)	**<0.001**
	Last	13.50 (4.95-20.38)	127.78 (30.08-227.13)	**0.015**
	% Change	43.48 (-16.85-253.10)	-20.03 (-64.01-84.71)	0.126
Non-study Toe	**Baseline**	38.98 (12.68-141.05)	54.80 (13.95-127.43)	0.689
	Last	18.83 (11.83-215.65)	35.33 (15.85-146.28)	0.596
	% Change	47.32 (-11.92-115.76)	2.92 (-72.27-196.30)	0.884
Non-study Dorsum	**Baseline**	15.40 (3.58-116.90)	70.68 (31.10-138.60)	0.149
	Last	15.40 (12.13-215.65)	119.03 (42.43-265.28)	0.122
	% Change	278.23 (-38.48-801.20)	-39.97 (-45.04-43.51)	0.221

*Independent-Samples Mann-Whitney U Test

Table 5.7-9: Post occlusive reactive hyperaemia Time to Maximum Flux (s) baseline visit compared to last visit

			Baseline Value Median (IQR)	Last Value Median (IQR)	p-value*
Study toe	**DTL**	**All (14/11)**	13.40 (3.68-73.85)	27.08 (8.50-154.38)	**0.021**
		Healed (6/6)	13.40 (6.33-73.85)	64.43 (22.05-114.20)	**0.028**
		Unhealed (8/5)	27.78 (3.44-83.36)	9.68 (8.50-154.38)	0.345
	PCA	**All (9/8)**	220.80 (200.20-288.78)	54.50 (34.73-158.00)	0.050
		Healed (6/6)	210.50 (72.18-231.00)	50.71 (27.38-105.18)	**0.046**
		Unhealed (3/2)	288.78 (204.98-288.78)	141.08 (49.65-232.50)	0.593
Study Dorsum	**DTL**	**All (14/11)**	8.05 (3.45-17.55)	13.50 (4.95-20.38)	**0.041**
		Healed (6/6)	5.23 (2.85-9.28)	13.15 (5.68-13.60)	0.345
		Unhealed (8/5)	11.25 (3.83-21.35)	20.38 (4.95-192.98)	0.080
	PCA	**All (9/7)**	220.28 (93.65-279.80)	127.78 (30.08-227.13)	0.398
		Healed (6/6)	198.79 (69.18-287.33)	175.76 (105.20-227.13)	0.753
		Unhealed (3/1)	220.28 (93.65-271.38)	30.08	0.655
Non-study Toe	**DTL**	**All (14/11)**	38.98 (12.68-141.05)	18.83 (11.83-215.65)	0.182
		Healed (6/6)	38.98 (12.68-262.10)	87.20 (18.83-291.13)	0.600
		Unhealed (8/5)	34.05 (10.68-123.23)	11.83 (11.68-15.53)	0.080
	PCA	**All (7/7)**	54.80 (13.95-127.43)	35.33 (15.85-146.28)	0.917
		Healed (4/5)	34.38 (8.66-62.28)	69.55 (35.33-146.28)	0.273
		Unhealed (3/2)	127.43 (54.72-237.73)	15.51 (15.18-15.85)	0.593
Non-study Dorsum	**DTL**	**All (14/11)**	15.40 (3.58-116.90)	70.68 (31.10-138.60)	0.149
		Healed (6/6)	10.20 (2.30-55.38)	17.05 (12.13-142.95)	0.463
		Unhealed (8/5)	15.56 (5.91-117.88)	13.90 (12.88-215.65)	0.225
	PCA	**All (7/6)**	70.68 (31.10-138.60)	119.03 (42.43-265.28)	0.686
		Healed (4/5)	91.74 (58.41-125.70)	161.88 (76.18-265.28)	0.715
		Unhealed (3/1)	31.1 (13.23-273.90)	17.23	0.180

*Related Samples Wilcoxon Signed Rank Test

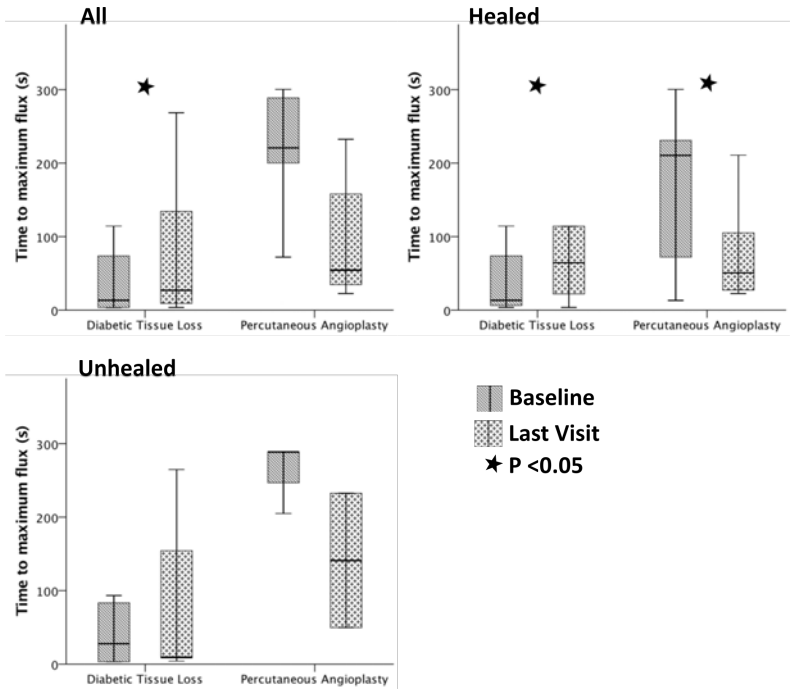

Figure 5.7-1: Time to maximum flux on study toe. Baseline compared to last visit.

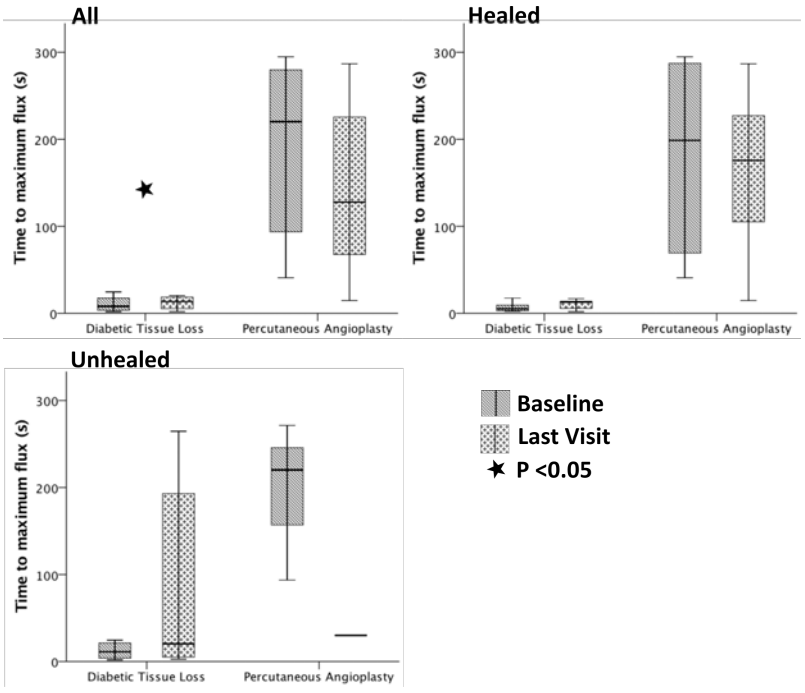

Figure 5.7-2: Time to maximum flux on study dorsum. Baseline compared to last visit.

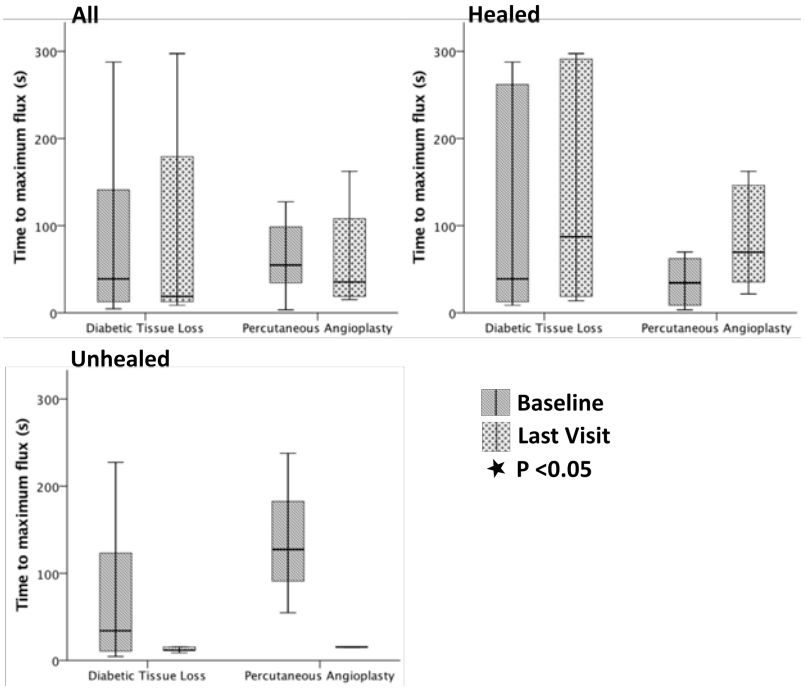

Figure 5.7-3: Time to maximum flux on non-study toe. Baseline compared to last visit.

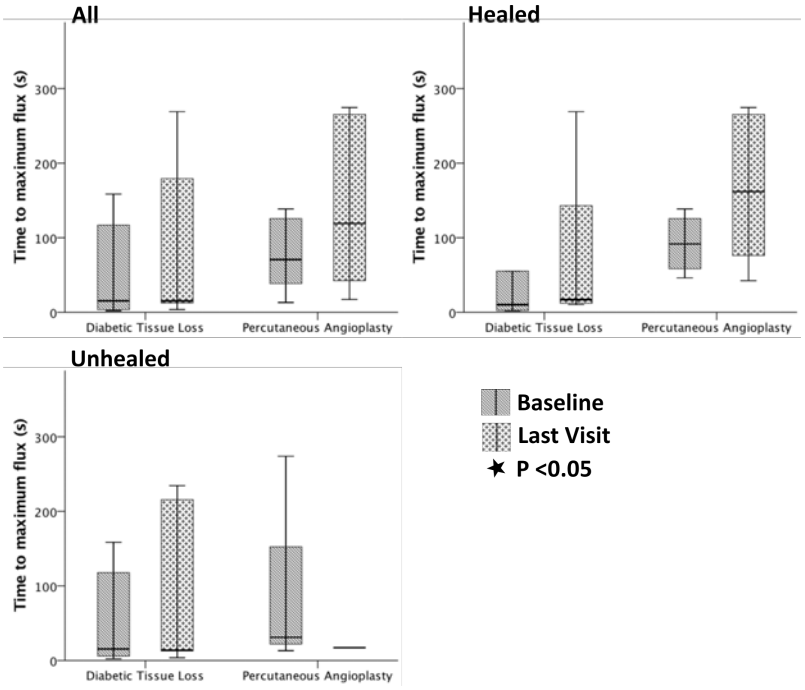

Figure 5.7-4: Time to maximum flux on non-study dorsum. Baseline compared to last visit.

Table 5.7-10: Post occlusive reactive hyperaemia Time to Maximum Flux (s) baseline visit compared to second visit

			Baseline Value Median (IQR)	Second Value Median (IQR)	p-value*
Study toe	**DTL**	**All (14/11)**	13.40 (3.68-73.85)	11.08 (4.40-27.08)	0.722
		Healed (6/6)	13.40 (6.33-73.85)	19.19 (6.83-27.08)	0.753
		Unhealed (8/5)	27.78 (3.44-83.36)	8.50 (4.28-11.08)	0.686
	PCA	**All (9/8)**	220.80 (200.20-288.78)	53.79 (18.09-205.16)	0.123
		Healed (6/6)	210.50 (72.18-231)	53.79 (27.38-177.83)	0.116
		Unhealed (3/2)	288.78 (204.98-288.78)	120.65 (8.8-232.5)	0.655
Study Dorsum	**DTL**	**All (14/11)**	8.05 (3.45-17.55)	5.28 (2.50-20.38)	0.182
		Healed (6/6)	5.23 (2.85-9.28)	3.09 (2.15-5.28)	0.600
		Unhealed (8/5)	11.25 (3.83-21.35)	20.38 (9.78-209.2)	**0.043**
	PCA	**All (9/7)**	220.28 (93.65-279.80)	127.78 (55.05-227.13)	0.398
		Healed (6/6)	198.79 (69.18-287.33)	142.33 (80.13-227.13)	0.753
		Unhealed (3/1)	220.28 (93.65-271.38)	55.05	0.317
Non-study Toe	**DTL**	**All (14/11)**	38.98 (12.68-141.05)	12.23 (8.73-120.95)	0.790
		Healed (6/6)	38.98 (12.68-262.10)	15.53 (6.98-120.95)	0.345
		Unhealed (8/5)	34.05 (10.68-123.23)	11.83 (11.68-105.38)	0.080
	PCA	**All (7/7)**	54.80 (13.95-127.43)	30.03 (15.18-69.55)	0.600
		Healed (4/5)	34.38 (8.66-62.28)	65.4 (30.03-69.55)	0.465
		Unhealed (3/2)	127.43 (54.73-237.73)	10.85 (6.53-15.18)	0.180
Non-study Dorsum	**DTL**	**All (14/10)**	15.40 (3.58-116.90)	24.15 (12.88-118.20)	0.114
		Healed (6/5)	10.20 (2.30-55.38)	18.7 (18.15-102.18)	0.500
		Unhealed (8/5)	15.56 (5.91-117.88)	29.6 (12.88-234.58)	0.138
	PCA	**All (7/6)**	70.68 (31.10-138.60)	70.63 (60.08-243.90)	0.500
		Healed (4/5)	91.74 (58.41-125.7)	76.18 (65.08-243.9)	1.000
		Unhealed (3/1)	31.1 (13.23-273.9)	60.08	0.317

*Related Samples Wilcoxon Signed Rank Test

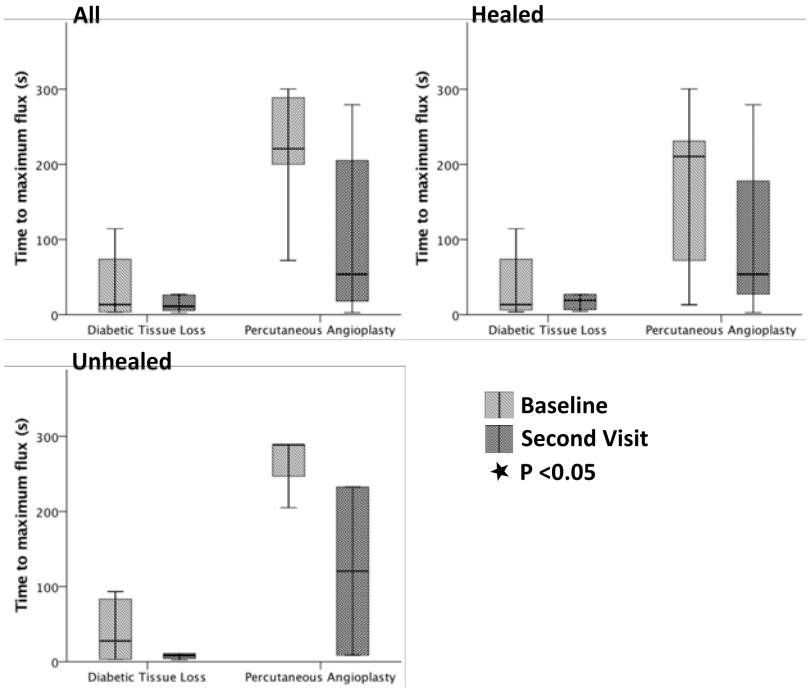

Figure 5.7-5: Time to maximum flux on study toe. Baseline compared to second visit.

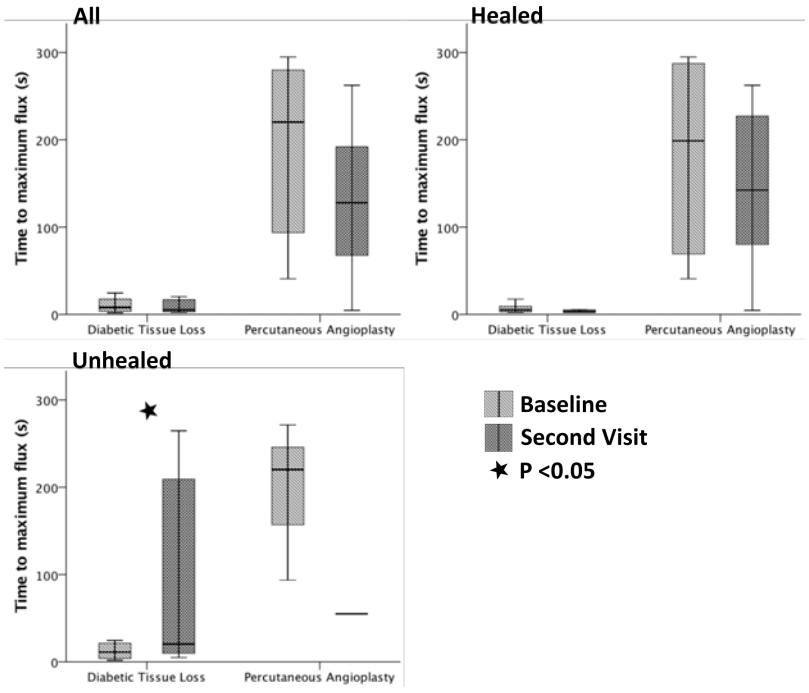

Figure 5.7-6: Time to maximum flux on study dorsum. Baseline compared to second visit.

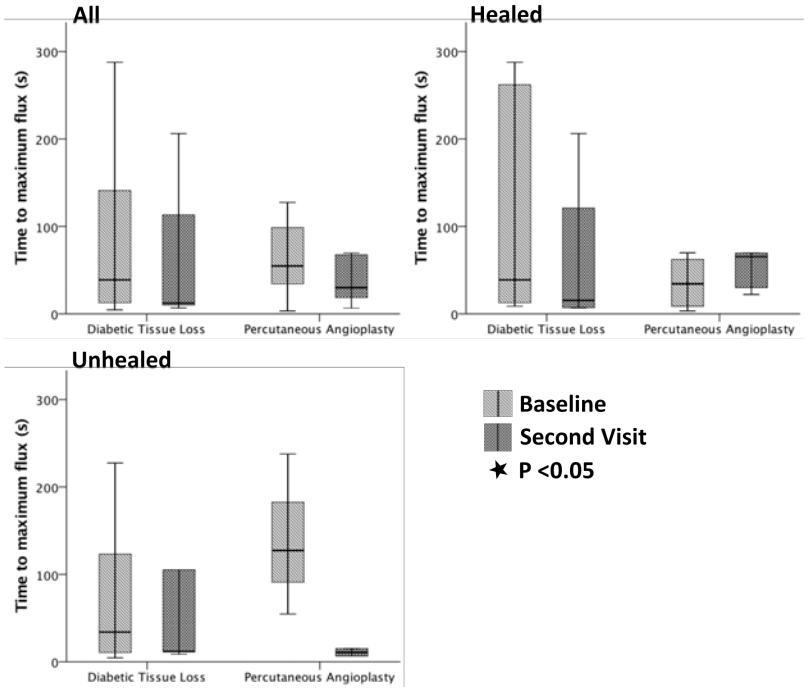

Figure 5.7-7: Time to maximum flux on non-study toe. Baseline compared to second visit.

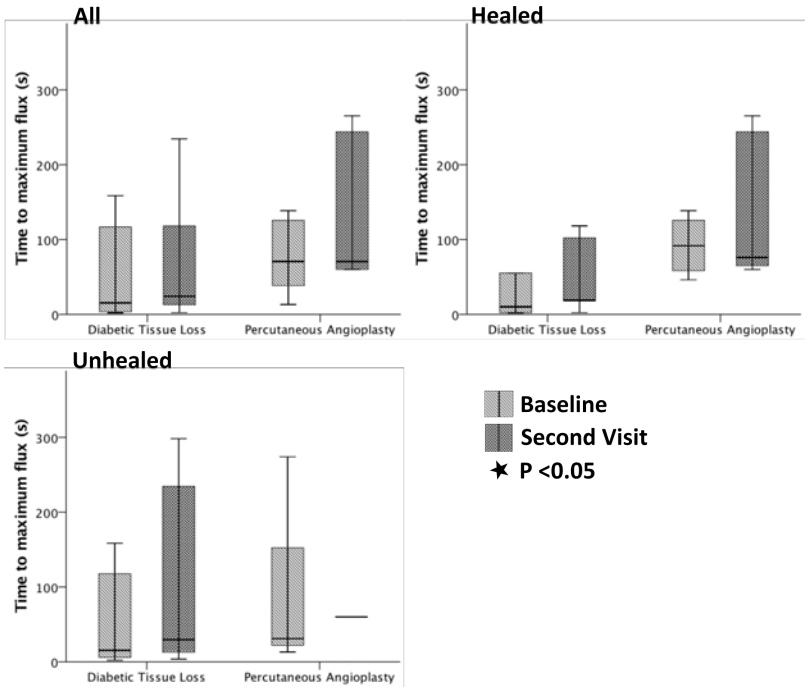

Figure 5.7-8: Time to maximum flux on non-study dorsum. Baseline compared to second visit.

Table 5.7-11: Post occlusive reactive hyperaemia Time to Maximum Flux (s), healed compared to unhealed patients

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study toe	DTL	Baseline (6/8)	13.40 (6.33-73.85)	27.78 (3.44-83.36)	0.662
		Second (6/5)	19.19 (6.83-27.08)	8.50 (4.28-11.08)	0.429
		Last (6/5)	64.43 (22.05-114.20)	9.68 (8.50-154.38)	0.792
	PCA	Baseline (6/3)	210.50 (72.18-231.00)	288.78 (204.98-288.78)	0.381
		Second (6/2)	53.79 (27.38-177.83)	120.65 (8.80-232.50)	1.000
		Last (6/2)	50.71 (27.38-105.18)	141.08 (49.65-232.50)	0.429
Study Dorsum	DTL	Baseline (6/8)	5.23 (2.85-9.28)	11.25 (3.83-21.35)	0.282
		Second (6/5)	3.09 (2.15-5.28)	20.38 (9.78-209.20)	**0.030**
		Last (6/5)	13.15 (5.68-13.60)	20.38 (4.95-192.98)	0.429
	PCA	Baseline (6/3)	198.79 (69.18-287.33)	220.28 (93.65-271.38)	1.000
		Second (6/1)	142.33 (80.13-227.13)	55.05	0.571
		Last (6/1)	175.76 (105.20-227.13)	30.08	0.571
Non-study Toe	DTL	Baseline (6/8)	38.98 (12.68-262.10)	34.05 (10.68-123.23)	0.662
		Second (6/5)	15.53 (6.98-120.95)	11.83 (11.68-105.38)	0.931
		Last (6/5)	87.20 (18.83-291.13)	11.83 (11.68-15.53)	0.082
	PCA	Baseline (4/3)	34.38 (8.66-62.28)	127.43 (54.73-237.73)	0.299
		Second (5/2)	65.40 (30.03-69.55)	10.85 (6.53-15.18)	0.095
		Last (5/2)	69.55 (35.33-146.28)	15.51 (15.18-15.85)	0.095
Non-study Dorsum	DTL	Baseline (6/8)	10.20 (2.30-55.38)	15.56 (5.91-117.88)	0.491
		Second (5/5)	18.70 (18.15-102.18)	29.60 (12.88-234.58)	0.690
		Last (6/5)	17.05 (12.13-142.95)	13.90 (12.88-215.65)	1.000
	PCA	Baseline (4/3)	91.74 (58.41-125.70)	31.1 (13.23-273.9)	0.629
		Second (5/1)	76.18 (65.08-243.90)	60.08	0.667
		Last (5/2)	161.88 (76.18-265.28)	17.23	0.333

*Independent Samples Mann-Whitney U Test, (n=healed/n=unhealed)

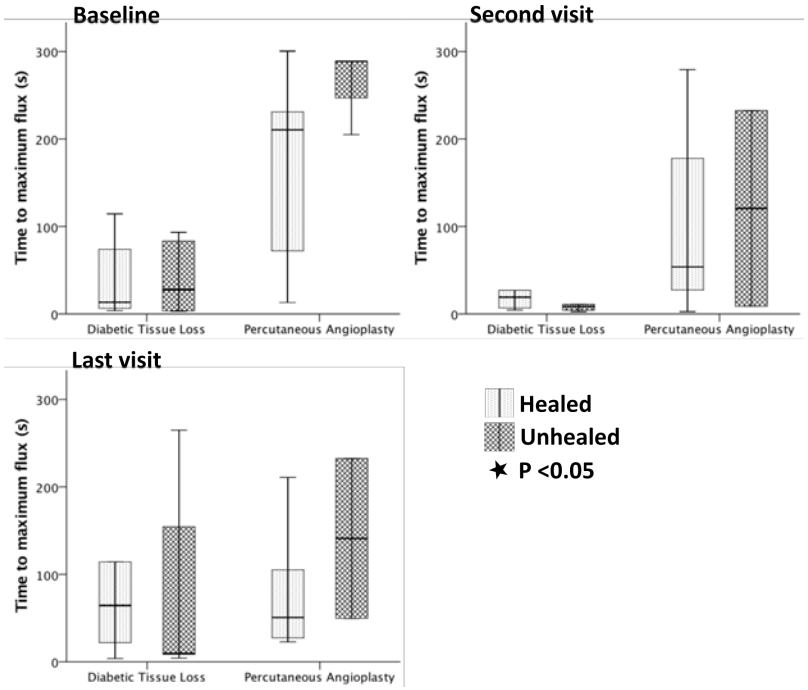

Figure 5.7-9: Healed patients compared to unhealed patients at baseline, second visit and last visit, study toe.

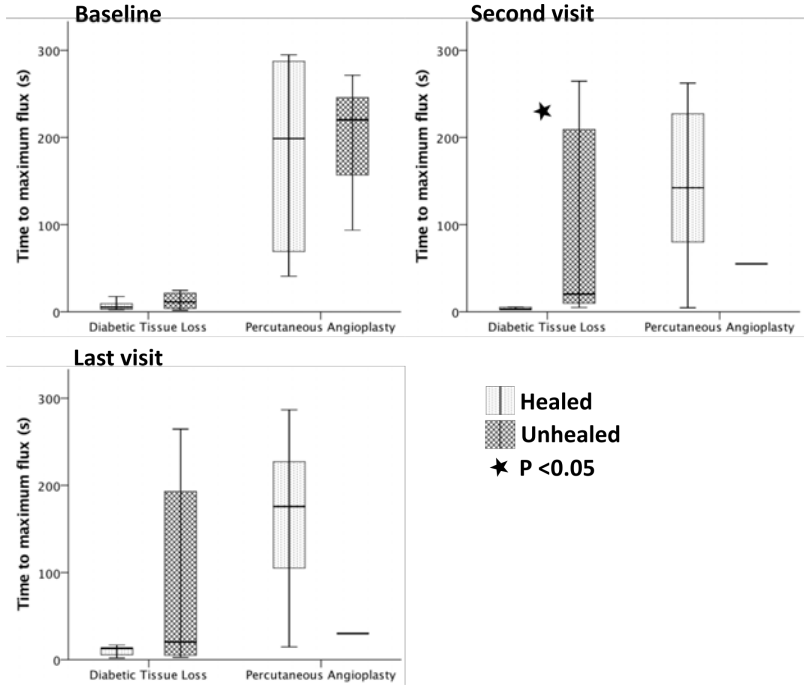

Figure 5.7-10: Healed patients compared to unhealed patients at baseline, second visit and last visit, study dorsum.

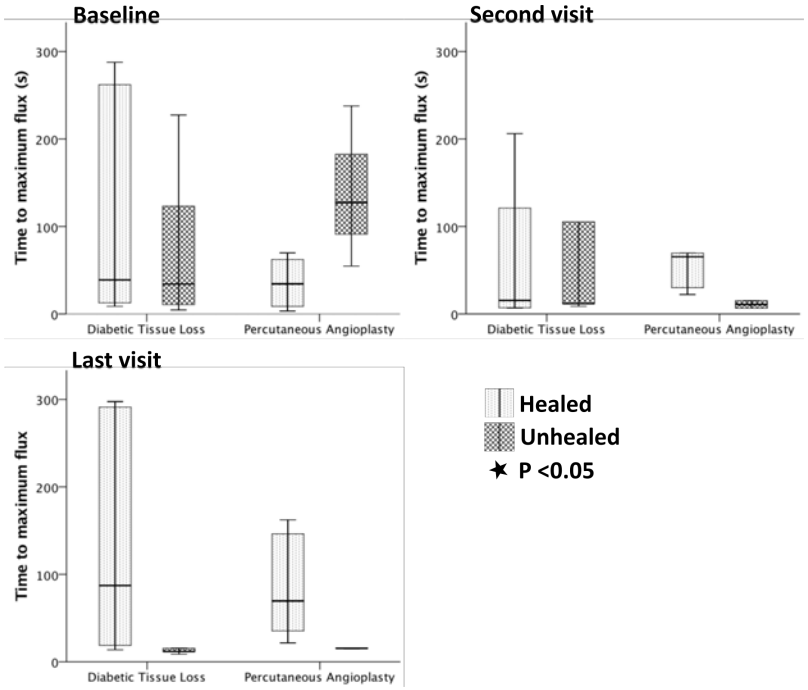

Figure 5.7-11: Healed patients compared to unhealed patients at baseline, second visit and last visit, non-study toe.

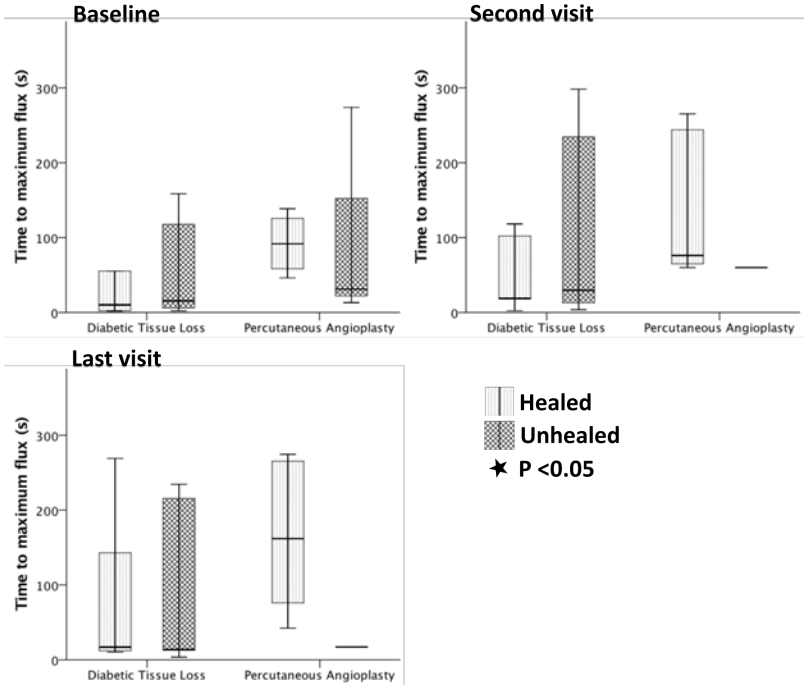

Figure 5.7-12: Healed patients compared to unhealed patients at baseline, second visit and last visit, non-study dorsum.

Table 5.7-12: Time to maximum flux on toe, study leg compared to non-study leg

		Study Leg Median (IQR)	Non-study Leg Median (IQR)	p-value*
DTL	**Baseline (14/14)**	13.40 (3.68-73.85)	38.98 (12.68-141.05)	0.158
	Last (11/11)	27.08 (8.50-154.38)	18.83 (11.83-215.65)	0.656
PCA	**Baseline (9/7)**	220.80 (200.20-288.78)	54.80 (13.95-127.43)	**0.028**
	Last (8/7)	54.50 (34.73-158.00)	35.33 (15.85-146.28)	0.612
*Related-Samples Wilcoxon Signed Rank Test, (n=study leg/n=non-study leg)				

Table 5.7-13: Time to maximum flux on dorsum, study leg compared to non-study leg

		Study Leg Median (IQR)	Non-study Leg Median (IQR)	p-value*
DTL	**Baseline (14/14)**	8.05 (3.45-17.55)	15.40 (3.58-116.90)	0.300
	Last (11/11)	13.50 (4.95-20.38)	15.40 (12.13-215.65)	0.374
PCA	**Baseline (9/7)**	220.28 (93.65-279.80_	70.68 (31.10-138.60)	0.237
	Last (7/6)	127.78 (30.08-227.13)	119.03 (42.43-265.28)	0.753
*Related-Samples Wilcoxon Signed Rank Test, (n=study leg/n=non-study leg)				

5.7.5. Skin Perfusion Pressure

At baseline, on both the study (76.2mmHg (60.2-102.1) vs 46.8mmHg (10.1-52.8), p=0.036) and non-study leg (82.8mmHg (72.2-88.8) vs 37.1 (10.1-57), p=0.007), the PCA group had a significantly lower SPP. The difference remained but was not significant by the last visit (Table 5.7-14)

There no significant difference between the baseline SPP and the second or last visit apart from in three areas. On the non-study leg in the DTL group the SPP was significantly higher for the unhealed patients at both the second (77.6mmHg (63-91.4) vs 91.5mmHg (54.1-103.2), p=0.043) and last visit (77.6mmHg (63-91.4) vs 97.6mmHg (91.5-103.2), p=0.043). On the study leg in the DTL group the healed patients had a significantly lower SPP at the

second visit (98.8mmHg (76.1-143.8) vs 66.7mmHg (33.6-86.5), p=0.046), significance was not reached at the last visit (98.8mmHg (76.1-143.8) vs 71.5mmHg (45.8-98.5), p=0.345) (Table 5.7-15 and Table 5.7-16).

There was no significant difference in SPP between those patients who healed and those who did not. The median SPP for those that healed in the DTL group was 98.8mmHg (76.1-143.8) and those that did not heal 74.6mmHg (52.3-86.6, p=0.138). In the PCA group the value for those that healed was 49.9mmHg (28.55-85.9) and for those that did not heal 28.3mmHg (10.1-46.5, p=0.267) (Table 5.7-17).

There was no significant difference between the study and non-study leg in the DTL group or the PCA group at baseline. In the PCA group, the SPP was significantly higher in the non-study leg at the last visit (Table 5.7-18).

Table 5.7-14: Skin Perfusion Pressure (mmHg) by group

		Diabetic Tissue Loss Median (IQR)	Percutaneous Angioplasty Median (IQR)	p-value*
Study Leg	**Baseline (13/6)**	76.20 (60.20-102.10)	46.80 (10.10-52.80)	**0.036**
	Last (11/6)	82.30 (45.80-99.50)	47.50 (38.70-75.60)	0.122
	% Change (11/4)	-6.37 (-68.15-30.75)	22.29 (-31.59-217.32)	0.571
Non-Study Leg	**Baseline (14/7)**	82.80 (72.20-88.80)	37.10 (10.10-57.00)	**0.007**
	Last (10/5)	94.30 (83.40-99.00)	60.30 (57.90-94.80)	0.594
	% Change (10/4)	13.49 (0.56-36.87)	171.60 (54.24-401.56)	0.054

*Independent-Samples Mann-Whitney U Test, (n=DTL/n=PCA)

Table 5.7-15: Skin Perfusion Pressure (mmHg) by baseline visit compared to second visit

			Baseline value Median (IQR)	Second value Median (IQR)	p-value*
Study Leg	**DTL**	**All (13/11)**	76.20 (60.20-102.10)	82.30 (37.50-95.60)	0.374
		Healed (6/6)	98.80 (76.10-143.80)	66.70 (33.60-86.50)	**0.046**
		Unhealed (7/5)	74.60 (52.03-85.60)	92.00 (82.30-95.60)	0.225
	PCA	**All (6/6)**	46.80 (10.10-52.80)	48.40 (44.00-61.60)	0.715
		Healed (4/6)	49.90 (28.55-85.90)	48.35 (44.00-61.60)	0.715
		Unhealed (2/0)	28.30 (10.10-46.50)	-	-
Non-Study Leg	**DTL**	**All (14/10)**	82.80 (72.20-88.80)	91.50 (60.80-103.20)	0.110
		Healed (6/5)	84.70 (82.00-88.80)	89.70 (83.00-96.30)	0.753
		Unhealed (8/5)	77.60 (63.00-91.40)	91.50 (54.10-103.20)	**0.043**
	PCA	**All (7/5)**	37.10 (10.10-57.00)	60.30 (48.40-64.80)	0.068
		Healed (4/5)	32.30 (18.80-49.70)	60.30 (48.40-64.80)	0.068
		Unhealed (3/0)	50.30 (10.10-57.00)	-	-

*Related-Samples Wilcoxon Signed Rank Test, (n=baseline/n=last value)

Table 5.7-16: Skin Perfusion Pressure (mmHg) by baseline visit compared to last visit

			Baseline value Median (IQR)	Last value Median (IQR)	p-value*
Study Leg	**DTL**	**All (13/11)**	76.20 (60.20-102.10)	82.30 (45.80-99.50)	0.722
		Healed (6/6)	98.80 (76.10-143.80)	71.50 (45.80-98.50)	0.345
		Unhealed (7/5)	74.60 (52.03-85.60)	95.60 (82.30-111.50)	0.500
	PCA	**All (6/6)**	46.80 (10.10-52.80)	47.50 (38.70-75.60)	1.000
		Healed (4/6)	49.90 (28.55-85.90)	47.45 (38.70-75.60)	1.000
		Unhealed (2/0)	28.30 (10.10-46.50)	-	-
Non-Study Leg	**DTL**	**All (14/10)**	82.80 (72.20-88.80)	94.30 (83.4-099.00)	0.169
		Healed (6/5)	84.70 (82.00-88.80)	92.10 (83.40-96.40)	0.686
		Unhealed (8/5)	77.60 (63.00-91.40)	97.60 (91.50-103.20)	**0.043**
	PCA	**All (7/5)**	37.10 (10.10-57.00)	60.30 (57.90-94.80)	0.068
		Healed (4/5)	32.30 (18.80-49.70)	60.30 (57.90-94.80)	0.068
		Unhealed (3/0)	50.30 (10.10-57.00)	-	-

*Related-Samples Wilcoxon Signed Rank Test, (n=baseline/n=last value)

Table 5.7-17: Skin Perfusion Pressure (mmHg) by healed patients compared to unhealed patients

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study Leg	**DTL**	**Baseline (6/7)**	98.80 (76.10-143.80)	74.60 (52.30-85.60)	0.138
		Second (6/5)	66.70 (33.60-86.50)	92.00 (82.30-95.60)	0.247
		Last (6/5)	71.50 (45.80-98.50)	95.60 (82.30-111.50)	0.429
	PCA	**Baseline (4/2)**	49.9 (28.55-85.9)	28.3 (10.1-46.5)	0.267
		Second (6/0)	48.35 (44-61.6)	-	-
		Last (6/0)	47.45 (38.7-75.6)	-	-
Non-Study Leg	**DTL**	**Baseline (6/8)**	84.70 (82.00-88.80)	77.60 (63.00-91.40)	0.414
		Second (6/5)	89.70 (83.00-96.30)	91.50 (54.10-103.20)	0.792
		Last (5/5)	92.10 (83.40-96.40)	97.60 (91.50-103.20)	0.421
	PCA	**Baseline (4/3)**	32.3 (18.8-49.7)	50.3 (10.1-57)	0.857
		Second (5/0)	60.3 (48.4-64.8)	-	-
		Last (5/0)	60.3 (57.9-94.8)	-	-

*Related-Samples Wilcoxon Signed Rank Test, (n=baseline/n=last value)

Table 5.7-18: Skin Perfusion Pressure (mmHg) study leg compared to non-study leg

		Study Leg Median (IQR)	Non-study Leg Median (IQR)	p-value*
DTL	**Baseline (13/14)**	76.20 (60.20-102.10)	82.80 (72.2-88.80)	0.402
	Last (11/10)	82.30 (45.80-99.50)	94.30 (83.40-99.00)	0.285
PCA	**Baseline (6/7)**	46.80 (10.10-52.80)	37.10 (10.10-57.00)	0.715
	Last (6/5)	47.50 (38.70-75.60)	60.30 (57.90-94.80)	**0.028**

*Related-Samples Wilcoxon Signed Rank Test, (n=study leg/n=non-study leg)

5.7.6. Toe Blood Pressure

At baseline the PCA group had significantly lower TBP in both the study leg (92.7mmHg (74.1-133.3) vs 50.3mmHg (31.4-79), p=0.031) and the non-study leg (100.3mmHg (67.5-122.2) vs 40.6mmHg (35.9-41.5), p=0.046). It remained lower at the last visit for both legs, but the difference was no longer significant in the study leg (Table 5.7-19).

There was no significant difference between the baseline and second visit or last visit in either group with one exception. In the PCA group on the non-study leg at the last visit, the median TBP was significantly higher than at baseline (37.1mmHg (10.1-57) vs 60.3mmHg (57.9-94.8), p=0.043) (Table 5.7-20 and Table 5.7-21).

Between healed and unhealed patients no significant difference was found. At baseline in the DTL group those who healed (117.4mmHg (107.2-133.3) had a higher median TBP than those who did not heal (74.8mmHg (64.2-124.2), p=0.142). This pattern was similar at the second visit, but by the last visit it had reversed (89.3mmHg (57.9-125.4) vs 119.9mmHg (96.9-144.3), p=0.556). In the PCA group, the values stayed very similar at all visits (Table 5.7-22).

When the study and non-study leg were compared in the PCA group, the non-study leg had a significantly lower result at the last visit (74.9mmHg (72.6-95.5) vs 59.7mmHg (55.7-70.8), p=0.028) (Table 5.7-23).

Table 5.7-19: Toe Blood Pressure (mmHg) by group

		Diabetic Tissue Loss Median (IQR)	Percutaneous Angioplasty Median (IQR)	p-value*
Study Leg	**Baseline (14/7)**	92.70 (74.10-133.30)	50.30 (31.40-79.00)	**0.031**
	Last (9/6)	112.60 (81.10-127.30)	74.90 (72.60-95.50)	0.388
	% Change (9/5)	5.56 (-25.21-25.88)	25.99 (-23.67-51.29)	0.438
Non-Study Leg	**Baseline (13/5)**	100.30 (67.50-122.20)	40.60 (35.90-41.50)	**0.046**
	Last (11/6)	105.90 (75.80-133.60)	59.70 (55.70-70.80)	**0.027**
	% Change (11/5)	9.33 (-13.56-53.04)	56.55 (24.88-70.60)	0.115

*Independent-Samples Mann-Whitney U Test, (n=DTL/n=PCA)

Table 5.7-20: Toe Blood Pressure (mmHg) by baseline visit compared to second visit

			Baseline value Median (IQR)	Second value Median (IQR)	p-value*
Study Leg	**DTL**	**All (14/10)**	92.70 (74.10-133.30)	104.20 (60.50-151.70)	0.958
		Healed (6/5)	117.40 (107.20-133.30)	132.60 (60.50-151.70)	0.500
		Unhealed (8/5)	74.80 (64.20-124.20)	81.10 (79.00-127.30)	0.893
	PCA	**All (7/6)**	50.30 (31.40-79.00)	76.90 (72.60-81.40)	0.345
		Healed (4/5)	63.10 (42.30-89.00)	73.70 (72.60-80.10)	0.465
		Unhealed (3/1)	31.40 (23.30-79.00)	81.40	0.317
Non-Study Leg	**DTL**	**All (13/11)**	100.30 (67.50-122.20)	104.70 (75.80-127.90)	0.722
		Healed (6/6)	119.80 (93.30-136.60)	114.90 (75.80-127.90)	0.600
		Unhealed (7/5)	99.80 (54.50-110.50)	86.70 (76.50-118.40)	0.500
	PCA	**All (7/6)**	40.60 (35.90-41.50	59.40 (53.40-73.90)	0.345
		Healed (4/5)	38.70 (34.00-65.70)	63.10 (55.70-73.90)	0.465
		Unhealed (3/1)	40.60 (40.60-40.60)	53.40	0.317

*Related-Samples Wilcoxon Signed Rank Test, (n=baseline/n=second value)

Table 5.7-21: Toe Blood Pressure (mmHg) by baseline visit compared to last visit

			Baseline value Median (IQR)	Last value Median (IQR)	p-value*
Study Leg	**DTL**	**All (14/9)**	92.70 (74.10-133.30)	112.60 (81.10-127.30)	0.859
		Healed (6/5)	117.40 (107.20-133.30)	89.30 (57.90-125.40)	0.345
		Unhealed (8/4)	74.80 (64.20-124.20)	119.90 (96.90-144.30)	0.465
	PCA	**All (6/6)**	50.30 (31.40-79.00)	74.90 (72.6-95.5)	0.500
		Healed (4/5)	63.10 (42.30-89.00)	76.10 (73.70-95.50)	0.465
		Unhealed (3/1)	31.40 (23.30-79.00)	60.30	0.317
Non-Study Leg	**DTL**	**All (13/11)**	100.30 (67.50-122.20)	105.90 (75.80-133.60)	0.285
		Healed (6/6)	119.80 (93.30-136.60)	119.80 (75.80-142.70)	0.345
		Unhealed (7/5)	99.80 (54.50-110.50)	86.70 (81.90-118.40)	0.225
	PCA	**All (7/6)**	40.60 (35.90-41.50)	59.70 (55.70-70.80)	**0.043**
		Healed (4/5)	38.70 (34.00-65.70)	63.10 (56.20-70.80)	0.068
		Unhealed (3/1)	40.60 (40.60-40.60)	50.70	0.317

*Related-Samples Wilcoxon Signed Rank Test, (n=baseline/n=last value)

Table 5.7-22: Toe Blood Pressure (mmHg) by healed patients compared to unhealed patients

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study Leg	**DTL**	**Baseline (6/8)**	117.40 (107.20-133.30)	74.80 (64.20-124.20)	0.142
		Second (5/5)	132.60 (60.50-151.70)	81.10 (79.00-127.30)	0.841
		Last (5/4)	89.30 (57.90-125.40)	119.90 (96.90-144.30)	0.556
	PCA	**Baseline (4/3)**	63.10 (42.30-89.00)	31.40 (23.30-79.00)	0.400
		Second (5/1)	73.70 (72.60-80.10)	81.40	0.667
		Last (5/1)	76.10 (73.70-95.50)	60.30	0.333
Non-Study Leg	**DTL**	**Baseline (6/7)**	119.80 (93.30-136.60)	99.80 (54.50-110.50)	0.295
		Second (6/5)	114.90 (75.80-127.90)	86.70 (76.50-118.40)	1.000
		Last (6/5)	119.80 (75.80-142.70)	86.70 (81.90-118.40)	0.537
	PCA	**Baseline (4/1)**	38.70 (34.00-65.70)	40.60	1.000
		Second (5/1)	63.10 (55.70-73.90)	53.40	0.667
		Last (5/1)	63.10 (56.20-70.80)	50.70	0.333

*Related-Samples Wilcoxon Signed Rank Test, (n=healed/n=unhealed)

Table 5.7-23: Toe Blood Pressure (mmHg) study leg compared to non-study leg

		Study Leg Median (IQR)	Non-study Leg Median (IQR)	p-value*
DTL	**Baseline (14/13)**	92.70 (74.10-133.30)	100.30 (67.50-122.20)	0.422
	Last (9/11)	112.60 (81.10-127.30)	105.90 (75.80-133.60)	0.859
PCA	**Baseline (7/5)**	50.30 (31.40-79.00)	40.60 (35.90-41.50)	0.345
	Last (6/6)	74.90 (72.60-95.50)	59.70 (55.70-70.80)	**0.028**

*Related-Samples Wilcoxon Signed Rank Test, (n=study leg/n=non-study leg)

5.7.7. Neuropathy

5.7.7.1. Vibration Perception Threshold

There was no significant difference in VPT between the groups at baseline or the last visit, the median VPT in the DTL group at baseline was 31.13V (29-38.5) compared to 42.25V (31.5-45) in the PCA group (p=0.141). The binary analysis demonstrated the large majority of patients in both groups had an abnormal VPT, 78.6% at baseline in the DTL group and 88.9% in the PCA group (p=0.237). The levels were similar at the last visit (DTL 90.9% and PCA 85.7%, p=0.237) and on the non-study leg (baseline 71.4% vs 87.5%, p=0.380 and last 81.8% vs 83.3%, p=0.728). Whether the patient went on to heal or not, there was no significant difference between the baseline and the last VPT (Table 5.7-24).

Between the study and the non-study leg, there was no significant difference in the results. The median VPT for the study leg at baseline in the DTL group was 31.13V (29.00-38.5) and 30.63V (23.25-37.5, p=0.196), in the non-study leg. In the PCA group, the baseline results were 42.25V (31.5-45) for the study leg and 34V (31.08-49, p=0.735) for the non-study. The median VPT for DTL patients at baseline who healed was 30.38V (29-35.5) and for

those that did not heal 32V (24.38-43.88, p=0.662). In the PCA group the results were 44.25V (31.5-46) and 41.5V (25.5-42.25, p=0.381) respectively. There was also no significant difference in the proportion of patients with abnormal VPT results in the healed and unhealed patients.

Table 5.7-24: Vibration Perception Threshold (V) healed patients compared to unhealed patients

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study Leg	**DTL**	**Baseline (6/8)**	30.38 (29.00-35.50)	32.00 (24.38-43.88)	0.662
		Last (6/5)	33.25 (30.75-33.785)	29.50 (28.00-37.25)	1.000
	PCA	**Baseline (6/3)**	44.25 (31.50-46.00)	41.50 (25.50-42.25)	0.381
		Last (6/1)	42.50 (34.50-49.00)	39.25	1.000
Non-Study Leg	**DTL**	**Baseline (6/8)**	24.13 (15.25-27.75)	37.25 (30.13-38.13)	**0.043**
		Last (6/5)	31.00 (26.75-34.00)	36.25 (26.80-38.00)	0.537
	PCA	**Baseline (5/3)**	49.00 (30.90-49.00)	32.00 (31.25-36.00)	0.786
		Last (5/1)	42.25 (38.25-49.00)	27.50	0.667

*Independent-Samples Mann-Whitney U Test, (n=healed/n=unhealed)

5.7.7.2. Neuropathy Total Symptom Score-6

The median NTSS-6 at baseline was 4.49 (3-8.99) in the DTL group and 2.33 (2-6.99, p=0.277) in the PCA group. At the last visit, the results were 3.33 (0-7.33) and 1.83 (0-8.66, p=0.840) respectively. At baseline five patients (35.7%) in the DTL group had a score of more than six (representing clinically significant symptoms) and three patients (33.3%) in the PCA group. By their last visit, this number had reduced to three (21.4%) in the DTL group and remained three (33.3%) in the PCA group (p=1.000). At both visits the differences between the groups were not statistically significant.

When the baseline NTSS-6 was compared to the last result, in the DTL group, those who healed had a significantly lower score at the last visit (3.66 (1-10.32) vs 0.00 (0-3.33), p=0.043). Those who did not heal had a higher score at the last visit although this did not reach significance (4.83 (3.83-7.99) vs 5.66 (3.66-10.32), p=0.458). In the PCA group, the results for the last visit (1.83 (0.00-8.66)) were lower than at baseline (4.50 (0.00-6.99)) but did not reach significance (p=0.500). In the DTL group 71% of patients who had a normal baseline result also had a normal last result, in the PCA group this was true of 60%.

In the DTL group, the NTSS-6 results for the unhealed patients were lower than the healed patients, although the results did not reach significance. In the PCA group the NTSS-6 for the unhealed patients was lower but, as in the DTL group did not reach significance (Table 5.7-25).

Table 5.7-25:Neuropathy Total Symptom Score-6 Healed compared to Unhealed

		Healed Median (IQR)	Unhealed Median (IQR)	p-value*
DTL	**Baseline (6/8)**	3.66 (1.00-10.32)	4.83 (3.83-7.99)	0.662
	Last (6/5)	0.00 (0.00-3.33)	5.66 (3.66-10.32)	0.126
PCA	**Baseline (6/3)**	4.50 (0.00-6.99)	2.33 (2.00-3.00)	1.000
	Last (6/2)	1.83 (0.00-10.33)	3.50 (0.00-6.99)	1.000
*Independent-Samples Mann-Whitney U Test, (n=healed/n=unhealed)				

5.7.7.3. 10g Monofilament

The results of the 10g monofilament testing were analysed as abnormal, unable to detect at least one point, compared to normal. There was significant neuropathy present with 50% of patients in the DTL group and 55% in the PCA group being unable to detect any points at baseline. There were no significant differences between the groups at baseline or at last visit on the study leg (Table 5.7-26).

There was no significant difference in the proportion of patients with abnormal results when the baseline result was compared to the last result. On the study leg, DTL group, 90% of patients who had an abnormal result at baseline also had an abnormal result at the last visit. In the PCA group, this proportion was 86% (p=1.000 for both groups). There was no significant difference between the study and non-study leg by group.

Table 5.7-26: Monofilament on study leg by group, baseline and last visit

			Group		p-value*
			Diabetic Tissue Loss (%)	Percutaneous Angioplasty (%)	
Baseline	**Correct responses**	**Abnormal**	13 (92.9)	8 (88.9)	1.000
		Normal	1 (7.1)	1 (11.1)	
Last	**Correct responses**	**Abnormal**	9 (81.8)	6 (75.0)	1.000
		Normal	2 (18.2)	2 (25.0)	

*Fishers Exact Test, Abnormal = ≥1 incorrect responses

5.7.7.4. Ipswich touch test

The results of the Ipswich touch test were treated in the same way as the monofilament with abnormal being defined as unable to detect at least one point. Similarly to the monofilament, the results suggested that there was significant neuropathy present. A lower proportion of patients, compared to the monofilament, were unable to detect any points at baseline, 29% in the DTL group and 33% in the PCA group. There were no significant differences between the groups at baseline or last visit on the study leg (Table 5.7-27).

There was no significant difference in the proportion of patients with abnormal results when the baseline result was compared to the last result. In both groups, 75% of patients who had an abnormal result at baseline also did at the last visit (p=1.000). This was similar for both the study leg and the non-study leg.

Table 5.7-27: Binary Ipswich Touch Test correct responses on study leg by group baseline and last visit

			Group		p-value*
			Diabetic Tissue Loss (%)	Percutaneous Angioplasty (%)	
Baseline	**Correct responses**	**Abnormal**	10 (71.4)	7 (77.8)	1.000
		Normal	4 (28.6)	2 (22.2)	
Last	**Correct responses**	**Abnormal**	9 (81.8)	5 (71.4)	1.000
		Normal	2 (18.2)	2 (28.6)	

*Fishers Exact Test, Abnormal = ≥1 incorrect responses

5.7.8. Outcomes

5.7.8.1. Procedures

In the PCA group seven of the procedures reported immediate technical success, Of the two patients who did not have immediate technical success, one patient was found to have good in-line flow to the foot and so no angioplasty was performed, the other underwent thrombolysis after a superficial femoral artery (SFA) occlusion was crossed and immediately occluded. After twenty-four hours thrombolysis and an SFA stent, in-line flow to the foot was achieved. Only two patients did not have in-line flow by the end of the procedure, they both had patent peroneal run-off.

One patient in each study group underwent a major amputation, there were no minor amputations or deaths in the study period.

5.7.8.2. Wound healing

Forty-three percent of patients in the DTL group healed by the end of the study and 56% of the PCA group (p=0.836). The median ulcer area at baseline in the DTL group was 343mm^2 (80-782) and 270mm^2 (180-1750, p=1.000) in the PCA group. There was no significant difference in the ulcer area between groups at either the second or final visit (Figure 5.7-13).

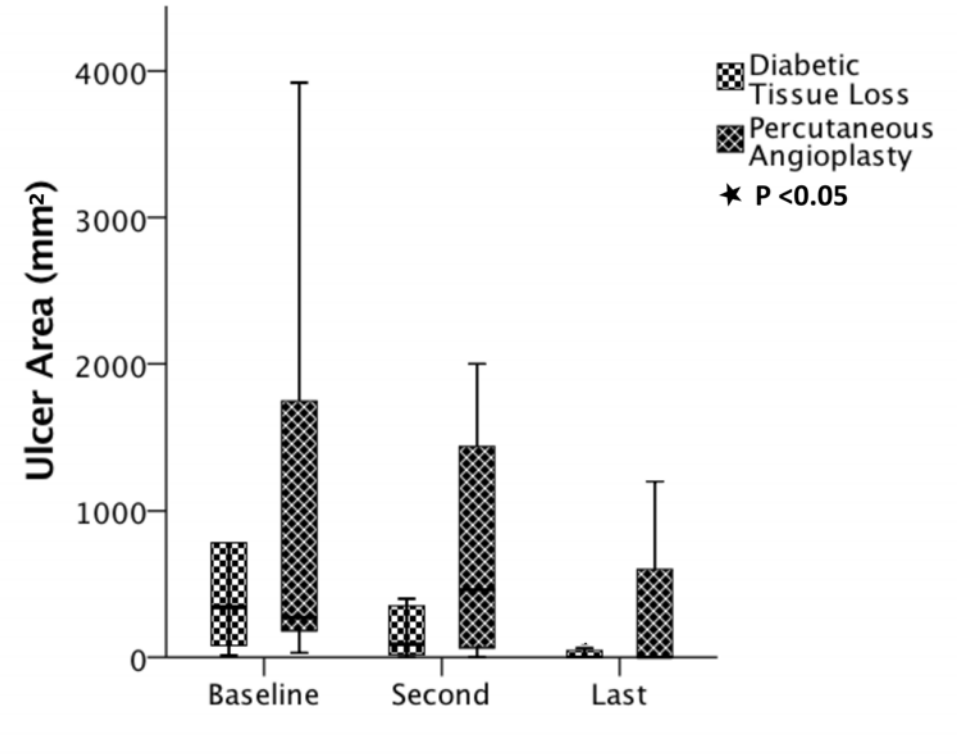

Figure 5.7-13: Ulcer area (mm^2) by group at baseline, second visit and last visit.

In the DTL group between the first and second visit there was a significant reduction in ulcer area (p=0.003) but not in the PCA group (p=1.000). By the last visit, there was a significant difference in both groups (Figure 5.7-14).

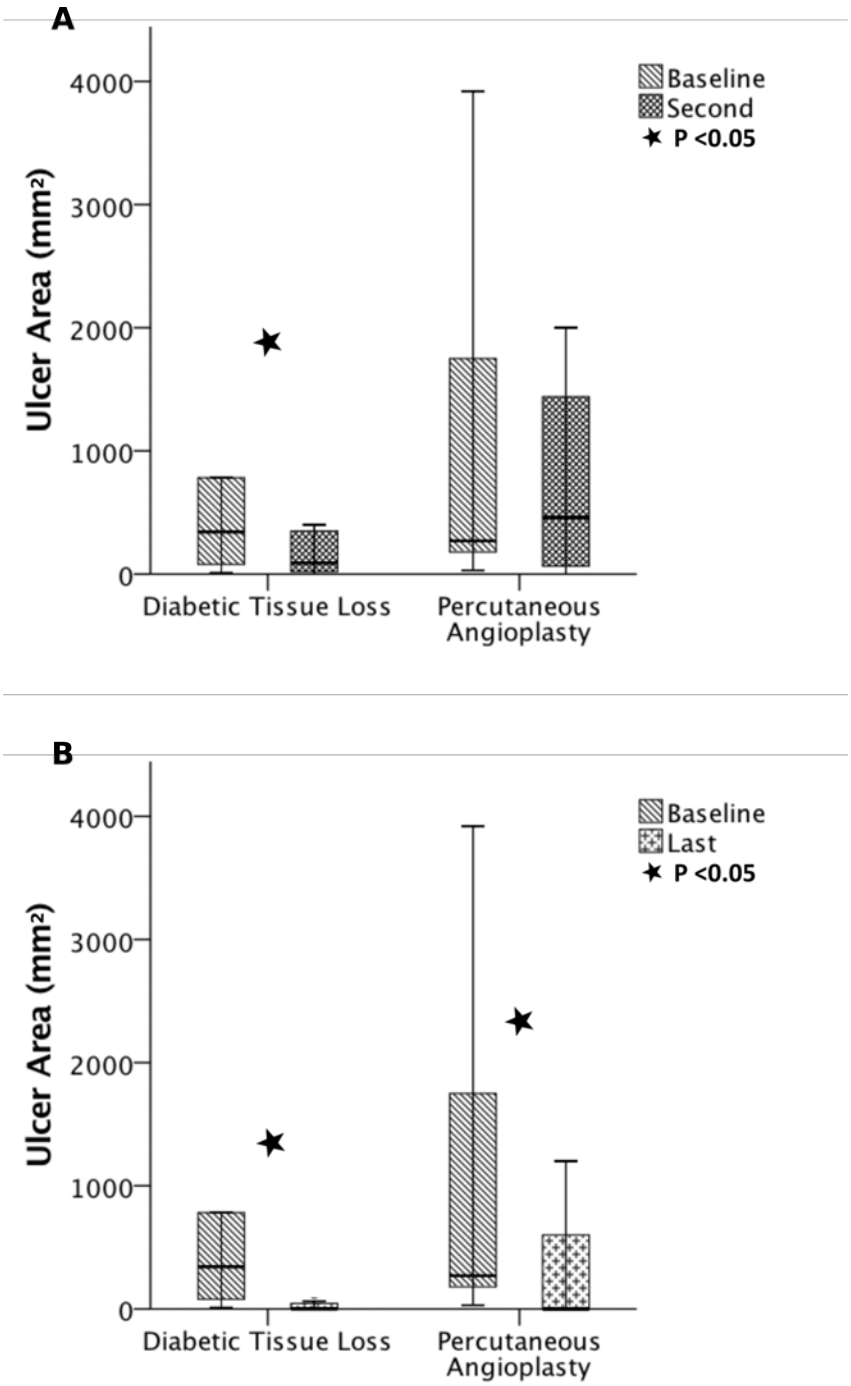

Figure 5.7-14: Ulcer area by group. A) Baseline compared to second visit. B) Baseline compared to last visit.

At baseline in the DTL group there was no significant difference in the ulcer area of those patients who eventually healed (343mm^2 (234-748)) compared to those who did not (340mm^2 (50-3384), p=0.755). Those in the PCA group who healed had significantly smaller ulcers at baseline (180mm^2 (150-270)) than those who did not heal (3920mm^2 (1750-4950), p=0.024) (Figure 5.7-15).

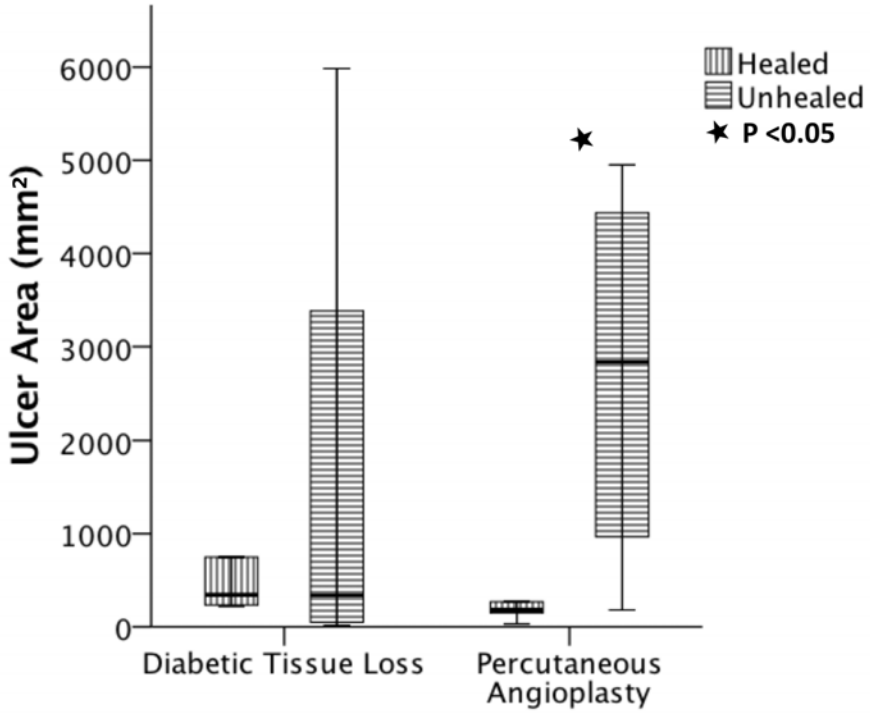

Figure 5.7-15: Ulcer area by group at baseline, patients who went on to heal compared to those who did not.

CHAPTER 6: DISCUSSION

In this chapter the results of the preceding studies are summarised and considered in the context of the literature. Avenues for future study are considered.

6.1. DISTRIBUTION OF ARTERIAL DISEASE IN DIABETES MELLITUS

The distribution of macrovascular disease in patients with diabetes mellitus (DM) was examined by applying a semi-quantitative scoring system to lower limb angiograms. In total 437 angiograms were examined, 222 in patients with diabetes and 215 in patients without diabetes.

6.1.1. Inter-observer reliability of the Bollinger score

Assessment of the reliability of the Bollinger data demonstrated good correlation between assessors with good internal consistency. The intra-observer reliability also demonstrated good correlation and internal consistency (Sections 3.4.1 and 3.4.2).

6.1.2. Primary Outcome: Difference between median Bollinger score in each arterial segment in patients with DM compared to patients without DM.

When the results were separated by indication for procedure the only vessels that had a significantly different burden of disease were the medial and lateral plantar vessels in patients with critical ischaemia (Table 3.8-4). Patients without DM had a higher burden of disease in these vessels.

Overall those with DM had a higher burden of disease throughout the infra-inguinal arterial tree (median total Bollinger score 88 vs 42) this difference was not significant (p=0.061).

6.1.3. Secondary outcome: Difference in short to medium outcomes

Those with DM had a significantly higher risk of major and minor amputation and death at one year. There was no significant difference in the rates of further revascularisation procedure (section 3.8.3).

6.2. WHAT IS THE RELATIONSHIP OF REVASCULARISATION AND IMPROVEMENT IN MICROCIRCULATION TO WOUND HEALING AND PERIPHERAL NEUROPATHY IN DIABETIC FOOT DISEASE? AN OBSERVATIONAL COHORT STUDY.

Despite the small recruitment numbers, it was possible to address some of the outcome measures of the cohort study. Regarding the demographics, the patients in the percutaneous angioplasty group (PCA) were significantly older than the diabetic tissue loss group (DTL). There was also significantly longer between visits in the PCA group. At the third visit, there appears to be an increase in the ulcer area (Table 5.7-7). This is more a reflection of those with larger ulcers continuing in the study longer than an actual increase in ulcer size, as Figure 6.2-1 demonstrates all but a few patients had an incremental decrease in their ulcer area. The three patients that had increased at the second visit were all in the PCA group.

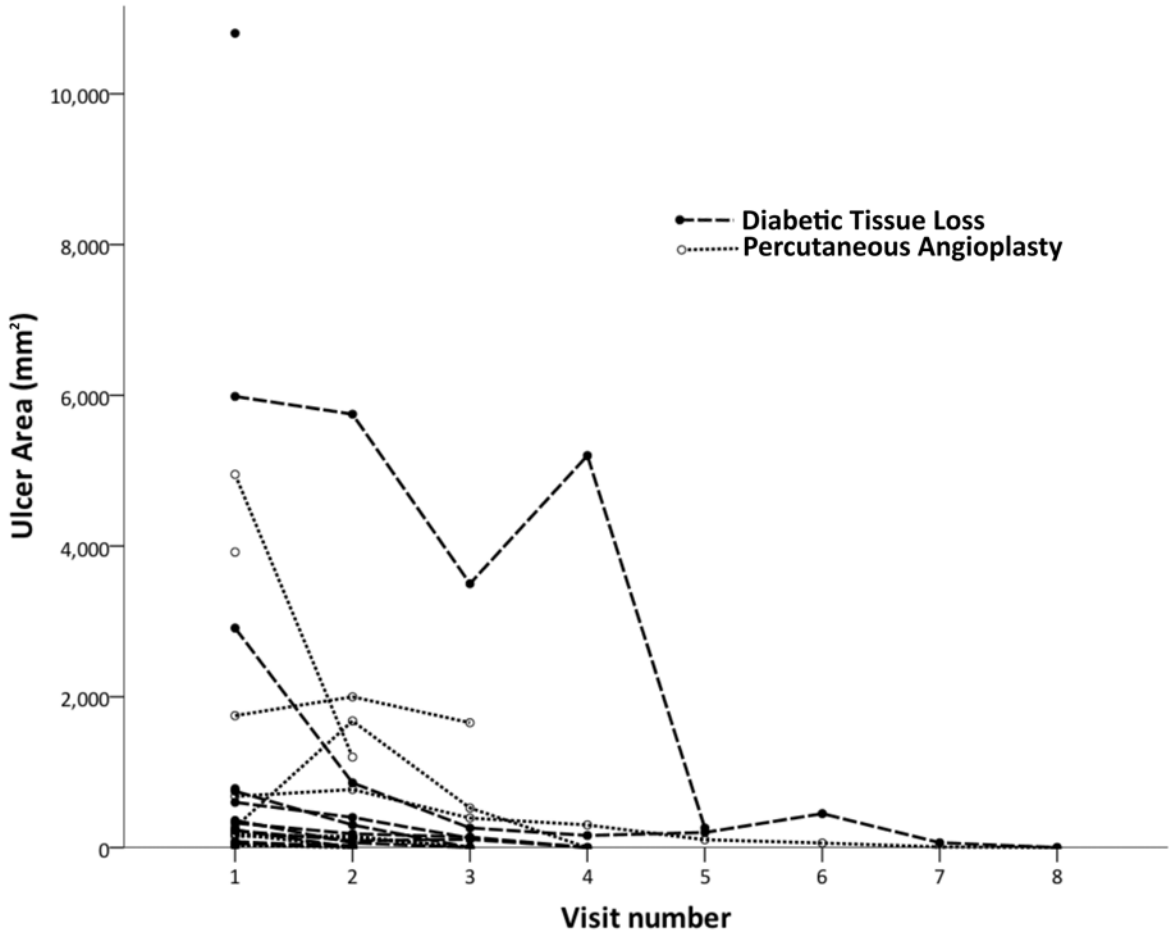

Figure 6.2-1: Ulcer area by visit for individual study participants

6.2.1. Primary Outcome: Evidence of difference in the level of change in the time to maximum flux between before and after PCA.

Despite the limitations of the study there was the suggestion that improving the macrocirculation, by way of PCA lead to an improvement in the microcirculation. On both the study toe and dorsum there was a reduction in the time to maximum flux (TtM) following angiography. By the last visit for the healed group on the study toe TtM had significantly reduced from 210.5s (72.18-231) to 50.71s (27.38-105.18, p=0.046). At baseline, those in the PCA group had a significantly longer TtM than those in the DTL group. By the

final visit, at the toe, there was no significant difference between to two groups, and there had been a large decrease in the TtM in the PCA group. On the dorsum, there did remain a significant difference between the groups at the last visit, but the TtM had also decreased (Table 5.7-8 and Table 5.7-9). This was also supported by the change in TtM when the study leg was compared to the non-study leg. At baseline on the toe in the PCA group, the TtM was significantly longer on the study leg; there was no significant difference by the last visit. On the dorsum, the trend was similar although statistical significance was not reached (Table 5.7-12 and Table 5.7-13). Combined these results suggest that PCA improves TtM to a level that is comparable to that of the DTL group. In the DTL group, the trends between baseline and last were in the opposite direction, of a smaller magnitude however the changes were statistically significant. Between the study leg and non-study leg, the trends were also reversed compared to the PCA group but statistical significance was not reached. A possible explanation for this is a reduction in inflammation related to having an active wound leading to a less pronounced hyperaemic response.

6.2.2. Secondary Outcomes

6.2.2.1. Evidence of difference in the level of change in the time to maximum flux between when ulceration active and when ulceration healed in patients with DM and no significant peripheral arterial disease.

By the end of the study, six patients in the DTL group had healed. Of these patients there had been a significant increase in the TtM by the last visit (13.4s vs 64.43s p=0.028) on the study toe and a non-significant increase on the study dorsum (5.23s vs 13.15s p=0.345)

(Table 5.7-9). Using TtM, it was not possible to identify patients at baseline who were more likely to heal; there was no significant difference in those who healed compared to those who did not. On both the study toe and dorsum, there was a trend towards a longer TtM in the unhealed patients at baseline (Table 5.7-11).

6.2.2.2. Time to wound healing

Six patients in each group reached the end point of a healed ulcer. The median time to healing in days was 118 (63-207) in the DTL group and 147 (93-162), p=0.937) in the PCA group. The PCA group appeared to be slightly slower to start healing than the DTL group as between the first and second visit there was no significant difference in ulcer size whereas there was a significant reduction by the last visit. In the DTL group, there was a significant reduction in size at both the second and last visits (Section 5.7.8).

6.2.2.3. Time to major amputation

Two patients underwent major amputation during the study period. The patient in the DTL group underwent the procedure thirty days following recruitment, prior to their second measurement for the study. The patient in the PCA group had one follow-up assessment and underwent the procedure at seventy-seven days.

6.2.2.4. The change in skin perfusion pressure (SPP) and toe blood pressure (TBP) at the end of the study

While SPP was significantly lower at baseline in the PCA group there was minimal change between the baseline and last values; this pattern was the same for TBP (Sections 5.7.5 and

5.7.6). There were no consistent trends between the groups or visits for both the SPP and TBP with similar levels of difference being seen on both the study and non-study leg.

6.2.2.5. Change in neuropathy measures at the end of the study

None of the tests used to assess neuropathy demonstrated any significant difference between the groups or visits. There were also no consistent trends within the numbers. The only comparison with a significant difference was in the Neuropathy Total Symptom Score – 6 (NTSS-6) results. The patients who healed in the DTL group had a significantly lower result at their last visit compared to baseline. However, when the results for those who healed were compared to those who did not heal there was no significant difference. Throughout the tests there was also no significant difference between the study leg and non-study leg (Section 5.7.7).

6.2.3. Overall Hypothesis: Improving the microcirculation of the foot in patients with diabetic foot disease improves wound healing and degree of peripheral neuropathy.

Due to the low numbers recruited it is not possible to accept or reject the overall hypothesis. When considering the questions asked in Section 5.3.1 the outcomes are as follows;

- Which of PCA or peripheral bypass surgery (PBS) provides a greater improvement in the microcirculation?
    - This will require further study with more recruits due to too few patients, in particular in the PBS group, being recruited

- What is the level of improvement in the microcirculation at the time of wound healing in patients with DM and PAD?
    - In the PCA group the patients that healed showed an improvement in post-occlusive reactive hyperaemia following intervention. There was also an improvement in the unhealed patients but to a lesser degree.
- Is there any improvement in the neurological status of patients with neuro-ischaemic ulceration who have undergone revascularisation?
    - This data set was unable to demonstrate any difference in neuropathy following PCA.

6.3. LIMITATIONS

6.3.1. Distribution of disease

The examination of the distribution of disease was based on a large prospectively maintained dataset. Following the pilot study, adjustments were made to the design including fine-tuning the arterial segments examined and collecting data on outcomes. The decision was also made to match the control and study groups for risk factors for peripheral arterial disease. This was done to control for these risk factors and also partly to rationalise the cohort size to a volume that was manageable for analysis by one investigator. A potential problem with closely matching cohorts is that they may no longer represent the general population. The demographics of the matched cohorts were compared to the raw dataset to examine this as a potential weakness. There was no significant difference in age in the DM group (matched 70.31 years $\pm$9.17 vs raw dataset 69.74$\pm$11.12, p=0.552) or in the no

diabetes mellitus group (NDM) (70.33±9.4 vs 69.26±13.7, p=0.256). A chi-squared goodness-of-fit test was performed to compare the proportions of the categorical demographics in the matched cohort to the raw dataset. When comparing the matched cohort, which contained no unknown data, to the raw dataset, which includes some unknown data, the unknown group was excluded. The summary of the areas of significant difference is presented in Table 6.3-1. The full breakdown of observed values, expected values and residuals can be found in Appendix VIII. Smoking was the only demographic that had significantly different proportions in both groups. The NDM group had more areas of significant difference than the DM group (four compared to three). This suggests that the NDM group had been more altered from the general population than the DM group.

Table 6.3-1: Significance results for comparisons of demographic proportions in the matched cohort compared to the raw dataset.

	p-value*	
	Diabetes mellitus	**No Diabetes mellitus**
Sex	0.326	**0.013**
Ethnicity	**<0.001**	0.300
Smoking	**0.003**	**0.001**
Hypertension	0.356	**<0.001**
High Cholesterol	0.407	0.181
Renal Function	**<0.001**	0.080
Timing of procedure	0.110	**0.001**

*Chi^2 Goodness-of-Fit Test

6.3.2. Cohort study

The study was designed to prospectively collect data on how the microcirculation around foot ulcers changed during their lifespan. The main technique aimed at achieving this was to perform repeated measures on the same ulcer. The majority of patients had two or more follow-up measurements after their initial assessment. Unfortunately, there were significant

problems with recruitment and only 22.5% of the number proposed in the power calculation (27/120) were recruited. This was particularly true in the peripheral bypass group where only two patients out of the proposed 48 were recruited (4.2%). In the PCA group 18.8% (9/48) were recruited and 58.3% (14/24) in the DTL group. Much of the problem with recruitment revolved around difficulties in identifying patients without DM as described in Section 1.1.1.1.

Of the twenty-six patients recruited only fifteen patients reached a defined endpoint during the period for recruitment and follow-up. Thirteen patients (50%) healed and two patients had a major amputation (7.7%). Six patients were lost to follow-up (23%), three requested to leave the study (11.5%), and two had continuing ulceration at the end of the study (7.7%). Of the three that requested to leave the study, one was an elderly patient who found the testing process exhausting and so did not wish to continue, one found the post occlusive reactive hyperaemia (PORH) painful at the baseline tests and the third had a family member become ill and they did not feel they had the time to continue in the study.

There was a wide timescale for the duration of the ulcer before recruitment of less than one month up to eighty-four months, in the DTL group the median duration was two months (1-13) and PCA group five months (3-7, p=0.336). A more consistent approach would have been only to recruit those attending for their first clinic appointment for the ulcer in question. Published data on time to first assessment in a specialist clinic suggests that this would have reduced the prior duration. In the most recent publication of the National Diabetes Foot Care Audit 71.9% of patients had their first assessment in thirteen days or less or self-referred (period of time not recorded but suggested to be shorter than other pathways) and only 8.6% waited over 2 months[264].

6.4. CONTEXT OF CURRENT EVIDENCE

6.4.1. Distribution of disease

The results of examination of the distribution disease generally agree with the results of the review presented in Chapter 2. Patients with DM had more disease overall compared to those without and this was particularly true below the knee. When our results were compared to the other two studies that had previously used the Bollinger score[83,90] the median scores were generally higher than ours. This was true of both study groups. Of the studies that separated out the infrageniculate vessels both Jude *et al.*[83] and Diehm *et al.* (2008)[90], using the Bollinger score, found lower scores in the peroneal artery (PEA) in both the control and the DM group. However, Menzoian *et al.*[159] and Ciavarella *et al.*[157], using an alternative scoring system and presence of occlusion respectively, were unable to demonstrate this.

It would seem that the Bollinger score is a more sensitive method of detecting the differences in degree of disease between vessels. An aim of our study was to further explore the differences between each arterial segment, which included dividing each of the tibial vessels into three segments. These segments demonstrated that, when all patients were considered, the only vessels in which there was significant difference across the segments were the anterior tibial artery (ATA) and PEA in patients with claudication. There was a non-significant trend towards worse disease distally (Table 6.4-1). There were, however, no significant differences between the DM group and the control group in the individual tibial segments (Section 3.8.2). For this reason, it can be argued that the division of the crural vessels into thirds has only added limited information. In Bollinger's original description of his scoring system, only the first 3cm of the ATA and 5cm of the PEA and posterior tibial

artery (PTA) were included. The results from these segments were significantly lower than the scores for the whole vessel (Table 6.4-2). This shows that extending the segments gives a fuller picture of the extent of disease present.

Table 6.4-1: Comparison of Bollinger score of proximal, middle and distal tibial arterial segments for all patients

		Proximal segment		Middle segment		Distal segment		p-value*
		n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	
Asymptomatic	**ATA**	23	4 (0-15)	23	4 (0-15)	22	13 (0-15)	0.201
	PEA	23	0 (0-2)	23	0 (0-3)	22	0 (0-3)	0.961
	PTA	23	2 (0-15)	23	4 (0-15)	22	11 (0-15)	0.832
Claudication	**ATA**	148	2 (0-6)	139	0 (0-13)	140	1 (0-15)	**0.005**
	PEA	148	0 (0-3)	140	0 (0-13)	138	0 (0-13)	**0.002**
	PTA	148	0 (0-4)	139	0 (0-4)	140	0 (0-7)	0.082
Critical Ischaemia	**ATA**	133	6 (0-13)	131	3 (0-15)	130	13 (0-15)	0.407
	PEA	132	3 (0-13)	131	1 (0-15)	130	0 (0-15)	0.598
	PTA	133	10 (1-15)	131	13 (0-15)	129	15 (0-15)	0.528

*Related samples Friedman's two-way analysis of variance by ranks, ATA Anterior tibial artery, PEA Peroneal artery, PTA Posterior tibial artery

Table 6.4-2: Comparison of Bollinger score for the originally described tibial segment to the score for the whole vessel

		Original segment		Score for whole vessel		p-value*
		n	Median (IQR)	n	Median (IQR)	
Asymptomatic	**Anterior tibial**	23	2 (0-13)	22	13 (0-15)	**0.011**
	Peroneal	23	0 (0-0)	22	0 (0-3)	0.077
	Posterior tibial	23	3 (0-15)	22	13 (0-15)	0.078
Claudication	**Anterior tibial**	148	0 (0-3)	140	5 (0-15)	**<0.001**
	Peroneal	148	0 (0-1)	140	2 (0-13)	**<0.001**
	Posterior tibial	148	0 (0-3)	143	0 (0-13)	**<0.001**
Critical Ischaemia	**Anterior tibial**	133	3 (0-7)	130	13 (2-15)	**<0.001**
	Peroneal	132	1 (0-13)	131	3 (0-15)	**<0.001**
	Posterior tibial	133	4 (0-15)	132	14 (2-15)	**<0.001**

*Mann-Whitney U Test

Our results suggested that patients without DM and presenting with critical ischaemia had more disease in the pedal vessels than patients with DM. The trend was also in this direction in the claudication group but not in the asymptomatic group (Section 3.8.2). In the literature, this has been an inconsistent finding. Conrad in 1967 examined casts of the vascular tree from amputated limbs. In this cohort of twenty patients, the NDM group had more pedal disease. The groups from this study are a bit unusual in that almost all of the NDM group presented with acute symptoms whereas the DM group had chronic presentations[169]. Ciavarella *et al* also found more plantar disease in the DM group in their 1993 study that examined patients with symptomatic PAD[157]. Menzoian *et al* in 1989 using LaMorte *et al's* scoring system (described in Section 2.3.2) found no significant difference between the groups[159,161]. Diehm *et al* in 2008 or Haine *et al* in 2018 were not able to demonstrate a significant difference between patients with and without DM either[90,171]. The trend in both studies was for patients with DM to have a higher burden of disease than patients without[90,171]. Both Haine and Diehm separated patients with DM from patients with chronic kidney disease (CKD) and found that these patients had a higher burden of pedal disease than patients with DM or patients with PAD and no DM[90,171]. In Ciavarella's cohort 61% of DM patients also had CKD, none of the patients in the NDM cohort did[157]. Conrad and Menzoian do not record the proportions of patients with CKD[169]. The assumption would be that a proportion of these patients would have had CKD and this may have influenced the results. From this evidence, it is not possible to conclude that there is any significant difference in the incidence of pedal disease between patients with and without DM. It has been suggested that patients with crural disease related to DM will have relative sparing of the pedal vessels. Graziani *et al* in 2007, in a cohort all with DM and tissue loss secondary to

ischaemia, found that of the 118 patients, whose crural vessels were all occluded, 88% had at least one patent pedal vessel[265]. However, Diehm, in a much smaller cohort (n=25), found that the DM patients had more disease in the foot compared to the calf. The presence, or not, of pedal disease is important as when there is significant crural disease the pedal vessels can be a potential target for bypass and, as endovascular techniques are improving, are a potential target or retrograde conduit for endovascular therapy[266-271]. In addition to that patients with pedal disease have been found to have higher rates of amputation and mortality and it is an independent predictor of failure of revascularisation[269].

Other studies have considered the outcomes of patients with DM following PCA or stenting. Many have not demonstrated any significant difference in the outcomes between patients with DM and those without[158,168,272-276]. With the exception of Melliere *et al*[275], these are all studies with small numbers. There are however studies that have found similar results to us, many of these also have small numbers[83,277-280]. However a recently published study[281], which included 714 patients (58.5% with DM) who underwent PCA for popliteal or infrapopliteal disease, found on survival analysis that patients with DM had poorer outcomes for major adverse events ($p<0.001$), all-cause mortality ($p=0.001$), major amputation ($p=0.001$) and further revascularisation ($p=0.03$). On multivariate analysis, DM remained associated with significantly higher rates of major adverse events, all-cause mortality and major amputation (hazard ratio (HR) 1.73 (95% confidence interval 1.35-2.23), 1.83 (1.33-2.52) and 5.52 (1.82-16.71) respectively). However further revascularisation did not have a statistically significant association (HR 1.35, 95% CI 0.98-1.87). Darling *et al.* separately analysed patients with insulin dependent diabetes (IDDM) from those who were non-insulin dependent (NIDDM)[282]. They found that the IDDM group (342 patients) had a higher risk of

incomplete wound healing (HR 1.4 95% CI 1.1-2.6), major amputation (HR 4.1, 95% CI 1.3-12.6) and RAS (a combination of re-intervention, major amputation and restenosis, HR 1.5, 95% CI 1.1-2.2). Compared to the NDM group (171 patients) there was no increased risk associated with NIDDM (133 patients), in fact it appeared to be associated with lower risk of mortality (HR 0.7, 95% CI 0.4-0.9). Abularrage *et al.* compared the outcomes for 533 limbs of patients with DM and 542 without DM[174]. They also found on survival analysis that patients with DM had worse limb salvage ($p<0.001$), and survival ($p=0.001$). In addition, there was worse primary patency (defined as the return of symptoms and worsening ankle-brachial indices or pulse volume recordings, $p=0.009$) but comparable assisted patency (further endovascular procedure, excluding surgical bypass, $p=0.18$).

Inter and intra-observer reliability for the Bollinger score was examined as part of the study to consider whether the adjustments to the scoring system were reproducible. The results showed good inter-observer agreement and good intra-observer agreement (Section 3.4.1). In Bollinger's original description of the scoring system, they examined agreement between five scorers on six angiograms using Kendall's coefficient of concordance (KCC)[82]. They state that the agreement appeared to be excellent with a p-value <0.001 but unfortunately do not quote the actual value of KCC, so the accuracy of this statement is hard to assess. Since then a few other studies have addressed this question using a variety of methods. Bradbury *et al.* (2010)[84] compared the mean score for the whole leg, above the knee and below the knee, for two observers. These scores were further divided into four groups (<3, 3-5, 6-8, ≥9) to allow comparison to the second Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease (TASC II) classification which was also being assessed. They found that in approximately 75% of cases the two

observers placed the patient in the same Bollinger score group. Morris *et al.* compared two observers scores of twenty patients using weighted κ values[283]. They found poor intra and inter-observer agreement for the profunda-femoris segment (-0.07(standard error 0.14) and 0.26 (0.16) respectively), but the rest of the values were moderate to very good and particularly strong on the tibial vessels. Müller-Bühl *et al.* calculated an intra-class correlation coefficient between three observers of 0.85[284]. This was only based on the iliac and femoro-popliteal segments though. Due to the variation in methods used it is difficult to compare our results to these, but it would seem that the agreement between observers was comparable to other studies.

6.4.2. Cohort study

6.4.2.1. Post occlusive reactive hyperaemia

There is a wide range of TtM values reported in the literature both for patients with DM and no PAD and those with PAD. Lanting *et al.*[107] in patients with type 2 DM found that patients with no history of complications had a mean TtM of 17.11 seconds whereas those with a history of ulceration or amputation took 47.49s and those with an active ulcer 19.17s. These figures are based on PORH tests being performed with occlusion of the hallux only. Our values from the study toe are shorter than Lanting's measurements in active ulceration (DTL all patients at baseline 13.4s (3.68-73.85) and longer in those that had healed at the final visit (comparable to those with a history of ulceration). Lanting *et al.* found that a longer TtM was associated with increased odds of a history of ulceration but not with active ulceration.

Morales *et al.*[231] compared patients with Fontaine class II or III peripheral arterial disease and no DM with healthy controls. Occlusion was performed at the thigh rather than the ankle and measurements were taken on the dorsum of the foot. Their median TtM was 56.3s (50.92-87.74) which is much shorter than our baseline values (220.28s (93.65-279.8)), similar to the post-treatment second values (53.79s (18.09-205.16)) but shorter than the last values particularly in the healed group (175.76s (105.2-227.13)).

Ray *et al.*[110] examined patients with severe PAD prior to undergoing revascularisation. Of those in their cohort with DM (n=16) the patients who had clinically improved at six months had a mean TtM of 79s and those who had not 183s. Those who healed, at baseline, in our PCA group had a median TtM of 198.79s (69.18-287.33). Those who did not heal median measurement was 220.28s (93.65-271.38). The trend is in the same direction but the time scales are much longer. Overall it is not possible to compare or draw conclusions from the evidence above as our study is small and underpowered and many of these studies can also be described that way. This and differences in cohort characteristics will explain, at least in part, why the values vary so much.

There are no published direct comparisons of patients with DM pre-and post-PCA using laser Doppler fluxmetry (LDF). Data previously obtained at our institution showed a mean improvement of fourteen seconds at six-weeks following PCA in ten patients, four of whom had DM (69$\pm$27 vs 55$\pm$30, p=non-significant)[261]. This is a much smaller change than observed in the current study with the smallest difference at the second visit being 92s. Using alternative methods to assess the microcirculation others have demonstrated this improvement following revascularisation[51,78]. Like Arora *et al.* I have been able to demonstrate that following PCA the treated leg has improved microcirculation but it has not

quite improved to the level of the leg that does not require revascularisation (Table 5.7-12 and Table 5.7-13)[51].

The only PORH variable that was presented in the results was TtM (see Figure 4.4-2 for examples of other variables). This was chosen as throughout the comparisons it most frequently demonstrated a significant difference between measures. In addition to this within the literature TtM has regularly been found to be able to discriminate between groups[107,231,232,285,286]. Other variables that have been found to discriminate include time to resting flux (time from release of cuff until value of resting flux reached), time to recovery (TtR), time to half recovery (Tt1/2), maximum flux and a ratio of maximum flux to resting flux[231,232,285,286]. Variables that have been found to be reliable and reproducible include resting flux, latency between cuff release and start of recovery, maximum flux, TtM, maximum flux above resting flux, a ratio of maximum flux to biological zero and reperfusion rate[93,234,259]. The other variables that we measured can be found in Appendix VII In this dataset, other than TtM, the variables that most commonly demonstrated a significant difference were Tt1/2 and TtR. The trends that they demonstrated mirrored that of TtM and so do not offer up any further insights into the relationship.

As discussed in Section 4.7 the relationship between the macro and microcirculation is complex and poorly understood. Few studies have examined the relationship between lower limb PAD, DM and their respective and related impacts on the microcirculation. Williams *et al* in 2006 compared the transcutaneous oxygen pressure ($TcPO_2$) in 130 limbs. Patients were divided into controls with no PAD or DM (n=27), PAD with no DM (n=14), DM with no PAD (n=25), DM and PAD (n=7), DM with peripheral neuropathy (DPN) (n=41) and DM with PAD and DPN (n=16)[287]. No patients had critical ischaemia or active tissue loss. The presence or

absence of PAD was confirmed with duplex ultrasound. Toe blood pressure index (TBPI) was measured and compared to $TcPO_2$ of the dorsum of the foot. They found that there was a significant positive correlation between TBPI and $TCPO_2$ in the patients with PAD. In the DM groups without PAD there was no strong correlation between the two variables apart from a negative correlation in patients with abnormally high TBPI (>1.2) and associated low $TcPO_2$. Whilst the groups with PAD had significantly lower TBPI than the DM groups the results for $TcPO_2$ were much more similar across the groups. The PAD group with no DM had normal $TcPO_2$ despite low TBPI. The only group with significantly lower $TcPO_2$ was those with all three pathologies. They concluded that there were two major influences on the cutaneous perfusion in patients with DM and PAD and those were the macrovascular disease and a global microcirculatory change related to DM[287]. Pardo *et al* in 2014 correlated ankle brachial pressure index (ABPI) with $TcPO_2$ in patients with both DM and PAD before, during and after PCA[288]. They found that both ABPI and $TcPO_2$ improved following PCA but the correlation between the two was poor[288]. No studies that correlated LDF parameters to degree of macrovascular disease were identified.

In our data the DM group had been clinically assessed to have no significant macrovascular disease and this was assumed to remain stable through the study. In the PCA group, one patient was found to not have significant PAD at angiography whilst all the others had a procedure that improved the macrovascular flow. In one case thrombolysis was required for twenty-four hours before flow was achieved and in two cases the flow achieved was not in-line to the foot but via the peroneal artery only (Section 5.7.8.1). The thrombolysis patient went on to heal and had a reduction from 231s to 48s in TtM at second visit. Unfortunately, both patients with peroneal run-off only left the study early for the

reasons discussed in Section 6.3.2. One only had baseline measurements the other had two measurements post-procedure and demonstrated a reduction in TtM from 289s to 9s at second visit. The patient with a diagnostic only procedure had more favourable parameters than the other members of the group at baseline, TtM 13.15s, there was also a reduction at second visit to 3s. Using Spearman's correlation coefficient, there was no significant correlation between TtM and SPP or TBP in either the DM or PCA group (Baseline SPP compared to toe DM correlation 0.47 p=0.117, PCA 0.35 p=0.499, baseline TBP compared to toe DM 0.04 p=0.161, PCA 0.12 p=0.818).

6.4.2.2. Skin perfusion pressure

Our results for SPP were higher than many published results in both the DTL and PCA group. Yotsu *et al.*[253], in their neuropathic group, who are comparable to our DTL group, quoted median SPP of 67mmHg (57-75) for those who healed and 65mmHg (40-69) for those who did not. Like us, they found no significant difference between the groups. The neuro-ischaemic group as a comparison to our PCA group results were 38mmHg (22-51) and 17mmHg (10-32) respectively. Utsunomiya *et al.*[289] recorded the SPP prior to and up to forty-eight hours after PCA on a cohort of patients with critical ischaemia and tissue loss of whom 72% had DM. Their mean SPP before the procedure was 24.3±13mmHg and 40.7±16.1mmHg following the procedure. Kawarada *et al.*[290] demonstrated a significant increase in SPP following PCA of the ATA in patients with DM or end-stage renal failure (35mmHg (28-41) vs 52mmHg (38-65), p=0.001). The increase following angioplasty of the posterior tibial artery was not significant (27mmHg (21-33) vs 42mmHg (31-56), p=0.005).

An SPP of less than 40mmHg has been proposed as a poor predictor of healing[237,255]. Only one patient (7.7%) in the DTL group had an SPP of less than 40mmHg; they did not heal. In the PCA group two patients (33.3%) had SPP of less than 40mmHg, one healed and the other did not.

The variability in results both within the literature and when compared to our results is probably related to the small numbers in the cohorts. This is going to increase the heterogeneity between groups. While reliability, sensitivity and specificity have been shown to be good, overall the quality of the evidence is poor[112,221]. Having said that there are very few patients in which is not possible to perform SPP, unlike TBP, where previous amputation can preclude many patients with diabetic foot disease.

6.4.2.3. Toe Blood pressure

Within published guidelines toe blood pressure index is advised in patients with ABPI of more that 1.4[15,74,184]. There is not, however, consensus on the parameters which represent critical ischaemia. TASC II considers that a TBP of <50mmHg is indicative of critical limb ischaemia (definition: ischaemic rest pain for more than two weeks, ulcers or gangrene related to arterial disease)[81]. The International Working Group on the Diabetic Foot felt that a TBP of <30mmHg was an indication for revascularisation and patients with a TBP of ≥30mmHg should heal[15]. The most recent European guidelines on the diagnosis and treatment of PAD includes the Wound, Ischaemia and Foot infection (WIfI) classification for risk stratification of chronic limb threatening ischaemia[184]. In this classification a TBP of <30mmHg puts a patient in the highest category of risk of amputation[291]. In this current cohort, only one patient had a TBP of <30mmHg at baseline, in the PCA group, they did not

heal. If <50mmHg was used as a cut off four patients were affected, only one of these was in the DTL group, and they did not heal. In the PCA group one healed and the other two did not.

The reliability of TBP using laser Doppler has been found to be good in patients with PAD[292]. In patients with diabetic foot ulcers, specifically, there are only two studies that have considered this, and it would seem that the sensitivity and specificity are slightly weaker in this cohort[221]. As with SPP, our values of TBP are higher than many of those that have been published. Most cohorts, of patients with tissue loss, in the published literature, include a mixture of patients with and without PAD and so are different to the cohorts presented here. In addition to this TBP values vary greatly depending on the device use and so it can be hard to compare between cohorts[293]. TBP whilst is a good marker for PAD it can be influenced by factors that cause vasoconstriction. For example, room temperature and consumption of caffeine. These factors were considered and controlled for in the design of the study, and there was no significant difference found between the groups or visits (Table 5.7-6 and Table 5.7-7). A cool room temperature or recent consumption of caffeine would be expected to reduce the TBP, and so it is unlikely these factors contributed to the higher results[294].

6.4.2.4. Neuropathy

The aetiology on diabetic peripheral neuropathy (DPN) is complex. Various pathways and mechanisms have been proposed involving metabolic defects, inflammatory and oxidative stress and vascular disease[295]. The only treatment that has been convincingly found to have an impact on the development and progression of DPN is good glycaemic control[63]. No

studies have been able to demonstrate a causal relationship between improvement in macrovascular disease and DPN.

The improvement that was seen in NTSS-6 in the DTL group can also be explained by a reduction in the symptoms of active ulceration or infection.

6.5. PLANS FOR FUTURE STUDY

From the studies presented above it is not possible to answer the overall hypothesis (Section 6.2.3). One option for future work would be to refine the cohort study and continue recruitment with a focus on recruiting patients without DM and those undergoing PBS. When considering this as an option the sample size calculation performed in the design of the pilot study (Section 5.3.2) was repeated using data gained from this study.

When the sample size calculation was originally performed there was no available data for the DTL group. The results from the pilot study make it possible to repeat the power calculation for the DTL and PCA groups. The results included in the calculation were the baseline mean values compared to the last value of time to maximum flux on the study toe

DTL Patients (N=14): Baseline = 53 (78); Last = 89 (102)

PCA Patients (N=10): Baseline = 202 (99); Last = 94 (83)

The average change was 36 seconds (89-53) in the DTL group and 108 seconds (202-94) in the PCA groups. Hence, the difference in the change between the two groups was 72 seconds (108-36).

The pooled standard deviation from this data is 103. If the baseline and last measurements on the same patient are assumed to be independent, the standard deviation of the change will be 103 times the square root of 2, i.e. 146

The calculation was powered based on a t-test, assuming a standard deviation of 146. For a detectable difference of 72, a sample size of 65 patients per group would be sufficient for 80% power at 5% alpha.

Due to the lack of recruitment in the PBS group the best data remains that which the initial power calculation was performed using. However, it would make sense to aim for equal group sizes if further recruitment was to take place. Therefore, if the same proportion of drop outs was to be expected (20%) the group size would increase to 80 meaning the target for recruitment would be 240 patients.

Based on the difficulties with the pilot study it is highly unlikely that this level of recruitment would be possible without involving a large number of sites. There are also questions whether the results would answer the hypothesis proposed and so it is felt that proceeding with this study would be fruitless.

To move forward with addressing the question of how the microcirculation relates to wound healing it is felt that a better understanding of how the macrocirculation relates to microcirculation is required first. In addition, how the microcirculation in the foot changes during ulcer healing needs to be better defined. To this end two new hypotheses are proposed below:

1. In patients with PAD with or without DM a higher burden of macrovascular disease, as measured by the Bollinger score, correlates to reduced skin perfusion of the foot.
2. As an ulcer of the lower extremity heals there is a reduction in skin perfusion.

For the first hypothesis the plan would be to recruit patients with Rutherford category 3 or 4 disease[296], i.e. significant symptomatic PAD but no tissue loss, who are to undergo PCA.

Patients would be grouped by a history of DM. Bollinger score would be calculated based on PCA and correlated to a measure of pedal skin perfusion performed within the twenty-four hours preceding PCA. This is a development of Williams's[287] and Pardo's[288] studies with a focus on the burden of macrovascular disease. A progression of this study could be to recalculate Bollinger score and repeat skin perfusion tests following PCA as a measure of success of the procedure.

The second hypothesis is aimed at further examining the finding that in our DTL group there was a significant reduction in TtM on the study toe between the baseline and last visit (Table 5.7-9). Patients with active diabetic foot ulcers but no significant PAD would be recruited. Patients would be recruited at first attendance for the index ulcer and accurate volumes of the ulcer measured at the same time as assessment of skin perfusion surrounding the ulcer and foot as a whole. To improve the cohort selection an objective measure of absence of PAD would need to be included within recruitment, possibly duplex ultrasound. There would be regular reassessment until ulcer healing. Potential comparison groups include patients with venous ulceration and no significant PAD.

For both these potential studies careful consideration needs to be made of whether LDF is the most appropriate measure of skin perfusion to utilise. This is particularly true of the second study due to the number of assessments that would be required. The process for performing PORH is quite lengthy and cumbersome. In addition, using the placement of single probes adjacent to the ulcers it is hard to form a generalised view of the status of the microcirculation surrounding the ulcer. A modality that images the whole of the ulcer area with surrounding skin from which it is possible to take an average of clearly defined areas may be more appropriate. Examples include laser Doppler perfusion imaging and laser

speckle contrast imaging[297]. $TcPO_2$ as an alternative measure should also be considered as this is the modality that is increasingly being included in guidelines on the management of PAD and diabetic foot disease, whereas LDF is not[74,184].

6.6. CONCLUSIONS

The results of both of these studies have to be taken in the context of issues with design and poor recruitment. In the examination of the arterial tree it has been demonstrated that it is possible to extend Bollinger's original segments down to include the whole of the crural vessels and the pedal vessels with good inter and intra-rater reliability. In well matched cohorts those without DM and critical ischaemia were found to have a higher burden of disease in the pedal vessels but patients with DM had a higher burden of disease overall. In addition to this, it has been shown that there is no difference in the need for further revascularisation in the DM patients despite having poorer outcomes overall. The studies then moved on to examine the microcirculation in patients with DM and active foot ulceration. This demonstrated that patients who had arterial disease and required a PCA had significantly impaired microcirculatory reactivity compared to patients without significant PAD. The study showed that microvascular reactivity significantly improved following a PCA bringing this group up to a level comparable to the DTL group.

Overall these studies have added to the literature considering the nature of PAD in patients with DM. Further work to investigate and clarify the relationship between the macro and microcirculation has been proposed.

REFERENCES

1. World Health Organisation. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia.* Geneva, Switzerland: World Health Organisation;2006.
2. World Health Organisation. *Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus: Abbreviated report of a WHO consultation.* Geneva, Switzerland: World Health Organisation;2011.
3. NCD Risk factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet.* 2016;387(10027):1513-1530.
4. Diabetes UK. Facts and Figures. https://www.diabetes.org.uk/Professionals/Position-statements-reports/Statistics. Published 2017. Accessed 23rd April 2018.
5. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. *Lancet.* 2016;387(10035):2331-2339.
6. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. *Lancet.* 2016;387(10035):2340-2348.
7. Han Cho N, Whiting D, Forouhi N, et al. *Diabetes Atlas.* www.diabetesatlas.org: International Diabetes Federation;2015. Accessed 7th June 2019
8. Tillin T, Hughes AD, Godsland IF, et al. Insulin resistance and truncal obesity as important determinants of the greater incidence of diabetes in Indian Asians and African Caribbeans compared with Europeans: the Southall And Brent REvisited (SABRE) cohort. *Diabetes Care.* 2013;36(2):383-393.
9. Defronzo RA. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes.* 2009;58(4):773-795.

10. Venables MC, Jeukendrup AE. Physical inactivity and obesity: links with insulin resistance and type 2 diabetes mellitus. *Diabetes Metab Res Rev.* 2009;25 Suppl 1:S18-23.

11. Nentwich MM, Ulbig MW. Diabetic retinopathy - ocular complications of diabetes mellitus. *World J Diabetes.* 2015;6(3):489-499.

12. Teng J, Dwyer KM, Hill P, et al. Spectrum of renal disease in diabetes. *Nephrology.* 2014;19(9):528-536.

13. Peltier A, Goutman SA, Callaghan BC. Painful diabetic neuropathy. *BMJ.* 2014;348:g1799.

14. Fox CS, Golden SH, Anderson C, et al. Update on Prevention of Cardiovascular Disease in Adults With Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement From the American Heart Association and the American Diabetes Association. *Circulation.* 2015;132(8):691-718.

15. Lipsky BA, Aragon-Sanchez J, Diggle M, et al. IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. *Diabetes Metab Res Rev.* 2016;32 Suppl 1:45-74.

16. World Health Organisation. *Global Report on Diabetes.* http://www.who.int/diabetes/global-report/en/ Accessed 7th June 2019

17. Fowkes FGR, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet.* 2013;382(9901):1329-1340.

18. Gregg EW, Sorlie P, Paulose-Ram R, et al. Prevalence of lower-extremity disease in the U.S. adult population ≥40 years of age with and without diabetes. *Diabetes Care.* 2004;27(7):1591-1597.

19. Tunstall-Pedoe H, Peters SAE, Woodward M, Struthers AD, Belch JJF. Twenty-Year Predictors of Peripheral Arterial Disease Compared With Coronary Heart Disease in the Scottish Heart Health Extended Cohort (SHHEC). *J Am Heart Assoc.* 2017;6(9):e005967.

20. Cooke JP, Wilson AM. Biomarkers of peripheral arterial disease. *J Am Coll Cardiol.* 2010;55(19):2017-2023.

21. Pande RL, Perlstein TS, Beckman JA, Creager MA. Association of insulin resistance and inflammation with peripheral arterial disease: the National Health and Nutrition Examination Survey, 1999 to 2004. *Circulation.* 2008;118(1):33-41.

22. Bansilal S, Farkouh ME, Fuster V. Role of insulin resistance and hyperglycemia in the development of atherosclerosis. *Am J Cardiol.* 2007;99(4a):6b-14b.

23. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: Epidemiology, pathophysiology, and management. *JAMA.* 2002;287(19):2570-2581.

24. Puri R, Kataoka Y, Uno K, Nicholls S. The Distinctive Nature of Atherosclerotic Vascular Disease in Diabetes: Pathophysiological and Morphological Insights. *Curr Diab Rep.* 2012;12(3):280-285.

25. Polovina MM, Potpara TS. Endothelial dysfunction in metabolic and vascular disorders. *Postgrad Med.* 2014;126(2):38-53.

26. Tousoulis D, Kampoli AM, Stefanadis C. Diabetes mellitus and vascular endothelial dysfunction: current perspectives. *Curr Vasc Pharmacol.* 2012;10(1):19-32.

27. Cronenwett JL, Johnston KW, Rutherford RB. *Rutherford's vascular surgery.* 7th ed. Philadelphia, PA: Saunders/Elsevier; 2010.

28. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation.* 2006;113(15):1888-1904.

29. Berne RM, Levy MN, eds. *Principles of physiology.* Third ed: Mosby Inc.; 2000.

30. Sena CM, Pereira AM, Seica R. Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta.* 2013;1832(12):2216-2231.

31. Tousoulis D, Tsarpalis K, Cokkinos D, Stefanadis C. Effects of insulin resistance on endothelial function: possible mechanisms and clinical implications. *Diabetes Obes Metab.* 2008;10(10):834-842.

32. Jenkins GW, Tortora GJ. *Anatomy and Physiology from Science to Life: International Student Version.* 3rd ed: John Wiley & Sons Inc; 2013.

33. Moore KL, Dalley AF. *Clincally Oriented Anatomy.* 4th ed. USA: Lippincott Williams & Wilkins; 1999.

34. Standring S. *Gray's Anatomy: The anatomical basis of clinical practice.* 41st ed: Elsevier; 2015.

35. Charkoudian N. Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol (1985).* 2010;109(4):1221-1228.

36. Charkoudian N. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc.* 2003;78(5):603-612.

37. LoGerfo FW, Coffman JD. Vascular and microvascular disease of the foot in diabetes: Implications for foot care. *N Engl J Med.* 1984;311(25):1615-1619.

38. Iozzo RV, Cohen IR, Grassel S, Murdoch AD. The biology of perlecan: the multifaceted heparan sulphate proteoglycan of basement membranes and pericellular matrices. *Biochem J.* 1994;302 (Pt 3):625-639.

39. Yasuda H, Taniguchi Y, Yamashita M, et al. Morphological characteristics of dermal diabetic microangiopathy. *Diabetes Res Clin Pract.* 1990;9(2):187-194.

40. Malik RA, Newrick PG, Sharma AK, et al. Microangiopathy in human diabetic neuropathy: relationship between abnormalities and the severity of neuropathy. *Diabetologia.* 1989;32(2):92-102.

41. Rayman G, Malik RA, Sharma AK, Day JL. Microvascular response to tissue injury and capillary ultrastructure in the foot skin of Type I diabetic patients. *Clin Sci (Lond).* 1995;89(5):467-474.

42. Rohrbach DH, Hassell JR, Kleinman HK, Martin GR. Alterations in the basement membrane (heparan sulfate) proteoglycan in diabetic mice. *Diabetes.* 1982;31(2):185-188.

43. Nikolova G, Strilic B, Lammert E. The vascular niche and its basement membrane. *Trends Cell Biol.* 2007;17(1):19-25.

44. Figueroa-Romero C, Sadidi M, Feldman EL. Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. *Rev Endocr Metab Disord.* 2008;9(4):301-314.

45. Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. *Pharmacol Ther.* 2008;120(1):1-34.

46. Tufro A, Veron D. VEGF and podocytes in diabetic nephropathy. *Semin Nephrol.* 2012;32(4):385-393.

47. Jorneskog G, Brismar K, Fagrell B. Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications. *Diabetologia.* 1995;38(4):474-480.

48. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation.* 1993;88(6):2510-2516.

49. Rayman G, Williams SA, Spencer PD, Smaje LH, Wise PH, Tooke JE. Impaired microvascular hyperaemic response to minor skin trauma in type I diabetes. *BMJ.* 1986;292(6531):1295-1298.

50. Sandeman DD, Pym CA, Green EM, Seamark C, A.C. Shore, Tooke JE. Microvascular vasodilatation in feet of newly diagnosed non-insulin dependent diabetic patients. *BMJ.* 1991;302(6785):1122-1123.

51. Arora S, Pomposelli F, LoGerfo FW, Veves A. Cutaneous microcirculation in the neuropathic diabetic foot improves significantly but not completely after successful lower extremity revascularization. *J Vasc Surg.* 2002;35(3):501-505.

52. Rogers LC, Armstrong DG. Podiatric Care. In: Sidawy AN, Perler BA, eds. *Rutherford's Vascular Surgery and Endovascular Therapy.* 9th Edition ed.: Elsevier; 2019:1557-1572.e1554.

53. Boulton AJ, Vinik AI, Arezzo JC, et al. Diabetic neuropathies: A statement by the American Diabetes Association. *Diabetes Care.* 2005;28(4):956-962.

54. Vinik A, Ullal J, Parson HK, Casellini CM. Diabetic neuropathies: clinical manifestations and current treatment options. *Nat Clin Pract Endocrinol Metab.* 2006;2(5):269-281.

55. Rahman MH, Jha MK, Suk K. Evolving Insights into the Pathophysiology of Diabetic Neuropathy: Implications of Malfunctioning Glia and Discovery of Novel Therapeutic Targets. *Curr Pharm Des.* 2016;22(6):738-757.

56. Papanas N, Ziegler D. Risk Factors and Comorbidities in Diabetic Neuropathy: An Update 2015. *Rev Diabet Stud.* 2015;12(1-2):48-62.

57. Dewanjee S, Das S, Das AK, et al. Molecular mechanism of diabetic neuropathy and its pharmacotherapeutic targets. *Eur J Pharmacol.* 2018;833:472-523.

58. Tesfaye S, Chaturvedi N, Eaton SEM, et al. Vascular Risk Factors and Diabetic Neuropathy. *N Engl J Med.* 2005;352(4):341-350.

59. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet.* 1998;352(9131):837-853.

60. Martin CL, Albers JW, Pop-Busui R. Neuropathy and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care.* 2014;37(1):31-38.

61. Dyck PJ, Albers JW, Andersen H, et al. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev.* 2011;27(7):620-628.

62. Boulton AJM, Gries FA, Jervell JA. Guidelines for the Diagnosis and Outpatient Management of Diabetic Peripheral Neuropathy. *Diabet Med.* 1998;15(6):508-514.

63. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol.* 2012;11(6):521-534.

64. Dinh TL, Veves A. A review of the mechanisms implicated in the pathogenesis of the diabetic foot. *Int J Low Extrem Wounds.* 2005;4(3):154-159.

65. Noor S, Zubair M, Ahmad J. Diabetic foot ulcer-A review on pathophysiology, classification and microbial etiology. *Diabetes Metab Syndr.* 2015;9(3):192-199.

66. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet.* 2005;366(9498):1736-1743.

67. Alavi A, Sibbald RG, Mayer D, et al. Diabetic foot ulcers: Part I. Pathophysiology and prevention. *J Am Acad Dermatol.* 2014;70(1):1.e1-18; quiz 19-20.

68. Baltzis D, Eleftheriadou I, Veves A. Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights. *Adv Ther.* 2014;31(8):817-836.

69. Dinh T, Tecilazich F, Kafanas A, et al. Mechanisms involved in the development and healing of diabetic foot ulceration. *Diabetes.* 2012;61(11):2937-2947.

70. Noor S, Khan RU, Ahmad J. Understanding Diabetic Foot Infection and its Management. *Diabetes Metab Syndr.* 2017;11(2):149-156.

71. Boada A. [Skin lesions in the diabetic foot]. *Actas Dermosifiliogr.* 2012;103(5):348-356.

72. Chisman R, Lowry D, Saeed MA, Tiwari A, David MD. Prescribing antibiotics in diabetic foot infection: what is the role of initial microscopy and culture of tissue samples? *Int Wound J.* 2017;14(4):685-690.

73. Apelqvist J, Bakker K, van Houtum WH, Schaper NC, International Working Group on the Diabetic Foot Editorial Board. Practical guidelines on the management and prevention of the diabetic foot: based upon the International Consensus on the Diabetic Foot (2007) Prepared by the International Working Group on the Diabetic Foot. *Diabetes Metab Res Rev.* 2008;24(S1):S181-187.

74. Hingorani A, LaMuraglia GM, Henke P, et al. The management of diabetic foot: A clinical practice guideline by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine. *J Vasc Surg.* 2016;63(2 Suppl):3s-21s.

75. National Institute for Health and Care Excellence: Clinical Guidelines. *Diabetic Foot Problems: Prevention and Management.* London: National Institute for Health and Care Excellence (UK). https://www.nice.org.uk/guidance/ng19 Accessed 13th June 2019

76. Soderstrom M, Arvela E, Alback A, Aho P, Lepantalo M. Healing of ischaemia tissue lesions after infrainquinal bypass surgery for critical leg iscaemia. *Eur J Vasc Endovasc Surg.* 2008;36(1):90-95.

77. Lee MS, Rha S-W, Han SK, et al. Comparison of Diabetic and Non-Diabetic Patients Undergoing Endovascular Revascularization for Peripheral Arterial Disease. *J Invasive Cardiol.* 2015;27(3):167-171.

78. Akbari CM, Gibbons GW, Habershaw GM, LoGerfo FW, Veves A. The Effect of Arterial Reconstruction on the Natural History of Diabetic Neuropathy. *Arch Surg.* 1997;132(2):148-152.

79. Toursarkissian B, Connaughton JC, D'Ayala M, et al. Does lower limb revascularization result in an improvement in sensory perception thresholds? *Ann Vasc Surg.* 2002;16(3):309-313.

80. Hinchliffe R.J, Andros G, Apelqvist J, et al. A systematic review of the effectiveness of revascularization of the ulcerated foot in patients with diabetes and peripheral arterial disease. *Diabetes Metab Res Rev.* 2012;28(S1):179-217.

81. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *Eur J Vasc Endovasc Surg.* 2007;33(S1):S1-S75.

82. Bollinger A, Breddin K, Hess H, et al. Semiquantitative assessment of lower limb atherosclerosis from routine angiographic images. *Atherosclerosis.* 1981;38(3-4):339-346.

83. Jude EBO, S.O.; Chalmers, N.; Boulton, A.J.M. Peripheral Arterial Disease in Diabetic and Nondiabetic Patients. *Diabetes Care.* 2001;24(8):1433-1437.

84. Bradbury AW, Adam DJ, Bell J, et al. Bypass versus Angioplasty in Severe Ischaemia of the Leg (BASIL) trial: A description of the severity and extent of disease using the Bollinger angiogram scoring method and the TransAtlantic Inter-Society Consensus II classification. *J Vasc Surg.* 2010;51(5 Suppl):32S-42S.

85. Fehervari M, Krepuska M, Szeplaki G, et al. The level of complement C3 is associated with the severity of atherosclerosis but not with arterial calcification in peripheral artery disease. *Int Angiol.* 2014;33(1):35-41.

86. Fehervari M, Sarkadi H, Krepuska M, et al. Bone mineral density is associated with site-specific atherosclerosis in patients with severe peripheral artery disease. *Calcif Tissue Int.* 2013;93(1):55-61.

87. Szeberin Z, Fehervari M, Krepuska M, et al. Serum fetuin-A levels inversely correlate with the severity of arterial calcification in patients with chronic lower extremity atherosclerosis without renal disease. *Int Angiol.* 2011;30(5):474-450.

88. Willenberg T, Diehm N, Zwahlen M, et al. Impact of long-term corticosteroid therapy on the distribution pattern of lower limb atherosclerosis. *Eur J Vasc Endovasc Surg.* 2010;39(4):441-446.

89. Willenberg T, Baumgartner I, Silvestro A, Do D-D, Zwahlen M, Diehm N. An angiographic analysis of atherosclerosis progression in below-the-knee arteries after femoropopliteal angioplasty in claudicants. *J Endovasc Ther.* 2010;17(1):39-45.

90. Diehm N, Rohrer S, Baumgartner I, Keo H, Do D, Kalka C. Distribution pattern of infrageniculate arterial obstructions in patients with diabetes mellitus and renal insufficiency - implications for revascularization. *Vasa.* 2008;37(3):265-273.

91. Bradbury AW, Adam DJ, Bell J, et al. Bypass versus Angioplasty in Severe Ischaemia of the Leg (BASIL) trial: A survival prediction model to facilitate clinical decision making. *J Vasc Surg.* 2010;51(5 Suppl):52S-68S.

92. de Mul FFM, Morales F, Smit AJ, Graaff R. A model for post-occlusive reactive hyperemia as measured with laser-Doppler perfusion monitoring. *IEEE Trans Biomed Eng.* 2005;52(2):184-190.

93. Klomp HM, Wittens CHA, van Urk H. Transcutaneous Oximetry, Laser Doppler Fluxmetry, and Capillary Microscopy: Variability in Patients with Advanced Atherosclerotic Disease of the Lower Extremity. *Vasc Endovascular Surg.* 2000;34(3):231-243.

94. Rossi M, Carpi A. Skin microcirculation in peripheral arterial obliterative disease. *Biomed Pharmacother.* 2004;58(8):427-431.

95. Chittenden SJ, Shami SK. Microvascular investigations in diabetes mellitus. *Postgrad Med J.* 1993;69(812):419-428.

96. Holloway GA, Watkins DW. Laser Doppler measurement of cutaneous blood flow. *J Invest Dermatol.* 1977;69(3):306-309.

97. Stern MD. In vivo evaluation of microcirculation by coherent light scattering. *Nature.* 1975;254(5495):56-58.

98. Tur E, Yosipovitch G, Bar-On Y. Skin reactive hyperemia in diabetic patients. A study by laser Doppler flowmetry. *Diabetes Care.* 1991;14(11):958-962.

99. Clough G, Chipperfield A, Byrne C, de Mul F, Gush R. Evaluation of a new high power, wide separation laser Doppler probe: Potential measurement of deeper tissue blood flow. *Microvasc Res.* 2009;78(2):155-161.

100. Roustit M, Blaise S, Millet C, Cracowski JL. Reproducibility and methodological issues of skin post-occlusive and thermal hyperemia assessed by single-point laser Doppler flowmetry. *Microvasc Res.* 2010;79(2):102-108.

101. Roustit M, Cracowski J-L. Assessment of endothelial and neurovascular function in human skin microcirculation. *Trends Pharmacol Sci.* 2013;34(7):373-384.

102. Veves A, Akbari CM, Primavera J, et al. Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. *Diabetes.* 1998;47(3):457-463.

103. Kondo Y, Muto A, Dardik A, Nishibe M, Nishibe T. Laser Doppler skin perfusion pressure in the diagnosis of limb ischemia in patients with diabetes mellitus and/or hemodialysis. *Int Angiol.* 2007;26(3):258-261.

104. Ambrozy E, Waczulikova I, Willfort A, et al. Healing process of venous ulcers: the role of microcirculation. *Int Wound J.* 2013;10(1):57-64.

105. Unal O, Karatepe O, Ugurlucan M, Koc B, Filizcan U, Aksoy M. Effects of lower extremity revascularization on the endothelial functions measured with noninvasive brachial artery flow-mediated dilatation. *Ann Vasc Surg.* 2011;25(7):969-974.

106. Saucy F, Dischl B, Delachaux A, et al. Foot skin blood flow following infrainguinal revascularization for critical lower limb ischemia. *Eur J Vasc Endovasc Surg.* 2006;31(4):401-406.

107. Lanting SM, Barwick AL, Twigg SM, et al. Post-occlusive reactive hyperaemia of skin microvasculature and foot complications in type 2 diabetes. *J Diabetes Complications.* 2017;31(8):1305-1310.

108. Jaffer U, Aslam M, Standfield N. Impaired Hyperaemic and Rhythmic Vasomotor Response in Type 1 Diabetes Mellitus Patients: A Predictor of Early Peripheral Vascular Disease. *Eur J Vasc Endovasc Surg.* 2008;35(5):603-606.

109. Bongard O, Krahenbuhl B. Predicting amputation in severe ischaemia. The value of transcutaneous PO2 measurement. *J Bone Joint Surg Br.* 1988;70-B(3):465-467.

110. Ray SA, Buckenham TM, Belli A-M, Taylor RS, Dormandy JA. The Predictive Value of Laser Doppler Fluxmetry and Transcutaneous Oximetry for Clinical Outcome in Patients Undergoing Revascularisation for Severe Leg Ischaemia. *Eur J Vasc Endovasc Surg.* 1997;13(1):54-59.

111. Pardo MA, M.; Breijo, F.R.; Bernal, F.L.; Felices, J.M.; Cateras, M. Increased transcutaneous oxygen pressure is an indicator of revascularization after peripheral transluminal angioplasty. *Acta Radiol.* 2010;51(9):990-993.

112. Yamada T, Ohta T, Ishibashi H, et al. Clinical reliability and utility of skin perfusion pressure measurement in ischemic limbs—Comparison with other noninvasive diagnostic methods. *J Vasc Surg.* 2008;47(2):318-323.

113. American Diabetes Association. Standards of Medical Care in Diabetes—2012. *Diabetes Care.* 2012;35(S1):S11-S63.

114. Argiana V, Eleftheriadou I, Tentolouris N. Screening for the High-risk Foot of Ulceration: Tests of Somatic and Autonomic Nerve Function. *Curr Diab Rep.* 2011;11(4):294-301.

115. Bloom S, Till S, Sönksen P, Smith S. Use of a biothesiometer to measure individual vibration thresholds and their variation in 519 non-diabetic subjects. *BMJ.* 1984;288(6433):1793-1795.

116. Bril V, Kojic J, Ngo M, Clark K. Comparison of a Neurothesiometer and Vibratron in Measuring Vibration Perception Thresholds and Relationship to Nerve Conduction Studies. *Diabetes Care.* 1997;20(9):1360-1362.

117. Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds: A prospective study. *Diabetes Care.* 1994;17(6):557-560.

118. Abbott CA, Vileikyte L, Williamson S, Carrington AL, Boulton AJM. Multicenter Study of the Incidence of and Predictive Risk Factors for Diabetic Neuropathic Foot Ulceration. *Diabetes Care.* 1998;21(7):1071-1075.

119. Martin CL, Waberski BH, Pop-Busui R, et al. Vibration perception threshold as a measure of distal symmetrical peripheral neuropathy in type 1 diabetes: results from the DCCT/EDIC study. *Diabetes Care.* 2010;33(12):2635-2641.

120. Feng Y, Schlösser FJ, Sumpio BE. The Semmes Weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy. *J Vasc Surg.* 2009;50(3):675-682.

121. Feng Y, Schlösser FJ, Sumpio BE. The Semmes Weinstein monofilament examination is a significant predictor of the risk of foot ulceration and amputation in patients with diabetes mellitus. *J Vasc Surg.* 2011;53(1):220-226.

122. Abbott CA, Carrington AL, Ashe H, et al. The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. *Diabet Med.* 2002;19(5):377-384.

123. Rayman G, Vas PR, Baker N, et al. The Ipswich Touch Test: a simple and novel method to identify inpatients with diabetes at risk of foot ulceration. *Diabetes Care.* 2011;34(7):1517-1518.

124. Jayaprakash P, Bhansali A, Bhansali S, et al. Validation of bedside methods in evaluation of diabetic peripheral neuropathy. *Indian J Med Res.* 2011;133:645-649.

125. Shehab DK, Al-Jarallah KF, Abraham M, Mojiminiyi OA, Al-Mohamedy H, Abdella NA. Back to basics: ankle reflex in the evaluation of peripheral neuropathy in type 2 diabetes mellitus. *QJM.* 2012;105(4):315-320.

126. McDermott MM, Sufit R, Nishida T, et al. Lower extremity nerve function in patients with lower extremity ischemia. *Arch Intern Med.* 2006;166(18):1986-1992.

127. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care.* 2010;33(10):2285-2293.

128. Dyck PJ, O'Brien PC. Meaningful degrees of prevention or Improvement of Nerve Conduction in Controlled Clinical Trials of Diabetic Neuropathy. *Diabetes Care.* 1989;12(9):649-652.

129. England JD, Gronseth GS, Franklin G, et al. Distal symmetric polyneuropathy: A definition for clinical research. *Neurology.* 2005;64(2):199-207.

130. Bastyr EJr, Price K, Bril V. Development and validity testing of the neuropathy total symptom score-6: questionnaire for the study of sensory symptoms of diabetic peripheral neuropathy. *Clin Ther.* 2005;27(8):1278-1294.

131. Ametov AS, Barinov A, Dyck PJ, et al. The Sensory Symptoms of Diabetic Polyneuropathy Are Improved With α-Lipoic Acid. *Diabetes Care.* 2003;26(3):771-776.

132. Bril V. NIS-LL: The Primary Measurement Scale for Clinical Trial Endpoints in Diabetic Peripheral Neuropathy. *Eur Neurol.* 1999;41(Suppliment 1):8-13.

133. Asad A, Hameed MA, Khan UA, Ahmed N, Butt M-u-RA. Reliability of the neurological scores for assessment of sensorimotor neuropathy in type 2 diabetics. *J Pak Med Assoc.* 2010;60(3):166-170.

134. Fonseca VA, Lavery LA, Thethi TK, et al. Metanx in Type 2 Diabetes with Peripheral Neuropathy: A Randomized Trial. *Am J Med.* 2013;126(2):141-149.

135. Tesfaye S, Tandan R, Bastyr EJI, et al. Factors That Impact Symptomatic Diabetic Peripheral Neuropathy in Placebo-Administered Patients From Two 1-Year Clinical Trials. *Diabetes Care.* 2007;30(10):2626-2632.

136. Casellini CM, Barlow PM, Rice AL, et al. A 6-Month, Randomized, Double-Masked, Placebo-Controlled Study Evaluating the Effects of the Protein Kinase C-β Inhibitor Ruboxistaurin on Skin Microvascular Blood Flow and Other Measures of Diabetic Peripheral Neuropathy. *Diabetes Care.* 2007;30(4):896-902.

137. Pittenger GL, Ray M, Burcus NI, McNulty P, Basta B, Vinik AI. Intraepidermal Nerve Fibers Are Indicators of Small-Fiber Neuropathy in Both Diabetic and Nondiabetic Patients. *Diabetes Care.* 2004;27(8):1974-1979.

138. Quattrini C, Tavakoli M, Jeziorska M, et al. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes.* 2007;56(8):2148-2154.

139. Arimura A, Deguchi T, Sugimoto K, et al. Intraepidermal nerve fiber density and nerve conduction study parameters correlate with clinical staging of diabetic polyneuropathy. *Diabetes Res Clin Pract.* 2013;99(1):24-29.

140. Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC. The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. *Brain.* 2004;127(7):1606-1615.

141. Becker F, Robert-Ebadi H, Ricco JB, et al. Chapter I: Definitions, epidemiology, clinical presentation and prognosis. *Eur J Vasc Endovasc Surg.* 2011;42 Suppl 2:S4-12.

142. Kok HK, Asadi H, Sheehan M, McGrath FP, Given MF, Lee MJ. Outcomes of infrapopliteal angioplasty for limb salvage based on the updated TASC II classification. *Diagn Interv Radiol.* 2017;23(5):360-364.

143. Teraa M, Conte MS, Moll FL, Verhaar MC. Critical Limb Ischemia: Current Trends and Future Directions. *J Am Heart Assoc.* 2016;5(2):pii: e002938.

144. Singh GD, Brinza EK, Hildebrand J, et al. Midterm Outcomes After Infrapopliteal Interventions in Patients With Critical Limb Ischemia Based on the TASC II Classification of Below-the-Knee Arteries. *J Endovasc Ther.* 2017;24(3):321-330.

145. Jaff MR, White CJ, Hiatt WR, et al. An update on methods for revascularization and expansion of the TASC lesion classification to include below-the-knee arteries: A supplement to the inter-society consensus for the management of peripheral arterial disease (TASC II): The TASC steering committee. *Catheter Cardiovasc Interv.* 2015;86(4):611-625.

146. Mustapha JA, Diaz-Sandoval LJ, Saab F. Infrapopliteal calcification patterns in critical limb ischemia: diagnostic, pathologic and therapeutic implications in the search for the endovascular holy grail. *J Cardiovasc Surg (Torino).* 2017;58(3):383-401.

147. Forsythe RO, Jones KG, Hinchliffe RJ. Distal bypasses in patients with diabetes and infrapopliteal disease: technical considerations to achieve success. *Int J Low Extrem Wounds.* 2014;13(4):347-362.

148. Wallaert JB, Nolan BW, Adams J, et al. The impact of diabetes on postoperative outcomes following lower-extremity bypass surgery. *J Vasc Surg.* 2012;56(5):1317-1323.

149. Haltmayer M, Mueller T, Horvath W, Luft C, Poelz W, Haidinger D. Impact of atherosclerotic risk factors on the anatomical distribution of peripheral arterial disease. *Int Angiol.* 2001;20(3):200-207.

150. Van Der Feen C, Neijens FS, Kanters SDJM, Mali WPTM, Stolk RP, Banga JD. Angiographic distribution of lower extremity atherosclerosis in patients with and without diabetes. *Diabet Med.* 2002;19(5):366-370.

151. De Bakey ME, Crawford ES, Garrett HE, Cooley DA, Morris GC, Jr., Abbott JP. Occlusive Disease of the Lower Extremities in Patients 16 to 37 Years of Age. *Ann Surg.* 1964;159:873-890.

152. Hansen ME, Valentine RJ, McIntire DD, Myers SI, Chervu A, Clagett GP. Age-related differences in the distribution of peripheral atherosclerosis: when is atherosclerosis truly premature? *Surgery.* 1995;118(5):834-839.

153. Kroger K, Buss C, Renzing-Kohler K, Santosa F, Rudofsky G. Segmental manifestation of peripheral atherosclerosis and its association to risk factors. *Vasa.* 2000;29(3):199-203.

154. Rueda CA, Nehler MR, Perry DJ, et al. Patterns of artery disease in 450 patients undergoing revascularization for critical limb ischemia: implications for clinical trial design. *J Vasc Surg.* 2008;47(5):995-999; discussion 999-1000.

155. Ozkan U, Oguzkurt L, Tercan F. Atherosclerotic risk factors and segmental distribution in symptomatic peripheral artery disease. *J Vasc Interv Radiol.* 2009;20(4):437-441.

156. Haimovici H. Patterns of arteriosclerotic lesions of the lower extremity. *Arch Surg.* 1967;95(6):918-933.

157. Ciavarella A, Silletti A, Mustacchio A, et al. Angiographic evaluation of the anatomic pattern of arterial obstructions in diabetic patients with critical limb ischaemia. *Diabete Metab.* 1993;19(6):586-589.

158. Lazaris AM, Tsiamis AC, Fishwick G, Bolia A, Bell PR. Clinical outcome of primary infrainguinal subintimal angioplasty in diabetic patients with critical lower limb ischemia. *J Endovasc Ther.* 2004;11(4):447-453.

159. Menzoian JO, LaMorte WW, Paniszyn CC, et al. Symptomatology and Anatomic Patterns of Peripheral Vascular Disease: Differing Impact of Smoking and Diabetes. *Ann Vasc Surg.* 1989;3(3):224-228.

160. Diehm N, Shang A, Silvestro A, et al. Association of cardiovascular risk factors with pattern of lower limb atherosclerosis in 2659 patients undergoing angioplasty. *Eur J Vasc Endovasc Surg.* 2006;31(1):59-63.

161. LaMorte WW, Menzoian JO, Sidawy A, Heeren T. A new method for the prediction of peripheral vascular resistance from the preoperative angiogram. *J Vasc Surg.* 1985;2(5):703-708.

162. Elliott BM, Robison JG, Brothers TE, Cross MA. Limitations of peroneal artery bypass grafting for limb salvage. *J Vasc Surg.* 1993;18(5):881-888.

163. Faglia E, Clerici G, Clerissi J, et al. When is a technically successful peripheral angioplasty effective in preventing above-the-ankle amputation in diabetic patients with critical limb ischaemia? *Diabet Med.* 2007;24(8):823-829.

164. Sumpio BE, Forsythe RO, Ziegler KR, van Baal JG, Lepantalo MJA, Hinchliffe RJ. Clinical implications of the angiosome model in peripheral vascular disease. *J Vasc Surg.* 2013;58(3):814-826.

165. Karmody AM, Leather RP, Shah DM, Corson JD, Naraynsingh V. Peroneal artery bypass: a reappraisal of its value in limb salvage. *J Vasc Surg.* 1984;1(6):809-816.

166. Ricco JB, Gargiulo M, Stella A, et al. Impact of angiosome- and nonangiosome-targeted peroneal bypass on limb salvage and healing in patients with chronic limb-threatening ischemia. *J Vasc Surg.* 2017;66(5):1479-1487.

167. Dosluoglu HH, Cherr GS, Lall P, Harris LM, Dryjski ML. Peroneal artery-only runoff following endovascular revascularizations is effective for limb salvage in patients with tissue loss. *J Vasc Surg.* 2008;48(1):137-143.

168. Abularrage CJ, Conrad MF, Haurani MJ, et al. Long-term outcomes of patients undergoing endovascular infrainguinal interventions with single-vessel peroneal artery runoff. *J Vasc Surg.* 2011;53(4):1007-1013.

169. Conrad MC. Large and small artery occlusion in diabetics and nondiabetics with severe vascular disease. *Circulation.* 1967;36(1):83-91.

170. Karacagil S, Almgren B, Bowald S, Eriksson I. Arterial lesions of the foot vessels in diabetic and non-diabetic patients undergoing lower limb revascularisation. *Eur J Vasc Surg.* 1989;3(3):239-244.

171. Haine A, Haynes AG, Limacher A, et al. Patency of the arterial pedal-plantar arch in patients with chronic kidney disease or diabetes mellitus. *Ther Adv Cardiovasc Dis.* 2018;12(5):145-153.

172. Pomposelli FB, Jr., Jepsen SJ, Gibbons GW, et al. Efficacy of the dorsal pedal bypass for limb salvage in diabetic patients: short-term observations. *J Vasc Surg.* 1990;11(6):745-751; discussion 751-742.

173. DeRubertis BG, Pierce M, Ryer EJ, Trocciola S, Kent KC, Faries PL. Reduced primary patency rate in diabetic patients after percutaneous intervention results from more frequent presentation with limb-threatening ischemia. *J Vasc Surg.* 2008;47(1):101-108.

174. Abularrage CJ, Conrad MF, Hackney LA, et al. Long-term outcomes of diabetic patients undergoing endovascular infrainguinal interventions. *J Vasc Surg.* 2010;52(2):314-322.e311-314.

175. Yang SL, Zhu LY, Han R, Sun LL, Li JX, Dou JT. Pathophysiology of peripheral arterial disease in diabetes mellitus. *J Diabetes.* 2017;9(2):133-140.

176. Stoner MC, Calligaro KD, Chaer RA, et al. Reporting standards of the Society for Vascular Surgery for endovascular treatment of chronic lower extremity peripheral artery disease: Executive summary. *J Vasc Surg.* 2016;64(1):227-228.

177. Saunders DJ, Bleasdale L, Summerton L, Hancock A, Homer-Vanniasinkam S, Russell DA. Assessment of the utility of a Vascular Early Warning System (VEWS) device in the assessment of peripheral arterial disease in patients with diabetes and incompressible vessels (published online ahead of print 13 February 2019). *Ann Vasc Surg.* 2019;S0890-5096(19):30142-30146.

178. Kang IS, Lee W, Choi BW, et al. Semiquantitative assessment of tibial artery calcification by computed tomography angiography and its ability to predict infrapopliteal angioplasty outcomes. *J Vasc Surg.* 2016;64(5):1335-1343.

179. Jones WS, Patel MR, Tsai TT, et al. Anatomic runoff score predicts cardiovascular outcomes in patients with lower extremity peripheral artery disease undergoing revascularization. *Am Heart J.* 2015;170(2):400-408.

180. Balshem H, Helfand M, Schunemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol.* 2011;64(4):401-406.

181. Wells G, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp Published 2010. Accessed 14th January 2018.

182. Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. Epidemiology of Diabetes Interventions and Complications (EDIC): Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. *Diabetes Care.* 1999;22(1):99-111.

183. Hirsch AT, Haskal ZJ, Hertzer NR, et al. ACC/AHA 2005 Practice Guidelines for the Management of Patients With Peripheral Arterial Disease (Lower Extremity, Renal, Mesenteric, and Abdominal Aortic): A Collaborative Report from the American Association for Vascular Surgery/Society for Vascular Surgery,* Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): Endorsed by the American Association of

Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. *Circulation.* 2006;113(11):e463-e654.

184. Aboyans V, Ricco JB, Bartelink MEL, et al. Editor's Choice - 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg.* 2018;55(3):305-368.

185. Cowell GW, Reid AW, Roditi GH. Changing trends in a decade of vascular radiology-the impact of technical developments of non-invasive techniques on vascular imaging. *Insights Imaging.* 2012;3(5):495-504.

186. de Vos MS, Hawkins AT, Hevelone ND, Hamming JF, Nguyen LL. National variation in the utilization of alternative imaging in peripheral arterial disease. *J Vasc Surg.* 2014;59(5):1315-1322.e1311.

187. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika.* 1965;52(3):591-611.

188. Akbari CM, LoGerfo FW. Diabetes and peripheral vascular disease. *J Vasc Surg.* 1999;30(2):373-384.

189. Houben A, Martens RJH, Stehouwer CDA. Assessing Microvascular Function in Humans from a Chronic Disease Perspective. *J Am Soc Nephrol.* 2017;28(12):3461-3472.

190. Gilje O, Kierland R, Baldes EJ. Capillary microscopy in the diagnosis of dermatologic diseases. *J Invest Dermatol.* 1954;22(3):199-206.

191. Anonymous A. Capillary microscopy. *Lancet.* 1946;248(6427):645-646.

192. Davis E. A Simple Technique for Capillary Microscopy. *Proc R Soc Med.* 1946;39(6):314.

193. Davis MJ, Lorincz AL. An improved technic for capillary microscopy of the skin. *J Invest Dermatol.* 1957;28(4):283-290.

194. Grassi W, Angelis R. Capillaroscopy: questions and answers. *Clin Rheumatol.* 2007;26(12):2009-2016.

195. Hofstee HMA, Serné EH, Roberts C, et al. A multicentre study on the reliability of qualitative and quantitative nail-fold videocapillaroscopy assessment. *Rheumatology.* 2012;51(4):749-755.

196. Fagrell B, Lundberg G. A simplified evaluation of vital capillary microscopy for predicting skin viability in patients with severe arterial insufficiency. *Clin Physiol.* 1984;4(5):403-411.

197. Mathura KR, Vollebregt KC, Boer K, De Graaff JC, Ubbink DT, Ince C. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. *J Appl Physiol.* 2001;91(1):74-78.

198. Ubbink DT, Jacobs MJ, Slaaf DW, Tangelder GJ, Reneman RS. Capillary recruitment and pain relief on leg dependency in patients with severe lower limb ischemia. *Circulation.* 1992;85(1):223-229.

199. Halfoun VLRC, Pires MLE, Fernandes TJ, Victer F, Rodrigues KK, Tavares R. Videocapillaroscopy and Diabetes mellitus: area of transverse segment in nailfold capillar loops reflects vascular reactivity. *Diabetes Res Clin Pract.* 2003;61(3):155-160.

200. Gschwandtner ME, Ambrozy E, Maric S, et al. Microcirculation is similar in ischemic and venous ulcers. *Microvasc Res.* 2001;62(3):226-235.

201. Dolezalova P, Young SP, Bacon PA, Southwood TR. Nailfold capillary microscopy in healthy children and in childhood rheumatic diseases: a prospective single blind observational study. *Ann Rheum Dis.* 2003;62(5):444-449.

202. Van Roon A, Abdulle AE, van Roon A, et al. Nailfold capillary microscopy and low body mass index in Raynaud's phenomenon patients. *Ann Rheum Dis.* 2017;76(s2):1265-1265.

203. Bertolazzi C, Cutolo M, Smith V, Gutierrez M. State of the art on nailfold capillaroscopy in dermatomyositis and polymyositis. *Semin Arthritis Rheum.* 2017;47(3):432-444.

204. Clemente-Coelho P. Interest of nailfold capillary microscopy in the differential diagnosis of systemic sclerosis, rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis.* 2005;64:555-555.

205. Sakaguchi S, Miyai N, Takemura S, et al. Morphologic Classification of Nailfold Capillary Microscopy in Workers Exposed to Hand-arm Vibration. *Ind Health.* 2011;49(5):614-618.

206. Ferri C, Pitaro N, Guiggioli D, Martini A. Nailfold capillary microscopy in lacunar infarction. *Stroke.* 1994;25(2):525.

207. Glickman FS. Quantitative nailfold capillary microscopy. *J Am Acad Dermatol.* 1992;26(5 Pt 1):790-791.

208. Gschwandtner ME, Ambrozy E, Schneider B, Fasching S, Willfort A, Ehringer H. Laser Doppler imaging and capillary microscopy in ischemic ulcers. *Atherosclerosis.* 1999;142(1):225-232.

209. Nabuurs-Franssen MH, Houben AJ, Tooke JE, Schaper NC. The effect of polyneuropathy on foot microcirculation in Type II diabetes. *Diabetologia.* 2002;45(8):1164-1171.

210. Forsythe RO, Hinchliffe RJ. Assessment of foot perfusion in patients with a diabetic foot ulcer. *Diabetes Metab Res Rev.* 2016;32(S1):232-238.

211. Delpy DT. Developments in oxygen monitoring. *J Biomed Eng.* 1988;10(6):533-540.

212. Parker D. Sensors for monitoring blood gases in intensive care. *J Phys E Sci Instrum.* 1987;20(9):1103-1112.

213. Mani R, Gorman FW, White JE. Transcutaneous measurements of oxygen tension at edges of leg ulcers: preliminary communication. *J R Soc Med.* 1986;79(11):650-654.

214. Dhanoa D, Baerlocher M, Benko AJ, et al. Position Statement on Noninvasive Imaging of Peripheral Arterial Disease by the Society of Interventional Radiology and the Canadian Interventional Radiology Association. *J Vasc Interv Radiol.* 2016;27(7):947-951.

215. Henni S, Semporé YW, Le Meliner T, Ouedraogo N, Hamel JF, Abraham P. Intra-test and test-retest reliability of exercise oximetry in arterial claudication. *Microvasc Res.* 2018;117:44-49.

216. Redlich U, Xiong YY, Pech M, et al. Superiority of transcutaneous oxygen tension measurements in predicting limb salvage after below-the-knee angioplasty: a prospective trial in diabetic patients with critical limb ischemia. *Cardiovasc Intervent Radiol.* 2011;34(2):271-279.

217. Hauser CJ, Klein SR, Mehringer CM, Appel P, Shoemaker WC. Superiority of transcutaneous oximetry in noninvasive vascular diagnosis in patients with diabetes. *Arch Surg.* 1984;119(6):690-694.

218. Franzeck UK, Talke P, Bernstein EF, Golbranson FL, Fronek A. Transcutaneous PO2 measurements in health and peripheral arterial occlusive disease. *Surgery.* 1982;91(2):156-163.

219. Wyss CR, Robertson C, Love SJ, Harrington RM, Matsen III AM. Relationship between transcutaneous oxygen tension, ankle blood pressure, and clinical outcome of vascular surgery in diabetic and nondiabetic patients. *Surgery.* 1987;101(1):56-62.

220. White RA, Nolan L, Harley D, et al. Noninvasive evaluation of peripheral vascular disease using transcutaneous oxygen tension. *Am J Surg.* 1982;144(1):68-75.

221. Wang Z, Hasan R, Firwana B, et al. A systematic review and meta-analysis of tests to predict wound healing in diabetic foot. *J Vasc Surg.* 2016;63(S2):29S-36S.e21-22.

222. Caselli A, Latini V, Lapenna A, et al. Transcutaneous oxygen tension monitoring after successful revascularization in diabetic patients with ischaemic foot ulcers. *Diabet Med.* 2005;22(4):460-465.

223. Rooke TW, Osmundson PJ. The influence of age, sex, smoking, and diabetes on lower limb transcutaneous oxygen tension in patients with arterial occlusive disease. *Arch Intern Med.* 1990;150(1):129-132.

224. Eleftheriadou I, Tentolouris A, Grigoropoulou P, et al. The association of diabetic microvascular and macrovascular disease with cutaneous circulation in patients with type 2 diabetes mellitus. *J Diabetes Complications.* 2019;33(2):165-170.

225. Pecoraro RE, Ahroni JH, Boyko EJ, Stensel VL. Chronology and Determinants of Tissue Repair in Diabetic Lower-Extremity Ulcers. *Diabetes.* 1991;40(10):1305-1313.

226. Ueno H, Fukumoto S, Koyama H, et al. Regions of arterial stenosis and clinical factors determining transcutaneous oxygen tension in patients with peripheral arterial disease. *J atheroscler thromb.* 2010;17(8):858-869.

227. Bouyé P, Picquet J, Jaquinandi V, Enon B. Reproducibility of proximal and distal transcutaneous oxygen pressure measurements during exercise in stage 2 arterial claudication. *Int Angio.* 2004;23(2):114-121.

228. Kalani M, Brismar K, Fagrell B, Ostergren J, Jorneskog G. Transcutaneous oxygen tension and toe blood pressure as predictors for outcome of diabetic foot ulcers. *Diabetes Care.* 1999;22(1):147-151.

229. Faglia E, Clerici G, Caminiti M, Quarantiello A, Curci V, Morabito A. Predictive Values of Transcutaneous Oxygen Tension for Above-the-ankle Amputation in Diabetic Patients with Critical Limb Ischemia. *Eur J Vasc Endovasc Surg.* 2007;33(6):731-736.

230. [Patent] Stern MD, Lappe DL, Inventors; US Health, assignee. Method and apparatus for measurement of blood flow using coherent light. 1978.

231. Morales F, Graaff R, Smit AJ, et al. How to assess post-occlusive reactive hyperaemia by means of laser Doppler perfusion monitoring: Application of a standardised protocol to patients with peripheral arterial obstructive disease. *Microvasc Res.* 2005;69(1–2):17-23.

232. Wahlberg E, Olofsson P, Swedenborg J, Fagrell B. Changes in postocclusive reactive hyperaemic values as measured with laser doppler fluxmetry after infrainguinal arterial reconstructions. *Eur J Vasc Endovasc Surg.* 1995;9(2):197-203.

233. Quattrini C, Harris ND, Malik RA, Tesfaye S. Impaired skin microvascular reactivity in painful diabetic neuropathy. *Diabetes Care.* 2007;30(3):655-659.

234. Yvonne-Tee GB, Rasool AHG, Halim AS, Rahman ARA. Reproducibility of different laser Doppler fluximetry parameters of postocclusive reactive hyperemia in human forearm skin. *J Pharmacol Toxicol Methods.* 2005;52(2):286-292.

235. Castronuovo Jr JJ, Adera HM, Smiell JM, Price RM. Skin perfusion pressure measurement is valuable in the diagnosis of critical limb ischemia. *J Vasc Surg.* 1997;26(4):629-637.

236. Tsai FW, Tulsyan N, Jones DN, Abdel-Al N, Castronuovo Jr JJ, Carter SA. Skin perfusion pressure of the foot is a good substitute for toe pressure in the assessment of limb ischemia. *J Vasc Surg.* 2000;32(1):32-36.

237. Faris I, Duncan H. Skin perfusion pressure in the prediction of healing in diabetic patients with ulcers or gangrene of the foot. *J Vasc Surg.* 1985;2(4):536-540.

238. Karanfilian RG, Lynch TG, Zirul VT. The value of laser Doppler velocimetry and transcutaneous oxygen tension determination in predicting healing of ischemic forefoot ulcerations and amputations in diabetic and nondiabetic patients. *J Vasc Surg.* 1986;4(5):511-516.

239. Jorneskog G, Brismar K, Fagrell B. Low molecular weight heparin seems to improve local capillary circulation and healing of chronic foot ulcers in diabetic patients. *Vasa.* 1993;22(2):137-142.

240. Padberg JFT, Back TL, Thompson PN, Hobson RW. Transcutaneous Oxygen (TcPO2) Estimates Probability of Healing in the Ischemic Extremity. *J Surg Res.* 1996;60(2):365-369.

241. Koblik T, Sieradzki J, Sendur R, et al. The effect of insulin and sulodexide (Vessel Due F) on diabetic foot syndrome - Pilot study in elderly patients. *J Diabetes Complications.* 2001;15(2):69-74.

242. Zimny S, Schatz H, Pfohl M. Determinants and estimation of healing times in diabetic foot ulcers. *J Diabetes Complications.* 2002;16(5):327-332.

243. Fife CE, Buyukcakir C, Otto GH, et al. The predictive value of transcutaneous oxygen tension measurement in diabetic lower extremity ulcers treated with hyperbaric oxygen therapy: a retrospective analysis of 1144 patients. *Wound Repair Regen.* 2002;10(4):198-207.

244. Newton DJ, Khan F, Belch JJF, Mitchell MR, Leese GP. Blood flow changes in diabetic foot ulcers treated with dermal replacement therapy. *J Foot Ankle Surg.* 2002;41(4):233-237.

245. Kalani M, Jorneskog G, Naderi N, Lind F, Brismar K. Hyperbaric oxygen (HBO) therapy in treatment of diabetic foot ulcers - Long-term follow-up. *J Diabetes Complications.* 2002;16(2):153-158.

246. Klingel R, Mumme C, Fassbender T, et al. Rheopheresis in patients with ischemic diabetic foot syndrome: Results of an open label prospective pilot trial. *Ther Apher Dial.* 2003;7(4):444-455.

247. Petrofsky JS, Lawson D, Suh HJ, et al. The influence of local versus global heat on the healing of chronic wounds in patients with diabetes. *Diabetes Technol Ther.* 2007;9(6):535-544.

248. Lawson D, Petrofsky JS. A randomized control study on the effect of biphasic electrical stimulation in a warm room on skin blood flow and healing rates in chronic wounds of patients with and without diabetes. *Med Sci Monit.* 2007;13(6):CR258-263.

249. Ichioka S, Yokogawa H, Sekiya N, et al. Determinants of wound healing in bone marrow-impregnated collagen matrix treatment: impact of microcirculatory response to surgical debridement. *Wound Repair Regen.* 2009;17(4):492-497.

250. Petrofsky JS, Lawson D, Berk L, Suh H. Enhanced healing of diabetic foot ulcers using local heat and electrical stimulation for 30 min three times per week. *J Diabetes.* 2010;2(1):41-46.

251. Yang C, Weng H, Chen L, et al. Transcutaneous Oxygen Pressure Measurement in Diabetic Foot Ulcers: Mean Values and Cut-Point for Wound Healing. *J Wound Ostomy Continence Nurs.* 2013;40(6):585-589.

252. Wang A, Sun X, Wang W, Jiang K. A study of prognostic factors in Chinese patients with diabetic foot ulcers. *Diabet Foot Ankle.* 2014;5:22936.

253. Yotsu RR, Pham NM, Oe M, et al. Comparison of characteristics and healing course of diabetic foot ulcers by etiological classification: Neuropathic, ischemic, and neuro-ischemic type. *J Diabetes Complications.* 2014;28(4):528-535.

254. Kluz J, Malecki R, Adamiec R. Practical importance and modern methods of the evaluation of skin microcirculation during chronic lower limb ischemia in patients with peripheral arterial occlusive disease and/or diabetes. *Int Angiol.* 2013;32(1):42-51.

255. Brownrigg JR, Hinchliffe RJ, Apelqvist J, et al. Performance of prognostic markers in the prediction of wound healing or amputation among patients with foot ulcers in diabetes: a systematic review. *Diabetes Metab Res Rev.* 2016;32(S1):128-135.

256. Holstein P, Lassen NA. Healing of ulcers on the feet correlated with distal blood pressure measurements in occlusive arterial disease. *Acta Orthop Scand.* 1980;51(6):995-1006.

257. Rubenstein DA, Yin W, Frame MD. Chapter 2 - Fundamentals of Fluid Mechanics. In: Rubenstein DA, Yin W, Frame MD, eds. *Biofluid Mechanics.* Boston: Academic Press; 2012:11-48.

258. Struijker Boudier HA, Cohuet GM, Baumann M, Safar ME. The heart, macrocirculation and microcirculation in hypertension: a unifying hypothesis. *J Hypertens Suppl.* 2003;21(3):S19-23.

259. Barwick A, Lanting S, Chuter V. Intra-tester and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux. *Microvasc Res.* 2015;99:67-71.

260. Yamamoto-Suganuma R, Aso Y. Relationship between post-occlusive forearm skin reactive hyperaemia and vascular disease in patients with Type 2 diabetes--a novel index for detecting micro- and macrovascular dysfunction using laser Doppler flowmetry. *Diabet Med.* 2009;26(1):83-88.

261. Murray AM. *Reperfusion as a marker of success of distal revascularisation [MD Thesis]*: School of Medicine, University of Birmingham; 2013.

262. Diabetes UK. How to perform the Touch the Toes Test. In. http://www.diabetes.org.uk/Guide-to-diabetes/Complications/Feet/Touch-the-Toes-test/ Accessed 7th June 2019

263. Prompers L, Huijberts M, Apelqvist J, et al. High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. *Diabetologia.* 2007;50(1):18-25.

264. Healthcare Quality Improvement Partnership. *National Diabetes Foot Care Audit - 2014-2016.* https://www.digital.nhs.uk/catalogue/PUB23525: NHS Digital;2017.

265. Graziani L, Silvestro A, Bertone V, et al. Vascular involvement in diabetic subjects with ischemic foot ulcer: a new morphologic categorization of disease severity. *Eur J Vasc Endovasc Surg.* 2007;33(4):453-460.

266. Slim H, Tiwari A, Ahmed A, Ritter JC, Zayed H, Rashid H. Distal versus Ultradistal Bypass Grafts: Amputation-free Survival and Patency Rates in Patients with Critical Leg Ischaemia. *Eur J Vasc Endovasc Surg.* 2011;42(1):83-88.

267. Uhl C, Hock C, Betz T, Topel I, Steinbauer M. Pedal bypass surgery after crural endovascular intervention. *J Vasc Surg.* 2014;59(6):1583-1587.

268. Nakama T, Watanabe N, Haraguchi T, et al. Clinical Outcomes of Pedal Artery Angioplasty for Patients With Ischemic Wounds: Results From the Multicenter RENDEZVOUS Registry. *JACC Cardiovasc Interv.* 2017;10(1):79-90.

269. Meloni M, Izzo V, Giurato L, Gandini R, Uccioli L. Below-the-ankle arterial disease severely impairs the outcomes of diabetic patients with ischemic foot ulcers. *Diabetes Res Clin Pract.* 2019;152:9-15.

270. Raskin D, Khaitovich B, Silverberg D, Halak M, Balan S, Rimon U. Primary Retrograde Dorsalis Pedis Artery Single Access for Revascularization of Chronic Total Occlusion in Patients with Critical Limb Ischemia. *J Vasc Interv Radiol.* 2019;30(4):554-559.

271. Meyer A, Schinz K, Lang W, Schmid A, Regus S, Rother U. Outcomes and Influence of the Pedal Arch in Below-the-Knee Angioplasty in Patients with End-Stage Renal Disease and Critical Limb Ischemia. *Ann Vasc Surg.* 2016;35:121-129.

272. Lazaris AM, Salas C, Tsiamis AC, et al. Factors affecting patency of subintimal infrainguinal angioplasty in patients with critical lower limb ischemia. *Eur J Vasc Endovasc Surg.* 2006;32(6):668-674.

273. Awad S, Karkos CD, Serrachino-Inglott F, et al. The impact of diabetes on current revascularisation practice and clinical outcome in patients with critical lower limb ischaemia. *Eur J Vasc Endovasc Surg.* 2006;32(1):51-59.

274. Kudo T, Chandra FA, Ahn SS. The effectiveness of percutaneous transluminal angioplasty for the treatment of critical limb ischemia: a 10-year experience. *J Vasc Surg.* 2005;41(3):423-435; discussion 435.

275. Melliere D, Berrahal D, Desgranges P, et al. Influence of diabetes on revascularisation procedures of the aorta and lower limb arteries: early results. *Eur J Vasc Endovasc Surg.* 1999;17(5):438-441.

276. An JH, Jang YM, Song KH, et al. Outcome of percutaneous transluminal angioplasty in diabetic patients with critical limb ischaemia. *Exp Clin Endocrinol Diabetes.* 2014;122(1):50-54.

277. Wahlgren CM, Kalin B, Lund K, Swedenborg J, Takolander R. Long-term outcome of infrainguinal percutaneous transluminal angioplasty. *J Endovasc Ther.* 2004;11(3):287-293.

278. Sabeti S, Mlekusch W, Amighi J, Minar E, Schillinger M. Primary patency of long-segment self-expanding nitinol stents in the femoropopliteal arteries. *J Endovasc Ther.* 2005;12(1):6-12.

279. Laxdal E, Jenssen GL, Pedersen G, Aune S. Subintimal angioplasty as a treatment of femoropopliteal artery occlusions. *Eur J Vasc Endovasc Surg.* 2003;25(6):578-582.

280. Laxdal E, Eide GE, Wirsching J, et al. Homocysteine levels, haemostatic risk factors and patency rates after endovascular treatment of the above-knee femoro-popliteal artery. *Eur J Vasc Endovasc Surg.* 2004;28(4):410-417.

281. Neupane S, Edla S, Maidona E, et al. Long-term outcomes of patients with diabetes mellitus undergoing percutaneous intervention for popliteal and infrapopliteal peripheral arterial disease. *Catheter Cardiovasc Interv.* 2018;92(1):117-123.

282. Darling JD, Bodewes TCF, Deery SE, et al. Outcomes after first-time lower extremity revascularization for chronic limb-threatening ischemia between patients with and without diabetes. *J Vasc Surg.* 2018;67(4):1159-1169.

283. Morris DR, Singh TP, Moxon JV, et al. Assessment and validation of a novel angiographic scoring system for peripheral artery disease. *Br J Surg.* 2017;104(5):544-554.

284. Muller-Buhl U, Wiesemann A, Oser B, Kirchberger I, Strecker EP. Correlation of hemodynamic and functional variables with the angiographic extent of peripheral arterial occlusive disease. *Vasc Med.* 1999;4(4):247-251.

285. Park C, Bathula R, Shore AC, et al. Impaired post-ischaemic microvascular hyperaemia in Indian Asians is unexplained by diabetes or other cardiovascular risk factors. *Atherosclerosis.* 2012;221(2):503-507.

286. Del Guercio R, Leonardo G, Arpaia MR. Evaluation of postischemic hyperemia on the skin using laser Doppler velocimetry: Study on patients with claudicatio intermittens. *Microvasc Res.* 1986;32(3):289-299.

287. Williams DT, Price P, Harding KG. The influence of diabetes and lower limb arterial disease on cutaneous foot perfusion. *J Vasc Surg.* 2006;44(4):770-775.

288. Pardo M, Alcaraz M, Bernal FL, Felices JM, Achel GD, Canteras M. Transcutaneous oxygen tension measurements following peripheral transluminal angioplasty procedure has more specificity and sensitivity than ankle brachial index. *Br J Radiol.* 2015;88(1046):20140571.

289. Utsunomiya M, Nakamura M, Nagashima Y, Sugi K. Predictive Value of Skin Perfusion Pressure After Endovascular Therapy for Wound Healing in Critical Limb Ischemia. *J Endovasc Ther.* 2014;21(5):662-670.

290. Kawarada O, Yasuda S, Nishimura K, et al. Effect of single tibial artery revascularization on microcirculation in the setting of critical limb ischemia. *Circ Cardiovasc Interv.* 2014;7(5):684-691.

291. Mills JL, Sr., Conte MS, Armstrong DG, et al. The Society for Vascular Surgery Lower Extremity Threatened Limb Classification System: risk stratification based on wound, ischemia, and foot infection (WIfI). *J Vasc Surg.* 2014;59(1):220-234.e221-222.

292. de Graaff JC, Ubbink DT, Legemate DA, de Haan RJ, Jacobs MJHM. The usefulness of a laser Doppler in the measurement of toe blood pressures. *J Vasc Surg.* 2000;32(6):1172-1179.

293. Widmer LW, Vikatmaa P, Aho P, Lepantalo M, Venermo M. Reliability and repeatability of toe pressures measured with laser Doppler and portable and stationary photoplethysmography devices. *Ann Vasc Surg.* 2012;26(3):404-410.

294. Bonham PA, Cappuccio M, Hulsey T, et al. Are ankle and toe brachial indices (ABI-TBI) obtained by a pocket Doppler interchangeable with those obtained by standard laboratory equipment? *J Wound Ostomy Continence Nurs.* 2007;34(1):35-44.

295. Yorek MA. Vascular Impairment of Epineurial Arterioles of the Sciatic Nerve: Implications for Diabetic Peripheral Neuropathy. *Rev Diabet Stud.* 2015;12(1-2):13-28.

296. Rutherford RB, Baker JD, Ernst C, et al. Recommended standards for reports dealing with lower extremity ischemia: revised version. *J Vasc Surg.* 1997;26(3):517-538.

297. Deegan AJ, Wang RK. Microvascular imaging of the skin. *Phys Med Biol.* 2019;64(7):07tr01.

APPENDIX I: SEARCH STRATEGY FOR DISTRIBUTION OF DISEASE REVIEW

Embase 1974 to 2016 July 26, Embase Classic 1947 to 1973 and Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid

Search	Search terms	Results
1	exp Diabetes Mellitus/	1113308
	Embase 1974 to 2016 July 26	731665
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	354254
	Embase Classic 1947 to 1973	27389
2	diabetes mellitus.mp.	1092202
	Embase 1974 to 2016 July 26	713842
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	351666
	Embase Classic 1947 to 1973	26694
3	diabet*.mp.	1453418
	Embase 1974 to 2016 July 26	870060
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	553197
	Embase Classic 1947 to 1973	30161
4	exp Diabetes Mellitus, Type 1/	157232
	Embase 1974 to 2016 July 26	90436
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	66723
	Embase Classic 1947 to 1973	73
5	exp Diabetes Mellitus, Type 2/	279449
	Embase 1974 to 2016 July 26	178182
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	101256
	Embase Classic 1947 to 1973	11
6	(type adj l).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	287951
	Embase 1974 to 2016 July 26	138859

	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	146051
	Embase Classic 1947 to 1973	3041
7	(type adj one).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	2584
	Embase 1974 to 2016 July 26	1392
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1050
	Embase Classic 1947 to 1973	142
8	(type adj II).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	227760
	Embase 1974 to 2016 July 26	101204
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	124206
	Embase Classic 1947 to 1973	2350
9	(type adj two).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	2116
	Embase 1974 to 2016 July 26	1156
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	868
	Embase Classic 1947 to 1973	92
10	type-2.mp.	425004
	Embase 1974 to 2016 July 26	224585
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	198017
	Embase Classic 1947 to 1973	2402
11	type-1.mp.	436355
	Embase 1974 to 2016 July 26	220133
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	213401
	Embase Classic 1947 to 1973	2821
12	(non-insulin adj dependant adj diabetes adj mellitus).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	39
	Embase 1974 to 2016 July 26	31
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	8
	Embase Classic 1947 to 1973	0
13	niddm.mp.	14940
	Embase 1974 to 2016 July 26	8064

	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	6876
	Embase Classic 1947 to 1973	0
14	(insulin adj dependant adj diabetes adj mellitus).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	88
	Embase 1974 to 2016 July 26	68
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	20
	Embase Classic 1947 to 1973	0
15	IDDM.mp.	14630
	Embase 1974 to 2016 July 26	7850
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	6780
	Embase Classic 1947 to 1973	0
16	(peripheral adj vascular adj disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	34961
	Embase 1974 to 2016 July 26	25532
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	7889
	Embase Classic 1947 to 1973	1540
17	exp Peripheral Vascular Diseases/	1624988
	Embase 1974 to 2016 July 26	1487439
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	48262
	Embase Classic 1947 to 1973	89287
18	PVD.mp.	4940
	Embase 1974 to 2016 July 26	2983
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1945
	Embase Classic 1947 to 1973	12
19	atherosclero*.mp.	380259
	Embase 1974 to 2016 July 26	233865
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	133990
	Embase Classic 1947 to 1973	12404
20	exp Atherosclerosis/	223673
	Embase 1974 to 2016 July 26	179940
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	31667

	Embase Classic 1947 to 1973	12066
21	(lower adj limb adj arterial adj disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	226
	Embase 1974 to 2016 July 26	126
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	98
	Embase Classic 1947 to 1973	2
22	(lower adj extremity adj arterial adj disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	440
	Embase 1974 to 2016 July 26	246
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	193
	Embase Classic 1947 to 1973	1
23	exp Peripheral Arterial Disease/	149371
	Embase 1974 to 2016 July 26	138066
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	3981
	Embase Classic 1947 to 1973	7324
24	(peripheral adj arterial adj disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	20716
	Embase 1974 to 2016 July 26	10977
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	9587
	Embase Classic 1947 to 1973	152
25	PAD.mp.	49705
	Embase 1974 to 2016 July 26	28910
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	18807
	Embase Classic 1947 to 1973	1988
26	claudication.mp.	30982
	Embase 1974 to 2016 July 26	17693
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	12016
	Embase Classic 1947 to 1973	1273
27	exp Intermittent Claudication/	17685
	Embase 1974 to 2016 July 26	9355
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	7350
	Embase Classic 1947 to 1973	980

28	(critical adj ischaemia).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	859
	Embase 1974 to 2016 July 26	481
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	375
	Embase Classic 1947 to 1973	3
29	exp Ischemia/	732052
	Embase 1974 to 2016 July 26	646932
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	53923
	Embase Classic 1947 to 1973	31197
30	ulceration.mp.	63936
	Embase 1974 to 2016 July 26	33445
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	25013
	Embase Classic 1947 to 1973	5478
31	exp Skin Ulcer/	100281
	Embase 1974 to 2016 July 26	58437
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	38675
	Embase Classic 1947 to 1973	3169
32	(anatomical adj1 distribution).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	3913
	Embase 1974 to 2016 July 26	2049
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1694
	Embase Classic 1947 to 1973	170
33	(distribution adj2 disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	3867
	Embase 1974 to 2016 July 26	2205
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1567
	Embase Classic 1947 to 1973	95
34	(extent adj2 disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	14300
	Embase 1974 to 2016 July 26	8190
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	5845
	Embase Classic 1947 to 1973	265

35	(disease adj2 location).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	3160
	Embase 1974 to 2016 July 26	2045
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1092
	Embase Classic 1947 to 1973	23
36	(distribution adj1 pattern).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	22369
	Embase 1974 to 2016 July 26	11520
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	10107
	Embase Classic 1947 to 1973	742
37	(pattern* adj2 arter* adj disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	16
	Embase 1974 to 2016 July 26	9
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	6
	Embase Classic 1947 to 1973	1
38	(distribution adj2 disease).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	3867
	Embase 1974 to 2016 July 26	2205
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1567
	Embase Classic 1947 to 1973	95
39	(anatomical adj distribution).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]2031	3879
	Embase 1974 to 2016 July 26	2031
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	1679
	Embase Classic 1947 to 1973	169
40	(computed adj tomography adj angiogra*).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	11979
	Embase 1974 to 2016 July 26	7019
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	4960
	Embase Classic 1947 to 1973	0
41	exp Tomography, X-Ray Computed/	1082569
	Embase 1974 to 2016 July 26	735608
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub	346944

	Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	
	Embase Classic 1947 to 1973	17
42	CTA.mp.	20590
	Embase 1974 to 2016 July 26	13007
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	7542
	Embase Classic 1947 to 1973	41
43	(magnetic adj resonance adj angiogr*).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	51966
	Embase 1974 to 2016 July 26	29759
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	2207
	Embase Classic 1947 to 1973	0
44	exp Magnetic Resonance Angiography/	47625
	Embase 1974 to 2016 July 26	28117
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	19508
	Embase Classic 1947 to 1973	0
45	MRA.mp.	17643
	Embase 1974 to 2016 July 26	10903
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	6731
	Embase Classic 1947 to 1973	9
46	exp Angioplasty/	132373
	Embase 1974 to 2016 July 26	74904
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	57373
	Embase Classic 1947 to 1973	96
47	angioplasty.mp.	155315
	Embase 1974 to 2016 July 26	84658
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	70561
	Embase Classic 1947 to 1973	96
48	angiogra*.mp.	607552
	Embase 1974 to 2016 July 26	330601
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	261871
	Embase Classic 1947 to 1973	15080
49	exp Angiography/	589727

	Embase 1974 to 2016 July 26	346952
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	216190
	Embase Classic 1947 to 1973	26585
50	exp Ultrasonography, Doppler/	92873
	Embase 1974 to 2016 July 26	30325
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	62329
	Embase Classic 1947 to 1973	219
51	(Doppler adj ultrasound).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	29208
	Embase 1974 to 2016 July 26	17388
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	11757
	Embase Classic 1947 to 1973	63
52	(arterial adj duplex).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, ui]	242
	Embase 1974 to 2016 July 26	149
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	93
	Embase Classic 1947 to 1973	0
53	exp Ultrasonography, Doppler, Duplex/	55717
	Embase 1974 to 2016 July 26	32340
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	23359
	Embase Classic 1947 to 1973	18
54	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15	2207660
	Embase 1974 to 2016 July 26	1233883
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	935467
	Embase Classic 1947 to 1973	38310
55	16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31	2037322
	Embase 1974 to 2016 July 26	1621995
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	316778
	Embase Classic 1947 to 1973	98549
56	32 or 33 or 34 or 35 or 36 or 37 or 38 or 39	1883742

	Embase 1974 to 2016 July 26	1157143
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	697678
	Embase Classic 1947 to 1973	28921
57	40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53	47198
	Embase 1974 to 2016 July 26	25758
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	20151
	Embase Classic 1947 to 1973	1289
58	**54 and 55 and 56 and 57**	**151**
	Embase 1974 to 2016 July 26	120
	Ovid MEDLINE(R) and MEDLINE(R) Daily 1946 to Present, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid	29
	Embase Classic 1947 to 1973	2

APPENDIX II: SEARCH STRATEGY FOR WOUND HEALING REVIEW

MESH Search

Search	Search terms
1	microcirculation
2	wound healing
3	diabetic foot
4	skin ulcer
5	Laser Doppler flowmetry
6	blood gas monitoring, transcutaneous
7	Microscopic angioscopy
8	Xenon radioisotopes
9	3 or 4
10	5 or 6 or 7 or 8
11	1 and 2 and 9 and 10
12	11 limited to English and humans

Keyword search

Search	Search terms
1	capillar* or venule* or arteriole* or small adj2 vessels or skin microcirculation or skin blood supply or skin blood flow or microangiopath* or microcircula* disturbance*
2	transcutaneous adj3 oxygen* or transcutaneous PO2 or transcutaneous oximetry or transcutaneous adj3 carbon dioxide or TcPO2 or TcPCO2
3	laser Doppler* or laser Doppler fluxmetry or laser Doppler Imaging or laser Doppler velocimetry or laser Doppler flux or LDF or LDI or Post occlusive reactive hyperaemia or PORH
4	capillary microscopy or capillary pressure or capillaroscopy
5	skin adj2 pressure or skin adj2 perfusion
6	xenon clearance or isotope clearance or haemodynamic test* or venoarteriolar response
7	2 or 3 or 4 or 5 or 6
8	wound* or ulcer* or ulcer healing or tissue loss or healing or wound complication* or non-healing or nonhealing or granulation tissue or amputat*
9	1 and 7 and 8
10	9 limited to English and humans only

APPENDIX III: PLAN FOR COHORT STUDY AS PRESENTED IN RESEARCH PROPOSAL

HYPOTHESIS

Improving the microcirculation of the foot in patients with diabetic foot disease improves wound healing and degree of peripheral neuropathy.

MAIN QUESTIONS TO BE ADDRESSED

To address the hypothesis, I am asking the following questions.

- What is the level of improvement in the microcirculation at the time of wound healing in patients with DM and PAD?
- Which of PCA or peripheral bypass surgery (PBS) provide a greater improvement in the microcirculation?
- Is there any improvement in the neurological status of patients with neuro-ischaemic ulceration who have undergone revascularisation?
- Is there a change in mean foot temperature immediately following revascularisation?

OUTCOME MEASURES

Primary outcome

- Evidence of difference in the level of change in the time to maximum flux between, before, and after PCA or PBS.

Secondary outcomes

- Evidence of difference in the level of change in the time to maximum flux between when ulceration active and when ulceration healed in patients with DM and no significant PAD.
- Time to wound healing
- Time to major amputation
- The change in skin perfusion pressure (SPP) at the end of the study
- The change in toe blood pressure index (TBPI) at the end of the study
- The change in VPT at the end of the study
- The change in monofilament detection at the end of the study

- The change in Ipswich touch test (ITT) at the end of the study
- The change in neuropathy total symptom score - 6 (NTSS-6) at the end of the study

End of the study is defined as either complete wound healing, major amputation of the study limb or time for the conduct of the study elapsing.

RESEARCH PLAN

To conduct an observational cohort study of patients with active diabetic foot disease who require lower limb revascularisation, either by arterial bypass graft or angioplasty. This will be based at three NHS hospital sites in the West Midlands.

STUDY GROUPS

Peripheral bypass surgery

Patients with active tissue loss and PAD confirmed clinically and on Duplex Ultrasound (DUS), Computed Tomography Angiography (CTA) or Magnetic Resonance Angiography (MRA) who on assessment by their named consultant require PBS. This group will be further divided into those with DM and those without.

Percutaneous angioplasty (PCA)

Patients with active tissue loss and PAD confirmed clinically and on DUS, CTA or MRA who on assessment by their named consultant require PCA. This group will be further divided into those with DM and those without.

Diabetic tissue loss without PAD (DTL)

Patients with type I or II DM and active tissue loss without clinically significant PAD.

INCLUSION CRITERIA

For participants requiring either PBS or PCA with DM and PAD.

- Type I or II DM requiring medical therapy
- Active tissue loss
- Require revascularisation (open or endovascular)
- Aged between 18 and 99 years
- Able to give informed consent

For participants requiring either PBS or PCA with PAD but no DM

- As the first group apart from no previous history of DM and a normal random glycated haemoglobin level (HBA1c) within the last six-months.

For participants with DM and no PAD

- As the first group apart from no evidence of PAD requiring revascularisation.

EXCLUSION CRITERIA

- Unwilling or unable to give informed consent
- Previous distal arterial bypass on the ulcerated leg.
- Angioplasty to the ulcerated leg during this period of active ulceration.
- Significant non-reconstructable PAD.
- Previously diagnosed peripheral neuropathy secondary to a cause other than DM.
- Medically unfit for the procedure.
- Pregnancy or breast-feeding
- Vitamin B_{12} deficiency.
- Alcohol dependency.
- Hypothyroidism.
- Renal failure requiring renal replacement therapy.

PATIENT IDENTIFICATION

Potential patients will be identified predominantly from diabetic foot clinics, vascular clinics and inpatient admissions at the study sites. The patients will be identified by the investigators and other doctors on the vascular and diabetes teams. As it will not be possible for a member of the study to be present in person at all relevant clinics review of referral documentation to clinics, theatre lists and angioplasty lists will be conducted. This will only be performed at the University Hospitals Birmingham site as I only have clinical responsibilities at this site and so am already in receipt of the relevant lists. If a potential patient is identified from these lists a patient invitation letter (Appendix IV) and a patient information sheet (Appendix V) will be posted to their home address. The patient invitation letter contains a reply slip and stamped addressed envelope which will allow the patient to express their desire or otherwise to be included in the study. If I do not receive a reply slip, that states the patient does not wish to be contacted, then, after 1-2 weeks the letter will be followed-up with a phone call. On the day of their procedure or next clinic appointment, the patient will be met by myself to answer any questions and confirmed that they still wish to participate. If appropriate, consent will be taken, and the first assessment carried out at this time.

CONSENT

All participants will be verbally invited to take part in the study by the clinical team providing their care. They will be given written information with regards to the purpose and design of the study (Appendix V). They will then be invited to participate and if agreed, consented using a standardised consent form (Appendix VI). This will be performed and undertaken by a member of the study team.

Consent to participate will include consent to the use of data obtained during their participation in the final analysis. Participants will be free to leave the study with only a verbal request, though their non-identifiable data will continue to remain within the study unless a written request is made to the contrary. This will be made clear at the time of consent.

ASSESSMENT

Timing of assessments

For the PBS and PCA groups, the initial assessment will be carried out within the 24 hours before their procedure. For the DTL group, the initial assessment will be carried out following informed consent being gained. If possible, this will be during the same attendance.

Post-procedure assessment for the PBS and PCA groups will occur at initial follow-up clinic appointment one month following their procedure. The infrared temperature measurement will be performed only once post procedure, within the first 24 hours after revascularisation. Subsequent re-examination will occur monthly (within a week) at the hospital appointment that falls nearest this time. This includes vascular, diabetes and podiatry appointments. Reassessment will continue until the ulcer is decided to have clinically healed, the limb is amputated, or the end of the study is reached.

The DTL group will have repeat assessments monthly until the ulcer is decided to have clinically healed, the limb is amputated, or the end of the study is reached.

Procedure for data collection

The patient will be asked to arrive for their appointment having not eaten for the last 2 hours and not had any caffeinated drinks since the proceeding night. They will be assessed in a temperature controlled clinic room (22^0C +/- 2^0C) or if for inpatients this is not possible the temperature will be recorded. The patient will be positioned on a couch in a semi-recumbent position so they can acclimatise to the temperature of the room. During the first fifteen minutes, the NTSS-6 questionnaire will be completed, and monofilament, ITT and VPT testing carried out on both feet. The characteristics of the ulcer/s will also be described. During the second fifteen minutes, the patient will be asked to relax, and the laser Doppler fluxmetry (LDF) probes will be attached. The protocol for each measurement is as follows.

10g Monofilament

Three areas on each foot will be tested. These are plantar aspects of the hallux, and base of the third and fifth metatarsals[18,120]. A positive or negative response for each area will be recorded. Abnormal will be defined as an inability to detect the monofilament in one area. If it is not possible to test any of the areas due to amputation or ulceration, this will be recorded.

Ipswich Touch Test

The tips of the 1st, 3rd and 5th toes of each foot will be tested in the order described by Diabetes UK in their leaflet "How to do the Touch the Toes Test"[262]. A positive or negative response for each area will be recorded. If it is not possible to test any of the areas due to amputation or ulceration, this will be recorded.

VPT

VPT will be assessed on the pulp of the hallux or next dominant toe if amputation has occurred in the past or the toe is necrotic. A neurothesiometer will be used. The first measurement will be taken increasing from zero to maximum and the level at which vibration is detected recorded. The second measurement will be taken from maximum to zero and the level at which vibration disappears recorded. An average of these two readings will be taken. If no vibration is detected, then 50V will be recorded.

Ulcer description

The ulcer/s will be described in the same way as used by the Eurodiale study [263]. Area of the ulcer will be determined by multiplying the largest diameter by the second largest diameter perpendicular to the first. Depth will be described as superficial or deep: a superficial ulcer is a full-thickness lesion of the skin not extending through the subcutis, and a deep ulcer is a lesion of the skin extending through the subcutis. An infection will be diagnosed if two or more of the following signs are present: frank purulence, local warmth, erythema, lymphangitis, oedema, pain, fever and foul smell. The anatomical location of the ulcer/s will also be described. The ulcer will be described as healed when complete epithelialisation has occurred.

Laser Doppler Fluxmetry

The patient's feet will be placed on a pillow to stabilise them and a laser Doppler probe placed on the pulp of the hallux or remaining dominant toe and dorsal surface of the foot between the 2nd and 3rd metatarsals. A cuff will be placed around the patient's ankle. The LDF will then be turned on and baseline flux measured for one minute, the cuff will then be

inflated for three minutes followed by rapid deflation and monitoring of the response for five minutes.

A toe cuff placed around the great toe or remaining dominant toe will then replace the cuff around the ankle. Toe blood pressure will then be measured.

Following this, the low-profile probe will be placed adjacent to the ulcerated tissue and a cuff placed over. SPP will then be measured with slow deflation of the cuff.

Probes will then be transferred to the other leg and the process repeated. Finally, the probes will be moved, one to the ventral surface of each forearm and a cuff placed around the upper arm and brachial pressure measured. This whole process will take approximately thirty minutes.

The data obtained will be processed using the Moor VMS-PC™ software.

Demographics

Demographics that will be collected at first appointment are listed below.

- Age
- Sex
- Type of DM
- Duration of DM
- Current medications
- Recent blood results including glycosylated haemoglobin, cholesterol, thyroid stimulating hormone, free T_4 and vitamin B_{12}.

DATA COLLECTION AND MANAGEMENT

Data will be recorded directly onto a standard data collection proforma, and the LDF data will be recorded onto an encrypted computer. The analysis will also take place on this computer.

Data storage

All identifiable patient data will be collected and stored on an encrypted computer to be kept locked within secured premises within the vascular department. This will be maintained as per data protection and GCP guidelines then stored for the required five years before destruction.

Source data

Source data will be kept within the source data file that will, in turn, be kept locked within a secure office locked and secure within the Vascular Department. It will be accessible only by those signatories within the research group and by the sponsors as requested.

DATA ANALYSIS PLAN

Sample size calculation

Data previously obtained at our institution[261] found the following values for the mean (Standard Deviation) times (in seconds) to maximum post-occlusive reactive hyperaemia:

Bypass Patients (N=29): Preoperative = 100 (22); Postoperative = 59 (38)

PCA Patients (N=9): Preoperative = 69 (27); Postoperative = 55 (30)

The average improvement was 41 seconds (100-59) in the bypass group and 14 seconds (69-55) in the PCA groups. Hence, the difference in the improvement between the two groups was 27 seconds (41-14).

The pooled standard deviation from this data is 30. If the pre and post-operative measurements on the same patient are assumed to be independent, the standard deviation of the change will be 30 times the square root of 2, i.e. 42

The study was powered based on a t-test, assuming a standard deviation of 42. For a detectable difference of 27, a sample size of 40 patients per group (i.e. 80 total) would be sufficient for 80% power at 5% alpha.

There is no equivalent data available with which it would be possible to calculate an appropriate sample size for the group with DM and no PAD. For this reason, they have not been included in the primary outcome. A sample size of 20 will be able to detect a difference of 33 seconds between the initial measurement and the point of healing. This was agreed to be reasonable as a consensus within the research group.

Due to the demographics of patients being investigated previous experience suggests that a high dropout rate (up to 20%) should be expected. This increases the size of our study groups to 48 for both the bypass and angioplasty groups and 24 for the control group. This brings our total sample size to 120 participants.

Proposed analyses

The initial comparison will be made between the three main study groups PCA, PBS and DTL, as described in Section 5.6.2. For the PCA and PBS group, there will then be a further comparison of those with DM and those without.

The outcome variables considered are made up of three different types of data, each of which will be analysed using a different approach (Table III-1).

Continuous data (e.g. PORH)

Initially, the data will be tested for normality, as this is an assumption underlying parametric analyses. If the data are normally distributed, then a t-test will be performed to compare means for mean temperature measurements and a repeated measures ANOVA model will be produced, to compare the serial measurements of the outcome between the three study arms. A second analysis will then be performed, including other potentially relevant variables, to account for the potentially confounding effects of baseline differences between the treatment arms.

Binary data (e.g. Wound healing)

For binary data, the outcome rates will initially be compared between the study arms using Fisher's exact tests. Multivariable binary logistic regression models will then be produced, to account for the effects of other potentially confounding factors.

Time to event data (e.g. Major amputation)

Time to event data will be analysed using survival analysis methods. Kaplan-Meier survival curves will be produced for each of the study arms, and comparisons made using log-rank tests. Cox regression models will then be used to account for the effects of other relevant factors.

Table III-1: Summary of proposed data analysis

Data type	Variables	Analysis
Baseline value	PORH VPT SPP TBPI	One way ANOVA/Kruskal-Wallis

	NTSS-6	
Repeated value/ Percentage change from baseline	PORH VPT SPP TBPI NTSS-6	Repeated measure ANOVA
Event	Wound healing Major amputation	Fisher exact test/binary logistic regression
Time to event	Wound healing Major amputation	Survival curve with Log-rank test/Cox regression
Categorical	VPT (≤25 to >25V) Monofilament (0 to ≥1 points detected) ITT (≤2 to >2 points detected) NTSS-6 (≤6 to >6 scored)	Binary logistic regression

PORH=Post-occlusive reactive hyperaemia, VPT=Vibration perception threshold, SPP=Skin perfusion pressure, TBPI=Toe blood pressure index, NTSS-6= Neuropathy Total Symptom Score – 6, ITT=Ipswich touch test

Missing data

All available data will be included in the analysis. This makes the assumption that missing values can be assumed to be missing at random. However, this may not be the case.

In order to verify this, comparisons will be made between those patients with data available and those with missing values. If no significant differences in the demographic or clinical factors of these groups are detected, then it will be assumed that the assumption of data missing at random has been met. If differences between those patients with and without missing data are present, then the degree of this bias will be compared between the treatment groups. If the levels of bias are similar in the three groups, then the effect of missing data on the conclusions of the final analysis can be assumed to be negligible.

If significant bias is present, and to a different degree in the three groups, this will be quantified as a limitation in the results, and the conclusions of the analyses interpreted with caution.

We anticipate that the data collection from follow-up appointments will be relatively complete, with the majority of missing data being as a result of patients either not attending, or choosing to be withdrawn from the study. If this proves to be the case, a sensitivity analysis will be performed. This will only consider data from a truncated period of follow-up, in which the number of missing values are minimal. Whilst this will not have as much statistical power as the main analysis (on account of the smaller amount of data), the effect sizes observed should be similar. Where this is the case, the conclusions of the main analysis will be accepted. However, if there are discrepancies between the main and sensitivity analyses, the conclusions will be interpreted in light of this potential bias.

ETHICAL CONSIDERATIONS

Local Research Ethics Committee approval was sought from the South Birmingham Research Ethics Committee. Approval was granted on 26th June 2014. Approval was also sought from the Research and Development departments of the three study sites, University Hospitals Birmingham NHS Foundation Trust, Dudley Group NHS Foundation Trust and Sandwell and West Birmingham Hospitals NHS Trust. These were approved on 24th July 2014, 18th July 2014 and 30th September 2014 respectively.

All research was conducted in line with the World Medical Association Declaration of Helsinki. All members of the research group received Good Clinical Practice Training as per the National Institute for Health Research, and the research was conducted in line with the

same principles. Funding to purchase the equipment (the laser Doppler equipment) for the study was sourced from the Vascular surgery research fund held by the Queen Elizabeth Hospital Birmingham Charity and not from commercial sources.

APPENDIX IV: PATIENT INVITATION LETTER

University Hospitals Birmingham NHS
NHS Foundation Trust

Mindelsohn Way,
Edgbaston,
Birmingham,
B15 2GW

Dear Sir/Madam,

As you may be aware University Hospitals Birmingham NHS Foundation Trust is actively involved in clinical research within many medical specialities. In the Vascular Surgery department we are currently conducting a study called **"What is the relationship of revascularisation and improvement in microcirculation to wound healing and peripheral neuropathy in diabetic foot disease? An observational cohort study."** You may be suitable to be included in this study and I have attached some further information to this letter.

I will be phoning you before your next hospital appointment to discuss this with you, or if you prefer please return the attached reply slip in the provided stamped addressed envelope. Alternatively you may contact me by the methods below.

Office:
Email:

Yours Sincerely

Danielle Lowry MBChB, MRCS
Vascular Research Registrar

Reply Slip

Name: _____

Date of Birth: ____/____/_____

Address: _____

_____

_____

_____

Postcode: _____

☐ I am happy to be contacted to discuss this study further

☐ I do not wish to participate in this study.

Signature: _____ Date: _____

APPENDIX V: PATIENT INFORMATION SHEET

University Hospitals Birmingham NHS
NHS Foundation Trust

Invitation to participate in research

What is the relationship of revascularisation and improvement in microcirculation to wound healing and peripheral neuropathy in diabetic foot disease? An observational cohort study.

1. Dear Sir / Madam

We would like to invite you to take part in the above study. This is because you have a foot ulcer and need a procedure (angioplasty or bypass surgery) to improve the blood supply to your leg. You may have also been asked if you have a foot ulcer and suffer from diabetes but your blood supply is good.

2. What is an angioplasty or bypass?

An angioplasty is where a small balloon is inserted in your artery and blown up at the section that is narrowed. This widens the affected section of artery, occasionally a stent, a fine wire cage, may be needed to help the artery stay open. The balloon is normally inserted at the groin and, on a wire, carefully threaded down to the affected area. This procedure is done in the x-ray department and you can usually go home on the same day.

Bypass surgery involves diverting blood around the blocked or narrowed section of artery. To do this the surgeon uses one of the veins in your leg and attaches it to the artery above and below the narrow section. This is done under a general anaesthetic (you are asleep during the operation) and you will be in hospital for about five days after the operation

3. What is this research on?

We are conducting research into diabetes and the risk of developing foot ulcers. To help us understand the changes that happen in the very small blood vessels of the skin when ulcers heal we are looking for volunteers who fall into three different groups. Patients in all three groups suffer from foot ulcers.

- Patients with diabetes who need either an angioplasty or bypass surgery to improve the blood supply in their leg.
- Patients with diabetes who already have good blood supply in their leg.
- Patients without diabetes who need either an angioplasty or bypass surgery to improve the blood supply in their leg.

We aim to recruit 120 patients in total. Volunteers in each group will all have the same tests performed. These tests look at how the very small vessels in the foot react to changes in the blood supply and also at sensation in the foot. By performing these tests before any procedure to improve the blood supply and then repeating them monthly until the ulcer heals we hope to gain information about the level of function of the small vessels required to make ulcers heal. If we know about the levels required to make ulcers heal then we may be able to predict when ulcers are about to occur and intervene earlier in the future.

4. What are the tests?

The tests involve putting sensors on the toes and around the ulcer. These measure the blood flow in the very small vessels in the skin. To see how these vessels respond to changes in flow to the larger vessels we measure how the flow changes after inflating a blood pressure cuff around the thigh or ankle. We need to keep the cuff inflated for 3 minutes after which it is deflated rapidly and we watch the change in blood flow in your foot for 5 minutes. After that another smaller cuff will be placed around your big toe (or a smaller one if you have had a previous amputation) and blown up for a short time, just like when you have your blood pressure on your arm. Finally a cuff will be placed over the area of your ulcer and only very briefly inflated and then deflated to see how much blood pressure is required to restore blood flow to the skin in this area. In addition to these tests we will also test how

well your nerves in the feet are working by checking sensation and ask you questions about symptoms of peripheral neuropathy (problems with nerves in the feet and legs) and check your blood pressure in both arms.

If you are going to have an angioplasty or bypass we will also take photos of your feet using a special infrared camera to help measure the temperature. These photos will be taken at the same time as the other tests and then again soon after your procedure, before you go home.

Picture of the cuff and a sensor in position. *Image courtesy of Moor Instruments.*

5. How long will the tests take?

The tests take about an hour to perform; this includes fifteen minutes in which you will be left to lie down relaxing in a quiet room. This is because the tests are more accurate if you have had time to become used to the temperature of the room and have not just been walking. To also aid with the accuracy of the tests we will ask that you arrive at your appointment having not had anything to eat or any caffeinated drinks for two hours. We also ask that you do not smoke (including e-cigarettes) for two hours before your appointment. If you have diabetes we will understand if it is not possible to avoid eating during this time but please let us know when you arrive. As soon as the tests are over you will be free to leave the clinic and carry on your day as normal.

6. What are the risks of participating?

There are no risks to you from participating. Your treatment and condition will remain unchanged by the tests we are performing; you will still see your consultant

as you would if you were not involved in the study. The tests are all safe and harmless tests, some of which you may have already experienced. Some people experience some mild discomfort while the cuff is inflated but this should not be any more than when you get your blood pressure checked.

7. What are the benefits of participating?

There are no medical benefits to you personally from participating in this study. Your current treatment remains unchanged. However, you will be given the chance to learn more about your condition and how it affects your body. Most importantly, you will be helping to improve our knowledge and understanding about how the blood vessels and nerves are affected in people with the condition. This in turn will hopefully help us to improve care and treatment for people with ulcers in the future.

8. How long will I be involved with the study?

You will be involved up until your ulcer heals or you require another operation on your foot. How long this takes is different with every person but the study is not currently planned to continue beyond 3 years. However if at any time you wish to leave the study then that is fine. If that happens we will ask that we be allowed to continue using any data we have already gathered. If you do not wish that to happen then you need to let us know in writing.

9. What happens to the information gathered?

All information given by you is confidential and anonymous. It is kept in conjunction with data protection regulations. The research team will only use it for the purposes of the study. At the end of the study you will be sent a brief summary of the results of the research and what it means, should you wish. The data and information will only be kept for three years following completion of the study after which it will be destroyed. The results of the study will be published in medical journals to enable doctors to better understand the risks of ulcers and how to reduce the risks in the future. I would like to personally thank you for your time so far and hope you will be keen to participate and help in this research. If you wish to discuss your potential

involvement in this research with somebody independent please contact the Patient Advice and Liaison Service (PALS).

Office: 0121 3713280

Email: PALS@uhb.nhs.uk

Miss Danielle Lowry MBChB, MRCS

Vascular Research Registrar

Email: [redacted]

Office: [redacted]

APPENDIX VI: PATIENT CONSENT FORM

University Hospitals Birmingham NHS
NHS Foundation Trust

Mindelsohn Way,
Edgbaston,
Birmingham,
B15 2WB

Patient Consent Form

What is the relationship of revascularisation and improvement in microcirculation to wound healing and peripheral neuropathy in diabetic foot disease? An observational cohort study.

Contacts

Research registrar (co-investigator): Danielle Lowry,

Office:

Email:

Patient Advice and Liaison Services (PALS)

Email: PALS@uhb.nhs.uk

Telephone: 0121 3713280

Patient ID:_____ Initials:_____ Date of Birth:_____

	Initials
1. I confirm that I have read and understand the information sheet dated _____version_____ for this study.	_____
2. I have had the opportunity to ask questions.	_____
3. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected.	_____
4. I understand that should I wish to withdraw from the study, the data collected from me will be used in analysing the results unless I specifically withdraw consent for this.	_____

5. I understand that records that identify me will be kept confidential and not be made publically available. If the results of the study are published my identity will remain confidential. _____

6. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. _____

7. I consent to the storage including electronic storage, of personal information for the purposes of the study only. _____

8. I agree to take part in this study _____

9. I agree that my GP, or any other doctor treating me will be notified of my participation in the study. _____

10. I would like to be contacted and informed of the findings of the study after its completion (please circle). Yes / No

Name of Patient	Patient's signature and date
_____	_____
Name of investigator receiving consent	Investigator's signature and date of signing
_____	_____

Original to be retained and filed in the site file. 1 copy to patient, 1 copy to be filed in patient notes.

APPENDIX VII: POST OCCLUSIVE REACTIVE HYPERAEMIA TABLES WITH ALL VARIABLES

Table I: Post occlusive reactive hyperaemia results at baseline by group

		Diabetic Tissue Loss Median (IQR) (n=14)	Percutaneous Angioplasty Median (IQR) (n=9)	p-value*
Study Toe	**Resting Flux (flux)**	53.15 (35.20-104.00)	74.50 (66.00-106.60)	0.277
	Biological Zero (flux)	4.70 (3.10-7.20)	3.80 (3.20-4.50)	0.781
	Maximum Flux (flux)	162.95 (107.70-212.40)	84.60 (74.50-168.30)	0.159
	Maximum Flux/Biological Zero	34.35 (21.21-58.72)	22.27 (15.26-41.18)	0.336
	Maximum Flux/Resting Level	2.76 (1.75-5.24)	1.27 (1.13-1.74)	**0.016**
	Time to Recovery (s)	1.26 (0.83-1.73)	3.58 (2.58-45.13)	**0.013**
	Time to Maximum Flux (s)	13.40 (3.68-73.85)	220.80 (200.20-288.78)	**0.002**
	Time to Half Recovery (s)	20.58 (13.45-78.28)	213.74 (140.11-261.53)	**0.005**
Study Dorsum	**Resting Flux (flux)**	16.80 (8.70-22.90)	28.70 (22.60-37.40)	**0.013**
	Biological Zero (flux)	4.60 (3.40-4.90)	5.80 (4.10-10.20)	0.201
	Maximum Flux (flux)	45.75 (23.30-63.20)	49.00 (37.80-86.50)	0.403
	Maximum Flux/Biological Zero	9.43 (6.98-17.11)	9.61 (8.04-15.09)	0.975
	Maximum Flux/Resting Level	2.63 (1.70-3.85)	1.76 (1.55-2.15)	0.124
	Time to Recovery (s)	1.03 (0.75-2.05)	2.68 (1.70-4.33)	**0.028**
	Time to Maximum Flux (s)	8.05 (3.45-17.55)	220.28 (93.65-279.80)	**<0.001**
	Time to Half Recovery (s)	13.64 (8.40-20.48)	171.00 (82.31-282.03)	**<0.002**
Non-study	**Resting Flux (flux)**	51.00 (32.80-95.60)	37.40 (21.90-78.50)	0.400

Toe	**Biological Zero (flux)**	3.70 (3.10-9.10)	3.80 (3.10-5.50)	0.971
	Maximum Flux (flux)	124.40 (49.10-178.60)	106.10 (38.70-147.10)	0.400
	Maximum Flux/Biological Zero	18.02 (5.40-29.78)	17.09 (7.04-40.87)	0.743
	Maximum Flux/Resting Level	2.26 (1.32-2.89)	2.09 (1.82-2.57)	0.856
	Time to Recovery (s)	1.51 (1.00-18.88)	2.45 (1.78-3.75)	0.287
	Time to Maximum Flux (s)	38.98 (12.68-141.05)	54.80 (13.95-127.43)	0.689
	Time to Half Recovery (s)	53.20 (23.48-141.25)	101.98 (17.4-133.98)	1.000
Non-study	**Resting Flux (flux)**	9.00 (7.60-10.90)	16.20 (12.60-47.50)	**0.003**
Dorsum	**Biological Zero (flux)**	4.30 (3.10-5.40)	5.60 (4.20-9.10)	0.110
	Maximum Flux (flux)	23.55 (13.60-36.40)	73.20 (22.30-97.30)	**0.016**
	Maximum Flux/Biological Zero	4.01 (3.61-6.91)	10.69 (5.30-13.92)	**0.046**
	Maximum Flux/Resting Level	2.21 (1.75-2.80)	2.23 (1.54-4.59)	0.856
	Time to Recovery (s)	0.91 (0.68-1.48)	1.75 (1.53-2.50)	**0.010**
	Time to Maximum Flux (s)	15.40 (3.58-116.90)	70.68 (31.10-138.60)	0.149
	Time to Half Recovery (s)	22.03 (7.43-56.18)	113.13 (53.18-139.78)	**0.037**

*Independent-Samples Mann-Whitney U Test

Table II: Post occlusive reactive hyperaemia results at last visit by group

		Diabetic Tissue Loss Median (IQR) (n=11)	Percutaneous Angioplasty Median (IQR) (n=7)	p-value*
Study Toe	**Resting Flux (flux)**	104.80 (32.30-138.90)	33.80 (24.70-84.45)	0.109
	Biological Zero (flux)	3.70 (2.70-7.30)	5.75 (3.70-7.80)	0.351
	Maximum Flux (flux)	170.00 (128.10-269.50)	80.35 (54.05-138.10)	**0.007**
	Maximum Flux/Biological Zero	37.69 (23.27-90.92)	22.21 (4.75-27.32)	**0.041**
	Maximum Flux/Resting Level	2.58 (1.39-3.97)	1.94 (1.27-2.62)	0.492
	Time to Recovery (s)	1.33 (0.98-6.35)	3.04 (1.89-7.20)	0.272
	Time to Maximum Flux (s)	27.08 (8.50-154.38)	54.50 (34.73-158.00)	0.442
	Time to Half Recovery (s)	36.13 (15.18-154.38)	72.31 (35.61-158.66)	0.545
Study Dorsum	**Resting Flux (flux)**	16.20 (10.70-29.40)	22.80 (15.00-30.40)	0.536
	Biological Zero (flux)	3.80 (3.30-4.60)	5.40 (4.60-6.50)	**0.027**
	Maximum Flux (flux)	32.90 (21.10-87.30)	38.00 (30.50-60.40)	1.000
	Maximum Flux/Biological Zero	7.14 (5.90-21.46)	7.06 (4.72-9.95)	0.536
	Maximum Flux/Resting Level	1.99 (1.68-3.01)	1.77 (1.20-3.34)	0.479
	Time to Recovery (s)	0.93 (0.80-1.53)	2.40 (0.78-4.53)	0.179
	Time to Maximum Flux (s)	13.50 (4.95-20.38)	127.78 (30.08-227.13)	**0.015**
	Time to Half Recovery (s)	16.28 (10.30-39.65)	128.93 (43.38-228.68)	0.020
Non-study Toe	**Resting Flux (flux)**	109.30 (21.00-184.50)	34.60 (25.10-54.30)	0.375
	Biological Zero (flux)	4.00 (2.60-11.50)	4.80 (3.20-6.50)	0.791
	Maximum Flux (flux)	220.00 (153.60-273.40)	83.90 (56.80-208.80)	0.085
	Maximum Flux/Biological Zero	38.40 (12.24-85.43)	22.43 (17.76-43.50)	0.425
	Maximum Flux/Resting Level	2.01 (1.41-4.04)	2.27 (1.50-3.85)	1.000

	Time to Recovery (s)	2.03 (1.20-2.83)	2.38 (1.75-3.93)	0.375
	Time to Maximum Flux (s)	18.83 (11.83-215.65)	35.33 (15.85-146.28)	0.596
	Time to Half Recovery (s)	23.70 (19.33-216.80)	76.13 (20.10-163.45)	0.791
Non-study Dorsum	**Resting Flux (flux)**	15.20 (8.00-17.90)	15.60 (13.70-20)	0.660
	Biological Zero (flux)	3.90 (3.50-5.00)	4.75 (3.90-5.90)	0.301
	Maximum Flux (flux)	24.80 (18.60-54.00)	30.00 (21.00-55.60)	0.884
	Maximum Flux/Biological Zero	6.09 (5.27-10.81)	5.41 (4.96-6.56)	0.404
	Maximum Flux/Resting Level	2.57 (1.22-3.67)	1.74 (1.32-4.30)	0.884
	Time to Recovery (s)	0.95 (0.30-1.43)	2.90 (1.50-3.58)	**0.048**
	Time to Maximum Flux (s)	15.40 (12.13-215.65)	119.03 (42.43-265.28)	0.122
	Time to Half Recovery (s)	29.60 (15.60-216.83)	120.69 (42.55-266.98)	0.149

*Independent-Samples Mann-Whitney U Test

Table III: Percentage change in Post occlusive reactive hyperaemia between baseline and last visit by group

		Diabetic Tissue Loss Median (IQR) (n=11)	Percutaneous Angioplasty Median (IQR) (n=7)	p-value*
Study Toe	**Resting Flux (flux)**	98.11 (-32.85-447.37)	-49.07 (-62.14-38.68)	0.051
	Biological Zero (flux)	-5.71 (-16.67-54.17)	18.32 (-15.97-87.18)	0.657
	Maximum Flux (flux)	-4.40 (-29.67-154.97)	-20.31 (-51.41-32.62)	0.310
	Maximum Flux/Biological Zero	5.14 (-29.38-123.73)	-33.54 (-67.06-6.59)	0.129
	Maximum Flux/Resting Level	-30.36 (-59.24-72.73)	32.61 (-2.24-74.07)	0.206
	Time to Recovery (s)	25.57 (-33.33-85.71)	-45.52 (-85.26--15.69)	0.101
	Time to Maximum Flux (s)	94.47 (18.87-328.06)	-42.14 (-80.90--2.17)	**0.001**
	Time to Half Recovery (s)	120.00 (58.47-290.89)	-53.72 (-81.39-25.27)	**0.003**
Study Dorsum	**Resting Flux (flux)**	22.99 (-22.54-86.21)	2.36 (-65.41-38.81)	0.285
	Biological Zero (flux)	-3.13 (-24.00-15.38)	-7.14 (-20.69-38.30)	0.724
	Maximum Flux (flux)	2.49 (-65.01-44.83)	-22.65 (-50.49--14.80)	0.536
	Maximum Flux/Biological Zero	-4.70 (-38.92-16.02)	-34.58 (-66.56-15.55)	0.375
	Maximum Flux/Resting Level	-21.89 (-35.51--1.18)	-35.22 (-45.95-67.00)	0.930
	Time to Recovery (s)	6.67 (-26.53-27.27)	-58.82 (-68.37-50.00)	0.126
	Time to Maximum Flux (s)	43.48 (-16.85-253.10)	-20.03 (-64.01-84.71)	0.126
	Time to Half Recovery (s)	9.09 (-10.44-93.75)	-33.66 (-71.97-84.18)	0.180
Non-study Toe	**Resting Flux (flux)**	112.97 (-29.46-212.71)	-16.92 (-37.67-45.19)	0.180
	Biological Zero (flux)	-21.21 (-33.33-155.56)	-9.50 (-25.81-30.77)	0.808
	Maximum Flux (flux)	101.55 (56.46-324.54)	8.11 (-42.96-81.76)	**0.037**
	Maximum Flux/Biological Zero	126.38 (-4.07-304.92)	-0.68 (-23.11-17.55)	0.122
	Maximum Flux/Resting Level	41.35 (-24.56-92.42)	12.97 (-8.24-35.56)	0.660

	Time to Recovery (s)	-4.58 (-40.98-40.00)	-17.72 (-74.65-32.31)	0.958
	Time to Maximum Flux (s)	47.32 (-11.92-115.76)	2.92 (-72.27-196.30)	0.884
	Time to Half Recovery (s)	1.32 (-48.56-136.36)	20.56 (-86.58-66.38)	0.660
Non-study Dorsum	**Resting Flux (flux)**	47.71 (0.00-123.68)	-18.70 (-43.65-13.57)	0.221
	Biological Zero (flux)	2.94 (-25.53-73.91)	5.36 (-20.41-30.56)	0.827
	Maximum Flux (flux)	56.62 (8.02-77.63)	-62.56 (-67.24--42.86)	0.052
	Maximum Flux/Biological Zero	52.63 (-3.82-89.77)	-58.82 (-63.42--28.96)	0.052
	Maximum Flux/Resting Level	19.64 (-44.19-65.32)	-53.63 (-66.44--24.03)	0.221
	Time to Recovery (s)	0.00 (-60.71-33.90)	86.96 (68.85-225.71)	0.180
	Time to Maximum Flux (s)	278.23 (-38.48-801.20)	-39.97 (-45.04-43.51)	0.221
	Time to Half Recovery (s)	133.66 (31.24-448.57)	-43.91 (-44.03-44.07)	0.075

*Independent-Samples Mann-Whitney U Test

Table IV: Post occlusive reactive hyperaemia, baseline results compared to last results

			Baseline Value Median (IQR)	Last Value Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	53.15 (35.20-104.00)	104.80 (32.30-138.90)	0.248
		Biological Zero (flux)	4.70 (3.10-7.20)	3.70 (2.70-7.30)	0.477
		Maximum Flux (flux)	162.95 (107.70-212.40)	170.00 (128.10-269.50)	0.790
		Maximum Flux/Biological Zero	34.35 (21.21-58.72)	37.69 (23.27-90.92)	0.859
		Maximum Flux/Resting Level	2.76 (1.75-5.24)	2.58 (1.39-3.97)	0.213
		Time to Recovery (s)	1.26 (0.83-1.73)	1.33 (0.98-6.35)	0.424
		Time to Maximum Flux (s)	13.40 (3.68-73.85)	27.08 (8.50-154.38)	**0.021**
		Time to Half Recovery (s)	20.58 (13.45-78.28)	36.13 (15.18-154.38)	**0.021**
	Percutaneous Angioplasty (n=9/8)	**Resting Flux (flux)**	74.50 (66.00-106.60)	33.80 (24.70-84.45)	0.093
		Biological Zero (flux)	3.80 (3.20-4.50)	5.75 (3.70-7.80)	0.575
		Maximum Flux (flux)	84.60 (74.50-168.30)	80.35 (54.05-138.10)	0.401
		Maximum Flux/Biological Zero	22.27 (15.26-41.18)	22.21 (4.75-27.32)	0.208
		Maximum Flux/Resting Level	1.27 (1.13-1.74)	1.94 (1.27-2.62)	0.161
		Time to Recovery (s)	3.58 (2.58-45.13)	3.04 (1.89-7.20)	0.069
		Time to Maximum Flux (s)	220.80 (200.20-288.78)	54.50 (34.73-158.00)	0.050
		Time to Half Recovery (s)	213.74 (140.11-261.53)	72.31 (35.61-158.66)	0.176
Study Dorsum	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	16.80 (8.7-22.90)	16.20 (10.70-29.40)	0.722
		Biological Zero (flux)	4.60 (3.4-4.90)	3.80 (3.30-4.60)	0.646
		Maximum Flux (flux)	45.75 (23.3-63.20)	32.90 (21.10-87.30)	0.859
		Maximum Flux/Biological Zero	9.43 (6.98-17.11)	7.14 (5.90-21.46)	0.477
		Maximum Flux/Resting Level	2.63 (1.70-3.85)	1.99 (1.68-3.01)	**0.021**

		Time to Recovery (s)	1.03 (0.75-2.05)	0.93 (0.80-1.53)	0.929
		Time to Maximum Flux (s)	8.05 (3.45-17.55)	13.50 (4.95-20.38)	**0.041**
		Time to Half Recovery (s)	13.64 (8.40-20.48)	16.28 (10.30-39.65)	0.091
	Percutaneous Angioplasty (n=9/7)	**Resting Flux (flux)**	28.70 (22.60-37.40)	22.80 (15.00-30.40)	0.735
		Biological Zero (flux)	5.80 (4.10-10.20)	5.40 (4.60-6.50)	0.735
		Maximum Flux (flux)	49.00 (37.80-86.50)	38.00 (30.50-60.40)	0.091
		Maximum Flux/Biological Zero	9.61 (8.04-15.09)	7.06 (4.72-9.95)	0.128
		Maximum Flux/Resting Level	1.76 (1.55-2.15)	1.77 (1.20-3.34)	0.866
		Time to Recovery (s)	2.68 (1.70-4.33)	2.40 (0.78-4.53)	0.237
		Time to Maximum Flux (s)	220.28 (93.65-279.80)	127.78 (30.08-227.13)	0.398
		Time to Half Recovery (s)	171.00 (82.31-282.03)	128.93 (43.38-228.68)	0.463
Non-study Toe	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	51.00 (32.80-95.60)	109.3 (21.00-184.50)	0.091
		Biological Zero (flux)	3.70 (3.10-9.10)	4.00 (2.60-11.50)	0.929
		Maximum Flux (flux)	124.40 (49.10-178.60)	220.00 (153.60-273.40)	**0.026**
		Maximum Flux/Biological Zero	18.02 (5.40-29.78)	38.40 (12.24-85.43)	0.075
		Maximum Flux/Resting Level	2.26 (1.32-2.89)	2.01 (1.41-4.04)	0.155
		Time to Recovery (s)	1.51 (1-18.88)	2.03 (1.20-2.83)	0.790
		Time to Maximum Flux (s)	38.98 (12.68-141.05)	18.83 (11.83-215.65)	0.182
		Time to Half Recovery (s)	53.20 (23.48-141.25)	23.70 (19.33-216.80)	0.477
	Percutaneous Angioplasty (n=7/7)	**Resting Flux (flux)**	37.40 (21.90-78.50)	34.60 (25.10-54.30)	0.463
		Biological Zero (flux)	3.80 (3.10-5.50)	4.80 (3.20-6.50)	0.753
		Maximum Flux (flux)	106.10 (38.70-147.10)	83.90 (56.80-208.80)	0.917
		Maximum Flux/Biological Zero	17.09 (7.04-40.87)	22.43 (17.76-43.50)	0.753
		Maximum Flux/Resting Level	2.09 (1.82-2.57)	2.27 (1.50-3.85)	0.345

		Time to Recovery (s)	2.45 (1.78-3.75)	2.38 (1.75-3.93)	0.600
		Time to Maximum Flux (s)	54.80 (13.95-127.43)	35.33 (15.85-146.28)	0.917
		Time to Half Recovery (s)	101.98 (17.40-133.98)	76.13 (20.10-163.45)	0.753
Non-study Dorsum	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	9.00 (7.60-10.90)	15.20 (8.00-17.90)	0.074
		Biological Zero (flux)	4.30 (3.10-5.40)	3.90 (3.50-5.00)	0.721
		Maximum Flux (flux)	23.55 (13.60-36.40)	24.80 (18.60-54.00)	**0.008**
		Maximum Flux/Biological Zero	4.01 (3.61-6.91)	6.09 (5.27-10.81)	0.248
		Maximum Flux/Resting Level	2.21 (1.75-2.80)	2.57 (1.22-3.67)	0.859
		Time to Recovery (s)	0.91 (0.68-1.48)	0.95 (0.30-1.43)	0.610
		Time to Maximum Flux (s)	15.40 (3.58-116.90)	15.40 (12.13-215.65)	0.182
		Time to Half Recovery (s)	22.03 (7.43-56.18)	29.60 (15.60-216.83)	**0.028**
	Percutaneous Angioplasty (n=7/6)	**Resting Flux (flux)**	16.20 (12.60-47.50)	15.60 (13.70-20.00)	0.500
		Biological Zero (flux)	5.60 (4.20-9.10)	4.75 (3.90-5.90)	0.893
		Maximum Flux (flux)	73.20 (22.30-97.30)	30.00 (21.00-55.60)	0.080
		Maximum Flux/Biological Zero	10.69 (5.30-13.92)	5.41 (4.96-6.56)	0.138
		Maximum Flux/Resting Level	2.23 (1.54-4.59)	1.74 (1.32-4.30)	0.225
		Time to Recovery (s)	1.75 (1.53-2.50)	2.90 (1.50-3.58)	0.500
		Time to Maximum Flux (s)	70.68 (31.10-138.60)	119.03 (42.43-265.28)	0.686
		Time to Half Recovery (s)	113.13 (53.18-139.78)	120.69 (42.55-266.98)	0.686

*Related Samples Wilcoxon Signed Rank Test

Table V: Post occlusive reactive hyperaemia, baseline results compared to last results. Healed patients only

			Baseline Value Median (IQR)	Last Value Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	83.50 (48.10-151.50)	59.75 (32.30-122.70)	0.600
		Biological Zero (flux)	5.00 (3.10-7.20)	5.20 (2.60-7.30)	0.917
		Maximum Flux (flux)	171.55 (140.00-254.60)	166.95 (128.00-190.90)	0.463
		Maximum Flux/Biological Zero	41.37 (21.21-58.72)	35.19 (23.27-49.22)	0.463
		Maximum Flux/Resting Level	1.87 (1.68-2.91)	3.00 (1.39-3.97)	0.600
		Time to Recovery (s)	1.50 (1.25-1.73)	1.50 (0.80-6.35)	0.463
		Time to Maximum Flux (s)	13.40 (6.33-73.85)	64.43 (22.05-114.20)	**0.028**
		Time to Half Recovery (s)	20.58 (13.45-78.28)	69.06 (28.53-121.43)	**0.028**
	Percutaneous Angioplasty (n=6/6)	**Resting Flux (flux)**	66.40 (15.3-172.80)	33.80 (29.7-56)	0.116
		Biological Zero (flux)	3.80 (3.20-4.20)	5.30 (3.30-9.10)	0.917
		Maximum Flux (flux)	81.40 (74.50-168.30)	80.40 (55.70-125.60)	0.600
		Maximum Flux/Biological Zero	20.01 (7.01-27.89)	22.21 (6.12-25.12)	0.463
		Maximum Flux/Resting Level	1.25 (1.13-1.74)	2.38 (1.56-2.79)	0.116
		Time to Recovery (s)	3.23 (2.25-45.13)	2.26 (1.68-4.88)	**0.028**
		Time to Maximum Flux (s)	210.50 (72.18-231.00)	50.71 (27.38-105.18)	**0.046**
		Time to Half Recovery (s)	200.20 (80.03-222.50)	67.24 (29.15-106.15)	0.225
Study Dorsum	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	18.85 (11.80-22.90)	11.15 (10.20-19.90)	0.600
		Biological Zero (flux)	4.85 (4.60-5.00)	4.00 (3.40-4.60)	0.686
		Maximum Flux (flux)	45.75 (32.10-63.20)	29.20 (20.10-37.40)	0.600
		Maximum Flux/Biological Zero	9.43 (6.98-9.66)	6.61 (5.90-8.92)	0.249
		Maximum Flux/Resting Level	2.79 (2.49-2.92)	2.19 (1.78-3.01)	0.249
		Time to Recovery (s)	0.83 (0.48-1.15)	0.86 (0.58-1.28)	0.916

		Time to Maximum Flux (s)	5.23 (2.85-9.28)	13.15 (5.68-13.60)	0.345
		Time to Half Recovery (s)	8.60 (5.13-14.48)	15.80 (9.60-18.48)	0.116
	Percutaneous Angioplasty (n=6/6)	**Resting Flux (flux)**	28.80 (21.90-43.30)	24.80 (15.00-30.40)	0.917
		Biological Zero (flux)	5.50 (4.10-10.20)	5.70 (4.60-6.50)	0.916
		Maximum Flux (flux)	65.60 (37.80-94.30)	45.90 (32.50-60.40)	0.173
		Maximum Flux/Biological Zero	12.35 (8.04-15.21)	8.18 (4.72-9.95)	0.249
		Maximum Flux/Resting Level	1.88 (1.59-2.22)	1.88 (1.20-3.34)	0.917
		Time to Recovery (s)	2.08 (1.60-2.68)	2.13 (0.78-4.53)	0.345
		Time to Maximum Flux (s)	198.79 (69.18-287.33)	175.76 (105.20-227.13)	0.753
		Time to Half Recovery (s)	119.83 (70.00-291.73)	176.43 (106.03-228.68)	0.893
Non-study Toe	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	65.65 (36.40-112.70)	122.10 (51.00-184.50)	0.463
		Biological Zero (flux)	4.55 (3.10-10.20)	5.55 (3.20-11.50)	0.600
		Maximum Flux (flux)	138.90 (120.00-303.20)	221.50 (173.90-273.40)	0.249
		Maximum Flux/Biological Zero	18.02 (13.95-36.81)	32.11 (22.57-85.43)	0.116
		Maximum Flux/Resting Level	1.75 (1.32-3.30)	1.98 (1.21-9.29)	0.345
		Time to Recovery (s)	1.64 (1.00-8.18)	2.06 (1.40-6.48)	0.600
		Time to Maximum Flux (s)	38.98 (12.68-262.10)	87.20 (18.83-291.13)	0.600
		Time to Half Recovery (s)	53.20 (38.25-268.18)	92.98 (19.68-292.73)	0.917
	Percutaneous Angioplasty (n=4/5)	**Resting Flux (flux)**	41.50 (32.60-63.20)	50.30 (34.60-54.30)	0.465
		Biological Zero (flux)	4.40 (3.40-5.90)	4.80 (3.20-6.50)	0.465
		Maximum Flux (flux)	111.70 (83.80-132.20)	145.30 (83.90-208.80)	0.715
		Maximum Flux/Biological Zero	19.83 (16.59-35.01)	36.49 (21.37-43.5)	1.000
		Maximum Flux/Resting Level	2.39 (2.02-2.71)	2.27 (1.67-3.85)	0.273
		Time to Recovery (s)	2.03 (1.70-10.95)	2.38 (2.15-3.93)	1.000
		Time to Maximum Flux (s)	34.38 (8.66-62.75)	69.55 (35.33-146.28)	0.273

		Time to Half Recovery (s)	55.79 (11.19-98.08)	126.33 (76.13-163.45)	0.273
Non-study Dorsum	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	8.65 (8.10-10.90)	17.00 (9.60-20.70)	0.028
		Biological Zero (flux)	4.15 (3.10-4.70)	4.85 (3.90-5.50)	0.249
		Maximum Flux (flux)	24.45 (12.10-39.90)	33.95 (20.10-54.00)	**0.028**
		Maximum Flux/Biological Zero	5.31 (3.40-10.22)	5.50 (4.26-10.81)	0.463
		Maximum Flux/Resting Level	1.91 (1.70-2.80)	2.38 (1.22-3.35)	0.753
		Time to Recovery (s)	0.74 (0.68-0.85)	0.95 (0.55-1.43)	0.249
		Time to Maximum Flux (s)	10.20 (2.30-55.38)	17.05 (12.13-142.95)	0.463
		Time to Half Recovery (s)	17.53 (5.85-23.05)	24.73 (15.60-143.58)	**0.043**
	Percutaneous Angioplasty (n=4/5)	**Resting Flux (flux)**	16.20 (14.00-24.60)	15.90 (13.70-20.00)	1.000
		Biological Zero (flux)	5.60 (4.90-9.10)	4.80 (4.70-5.90)	0.273
		Maximum Flux (flux)	78.00 (64.10-97.30)	30.80 (29.20-55.60)	0.144
		Maximum Flux/Biological Zero	13.09 (10.69-13.92)	5.39 (4.96-6.56)	0.144
		Maximum Flux/Resting Level	4.59 (3.17-5.99)	2.01 (1.47-4.30)	0.273
		Time to Recovery (s)	1.73 (0.53-1.75)	3.23 (1.50-3.58)	0.715
		Time to Maximum Flux (s)	70.68 (46.15-112.80)	161.88 (76.18-265.28)	0.715
		Time to Half Recovery (s)	76.03 (53.18-113.13)	162.98 (78.4-266.98)	0.715

*Related Samples Wilcoxon Signed Rank Test

Table VI: Post occlusive reactive hyperaemia, baseline results compared to last results. Unhealed patients only

			Baseline Value Median (IQR)	Last Value Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	50.10 (13.05-67.35)	107.70 (104.80-138.90)	**0.043**
		Biological Zero (flux)	4.70 (2.75-7.10)	3.40 (3.30-3.70)	0.225
		Maximum Flux (flux)	149.40 (105.95-204.50)	192.90 (138.30-277.80)	0.225
		Maximum Flux/Biological Zero	30.46 (18.60-56.96)	58.46 (37.69-97.10)	0.225
		Maximum Flux/Resting Level	4.16 (2.24-11.82)	2.23 (1.84-2.58)	0.080
		Time to Recovery (s)	0.85 (0.68-2.25)	1.18 (1.03-1.38)	0.500
		Time to Maximum Flux (s)	27.78 (3.44-83.36)	9.68 (8.50-154.38)	0.345
		Time to Half Recovery (s)	36.75 (13.68-92.81)	36.13 (15.18-154.38)	0.345
	Percutaneous Angioplasty (n=3/2)	**Resting Flux (flux)**	84.00 (74.50-106.60)	69.30 (16.40-122.10)	0.655
		Biological Zero (flux)	4.50 (2.90-4.70)	5.80 (5.10-6.40)	0.180
		Maximum Flux (flux)	137.40 (68.60-193.50)	86.10 (21.50-150.60)	0.180
		Maximum Flux/Biological Zero	41.18 (15.26-47.39)	16.45 (3.37-29.52)	0.180
		Maximum Flux/Resting Level	1.64 (0.92-1.82)	1.27 (1.23-1.31)	0.655
		Time to Recovery (s)	3.58 (2.93-117.55)	8.35 (3.65-13.05)	0.655
		Time to Maximum Flux (s)	288.78 (204.98-288.78)	141.08 (49.65-232.50)	0.655
		Time to Half Recovery (s)	290.63 (204.98-295.23)	142.73 (52.23-233.23)	0.655
Study Dorsum	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	16.05 (8.25-21.95)	23.60 (16.20-29.40)	0.345
		Biological Zero (flux)	3.75 (3.05-4.70)	3.30 (3.10-4.50)	0.892
		Maximum Flux (flux)	45.35 (21.90-63.50)	72.40 (24.80-87.30)	0.686
		Maximum Flux/Biological Zero	12.93 (6.07-23.75)	21.46 (6.82-26.45)	0.893
		Maximum Flux/Resting Level	2.06 (1.64-6.05)	1.84 (1.68-2.46)	**0.043**
		Time to Recovery (s)	1.44 (0.84-2.50)	1.05 (0.90-1.58)	0.893

		Time to Maximum Flux (s)	11.25 (3.83-21.35)	20.38 (4.95-192.98)	0.080
		Time to Half Recovery (s)	16.69 (11.93-27.03)	39.65 (12.40-193.23)	0.225
	Percutaneous Angioplasty (n=3/1)	**Resting Flux (flux)**	28.70 (22.60-37.40)	22.80	0.317
		Biological Zero (flux)	5.80 (4.00-10.70)	4.60	0.317
		Maximum Flux (flux)	49.00 (35.00-61.60)	30.50	0.317
		Maximum Flux/Biological Zero	8.76 (4.58-10.62)	6.63	0.317
		Maximum Flux/Resting Level	1.55 (1.31-2.15)	1.34	0.317
		Time to Recovery (s)	4.33 (3.08-5.93)	2.40	0.317
		Time to Maximum Flux (s)	220.28 (93.65-271.38)	30.08	0.317
		Time to Half Recovery (s)	222.18 (94.63-272.33)	43.38	0.317
Non-study Toe	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	46.25 (17.25-75.10)	109.30 (21.00-144.20)	**0.043**
		Biological Zero (flux)	3.60 (3.15-7.20)	3.10 (2.60-6.90)	0.686
		Maximum Flux (flux)	95.20 (32.30-168.20)	220.00 (84.80-269.60)	**0.043**
		Maximum Flux/Biological Zero	19.10 (5.05-28.47)	42.39 (10.64-50.76)	0.345
		Maximum Flux/Resting Level	2.33 (1.66-2.68)	2.01 (1.87-3.35)	0.500
		Time to Recovery (s)	1.31 (0.95-27.29)	1.48 (1.20-2.03)	0.893
		Time to Maximum Flux (s)	34.05 (10.68-123.23)	11.83 (11.68-15.53)	0.080
		Time to Half Recovery (s)	66.96 (14.99-128.04)	22.88 (16.63-23.70)	0.138
	Percutaneous Angioplasty (n=3/2)	**Resting Flux (flux)**	21.90 (8.10-78.50)	26.40 (20.60-32.10)	0.655
		Biological Zero (flux)	3.80 (3.10-5.50)	4.20 (3.50-4.80)	0.655
		Maximum Flux (flux)	38.70 (17.00-155.30)	54.70 (30.90-78.50)	0.655
		Maximum Flux/Biological Zero	7.04 (5.47-40.87)	14.43 (6.43-22.43)	0.655
		Maximum Flux/Resting Level	1.98 (1.77-2.09)	1.97 (1.5-2.44)	0.655
		Time to Recovery (s)	2.55 (2.45-3.75)	2.06 (1.75-2.38)	0.180
		Time to Maximum Flux (s)	127.43 (54.73-237.73)	15.51 (15.18-15.85)	0.180

		Time to Half Recovery (s)	133.98 (128.93-240.75)	19.04 (17.98-20.10)	0.180
Non-study Dorsum	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	9.25 (7.05-12.35)	8.00 (6.80-15.20)	1.000
		Biological Zero (flux)	4.80 (2.85-6.95)	3.50 (2.50-3.80)	0.465
		Maximum Flux (flux)	23.55 (15.25-34.55)	21.30 (18.60-32.30)	0.225
		Maximum Flux/Biological Zero	4.01 (3.76-5.74)	7.42 (6.09-8.07)	0.345
		Maximum Flux/Resting Level	2.27 (1.99-3.27)	3.11 (1.90-3.67)	0.500
		Time to Recovery (s)	1.21 (0.75-1.51)	1.10 (0.30-1.43)	0.144
		Time to Maximum Flux (s)	15.56 (5.91-117.88)	13.90 (12.88-215.65)	0.225
		Time to Half Recovery (s)	31.31 (14.40-141.03)	29.60 (17.85-216.83)	0.345
	Percutaneous Angioplasty (n=3/1)	**Resting Flux (flux)**	47.50 (11.30-86.60)	15.30	0.317
		Biological Zero (flux)	6.40 (4.20-9.60)	3.30	0.317
		Maximum Flux (flux)	73.20 (22.30-192.90)	17.90	0.317
		Maximum Flux/Biological Zero	7.63 (5.30-30.15)	5.42	0.317
		Maximum Flux/Resting Level	1.97 (1.54-2.23)	1.17	0.317
		Time to Recovery (s)	2 (1.53-2.50)	2.58	0.317
		Time to Maximum Flux (s)	31.10 (13.23-273.90)	17.23	0.317
		Time to Half Recovery (s)	120.08 (34.78-282.78)	21.45	0.317

*Related Samples Wilcoxon Signed Rank Test

Table VII: Post occlusive reactive hyperaemia, baseline results compared to second visit

			Baseline Value Median (IQR)	Second Value Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	53.15 (35.20-104.00)	98.00 (14.80-107.70)	0.286
		Biological Zero (flux)	4.70 (3.10-7.20)	3.30 (2.70-7.80)	0.182
		Maximum Flux (flux)	162.95 (107.70-212.40)	190.90 (75.40-258.40)	1.000
		Maximum Flux/Biological Zero	34.35 (21.21-58.72)	56.06 (25.33-90.92)	0.534
		Maximum Flux/Resting Level	2.76 (1.75-5.24)	2.58 (1.84-5.03)	0.286
		Time to Recovery (s)	1.26 (0.83-1.73)	1.18 (0.88-1.38)	0.540
		Time to Maximum Flux (s)	13.40 (3.68-73.85)	11.08 (4.40-27.08)	0.722
		Time to Half Recovery (s)	20.58 (13.45-78.28)	16.65 (12.55-36.13)	0.929
	Percutaneous Angioplasty (n=9/8)	**Resting Flux (flux)**	74.50 (66.00-106.60)	38.35 (23.05-76.05)	0.123
		Biological Zero (flux)	3.80 (3.20-4.50)	4.40 (4.15-8.65)	0.575
		Maximum Flux (flux)	84.60 (74.50-168.30)	102.85 (63.20-142.35)	0.575
		Maximum Flux/Biological Zero	22.27 (15.26-41.18)	22.18 (8.68-29.00)	0.263
		Maximum Flux/Resting Level	1.27 (1.13-1.74)	2.20 (1.37-3.78)	0.263
		Time to Recovery (s)	3.58 (2.58-45.13)	2.01 (1.13-3.49)	**0.017**
		Time to Maximum Flux (s)	220.80 (200.20-288.78)	53.79 (18.09-205.16)	0.123
		Time to Half Recovery (s)	213.74 (140.11-261.53)	74.01 (22.94-206.06)	0.345
Study Dorsum	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	53.15 (35.20-104.00)	98.00 (14.80-107.70)	0.505
		Biological Zero (flux)	4.70 (3.10-7.20)	3.30 (2.70-7.80)	0.373
		Maximum Flux (flux)	162.95 (107.70-212.40)	190.90 (75.40-258.40)	0.722
		Maximum Flux/Biological Zero	34.35 (21.21-58.72)	56.06 (25.33-90.92)	0.477
		Maximum Flux/Resting Level	2.76 (1.75-5.24)	2.58 (1.84-5.03)	0.328
		Time to Recovery (s)	1.26 (0.83-1.73)	1.18 (0.88-1.38)	0.374

		Time to Maximum Flux (s)	13.40 (3.68-73.85)	11.08 (4.40-27.08)	0.182
		Time to Half Recovery (s)	20.58 (13.45-78.28)	16.65 (12.55-36.13)	0.722
	Percutaneous Angioplasty (n=9/7)	**Resting Flux (flux)**	74.50 (66.00-106.60)	38.35 (23.05-76.05)	0.398
		Biological Zero (flux)	3.80 (3.20-4.50)	4.40 (4.15-8.65)	0.128
		Maximum Flux (flux)	84.60 (74.50-168.30)	102.85 (63.20-142.35)	0.499
		Maximum Flux/Biological Zero	22.27 (15.26-41.18)	22.18 (8.68-29.00)	0.735
		Maximum Flux/Resting Level	1.27 (1.13-1.74)	2.20 (1.37-3.78)	0.263
		Time to Recovery (s)	3.58 (2.58-45.13)	2.01 (1.13-3.49)	0.176
		Time to Maximum Flux (s)	220.80 (200.20-288.78)	53.79 (18.09-205.16)	0.398
		Time to Half Recovery (s)	213.74 (140.11-261.53)	74.01 (22.94-206.06)	0.600
Non-study Toe	**Diabetic Tissue Loss (n=14/11)**	**Resting Flux (flux)**	16.80 (8.70-22.90)	16.60 (11.60-29.40)	0.533
		Biological Zero (flux)	4.60 (3.40-4.90)	4.00 (3.10-4.70)	0.248
		Maximum Flux (flux)	45.75 (23.30-63.20)	46.10 (35.70-87.30)	**0.021**
		Maximum Flux/Biological Zero	9.43 (6.98-17.11)	11.01 (6.89-26.45)	**0.041**
		Maximum Flux/Resting Level	2.63 (1.70-3.85)	2.60 (2.15-3.22)	0.328
		Time to Recovery (s)	1.03 (0.75-2.05)	0.93 (0.85-1.05)	0.286
		Time to Maximum Flux (s)	8.05 (3.45-17.55)	5.28 (2.50-20.38)	0.790
		Time to Half Recovery (s)	13.64 (8.40-20.48)	12.40 (3.70-39.65)	0.790
	Percutaneous Angioplasty (n=7/7)	**Resting Flux (flux)**	28.70 (22.60-37.40)	15.00 (10.60-30.40)	0.249
		Biological Zero (flux)	5.80 (4.10-10.20)	5.00 (3.10-5.40)	0.128
		Maximum Flux (flux)	49.00 (37.80-86.50)	35.50 (22.40-68.50)	0.753
		Maximum Flux/Biological Zero	9.61 (8.04-15.09)	9.95 (4.72-13.71)	0.735
		Maximum Flux/Resting Level	1.76 (1.55-2.15)	2.11 (1.33-3.12)	0.116
		Time to Recovery (s)	2.68 (1.70-4.33)	1.85 (0.93-4.08)	0.600
		Time to Maximum Flux (s)	220.28 (93.65-279.80)	127.78 (55.05-227.13)	0.600

		Time to Half Recovery (s)	171.00 (82.31-282.03)	128.93 (60.43-228.68)	0.345
Non-study Dorsum	**Diabetic Tissue Loss (n=14/10)**	**Resting Flux (flux)**	9.00 (7.60-10.90)	8.80 (6.50-17)	0.953
		Biological Zero (flux)	4.30 (3.10-5.40)	3.80 (3.50-4.00)	0.767
		Maximum Flux (flux)	23.55 (13.60-36.40)	24.20 (14.40-68.60)	**0.032**
		Maximum Flux/Biological Zero	4.01 (3.61-6.91)	6.70 (4.73-9.94)	0.114
		Maximum Flux/Resting Level	2.21 (1.75-2.80)	3.12 (2.65-3.94)	0.241
		Time to Recovery (s)	0.91 (0.68-1.48)	0.64 (0.33-1.10)	0.161
		Time to Maximum Flux (s)	15.40 (3.58-116.90)	24.15 (12.88-118.20)	0.114
		Time to Half Recovery (s)	22.03 (7.43-56.18)	32.25 (19.20-121.53)	**0.021**
	Percutaneous Angioplasty (n=7/6)	**Resting Flux (flux)**	16.20 (12.60-47.50)	16.90 (8.70-23.30)	0.893
		Biological Zero (flux)	5.60 (4.20-9.10)	5.10 (4.80-6.70)	0.684
		Maximum Flux (flux)	73.20 (22.30-97.30)	38.35 (29.5-59.00)	0.138
		Maximum Flux/Biological Zero	10.69 (5.30-13.92)	7.53 (6.14-11.56)	0.225
		Maximum Flux/Resting Level	2.23 (1.54-4.59)	3.12 (1.47-4.35)	0.686
		Time to Recovery (s)	1.75 (1.53-2.50)	1.88 (1.25-2.30)	0.500
		Time to Maximum Flux (s)	70.68 (31.10-138.60)	70.63 (60.08-243.90)	0.500
		Time to Half Recovery (s)	113.13 (53.18-139.78)	90.81 (65.73-245.00)	0.686

*Related Samples Wilcoxon Signed Rank Test

Table VIII: Post occlusive reactive hyperaemia, baseline results compared to second visit. Healed only

			Baseline Value Median (IQR)	Second Value Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	83.50 (48.10-151.50)	77.00 (33.40-104.20)	0.917
		Biological Zero (flux)	5.00 (3.10-7.20)	3.40 (2.70-7.80)	0.173
		Maximum Flux (flux)	171.50 (140.00-254.60)	184.00 (168.20-197.60)	0.753
		Maximum Flux/Biological Zero	41.37 (21.21-58.72)	40.95 (25.33-65.56)	0.917
		Maximum Flux/Resting Level	1.87 (1.68-2.91)	2.66 (1.81-4.32)	0.463
		Time to Recovery (s)	1.50 (1.25-1.73)	1.25 (1.05-4.60)	0.527
		Time to Maximum Flux (s)	13.40 (6.33-73.85)	19.19 (6.83-27.08)	0.753
		Time to Half Recovery (s)	20.58 (13.45-78.28)	22.59 (12.55-29.20)	0.917
	Percutaneous Angioplasty (n=6/6)	**Resting Flux (flux)**	66.40 (15.30-172.80)	38.40 (29.70-59.00)	0.249
		Biological Zero (flux)	3.80 (3.20-4.20)	4.30 (4.10-10.90)	0.753
		Maximum Flux (flux)	81.40 (74.50-168.30)	102.90 (80.80-152.40)	0.917
		Maximum Flux/Biological Zero	20.01 (7.01-27.89)	22.18 (13.99-27.92)	0.600
		Maximum Flux/Resting Level	1.25 (1.13-1.74)	2.55 (2.08-4.76)	0.249
		Time to Recovery (s)	3.23 (2.25-45.13)	1.80 (0.58-2.10)	**0.028**
		Time to Maximum Flux (s)	210.50 (72.18-231.00)	53.79 (27.38-177.83)	0.116
		Time to Half Recovery (s)	200.20 (80.03-222.50)	74.01 (29.00-178.9)	0.465
Study Dorsum	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	18.90 (11.80-22.9)	18.50 (11.60-23.10)	0.833
		Biological Zero (flux)	4.90 (4.60-5.00)	4.00 (3.40-4.70)	0.249
		Maximum Flux (flux)	45.80 (32.10-63.20)	37.40 (35.70-46.10)	0.916
		Maximum Flux/Biological Zero	9.43 (6.98-9.66)	9.97 (6.89-14.88)	0.463
		Maximum Flux/Resting Level	2.79 (2.49-2.92)	2.50 (2.15-3.22)	0.917
		Time to Recovery (s)	0.83 (0.48-1.15)	0.89 (0.75-0.95)	0.917

		Time to Maximum Flux (s)	5.23 (2.85-9.28)	3.09 (2.15-5.28)	0.600
		Time to Half Recovery (s)	8.60 (5.13-14.48)	5.79 (3.53-15.33)	0.345
	Percutaneous Angioplasty (n=6/6)	**Resting Flux (flux)**	28.80 (21.90-43.30)	13.40 (10.60-26.80)	0.249
		Biological Zero (flux)	5.50 (4.10-10.20)	4.40 (3.10-5.40)	0.173
		Maximum Flux (flux)	65.60 (37.80-94.30)	29.10 (22.40-53.80)	0.345
		Maximum Flux/Biological Zero	12.35 (8.04-15.21)	9.88 (4.72-11.46)	0.600
		Maximum Flux/Resting Level	1.88 (1.59-2.22)	1.94 (1.33-3.12)	0.600
		Time to Recovery (s)	2.08 (1.60-2.68)	1.54 (0.93-4.08)	0.249
		Time to Maximum Flux (s)	198.79 (69.18-287.33)	142.33 (80.13-227.13)	0.753
		Time to Half Recovery (s)	119.83 (70.00-291.73)	143.80 (80.35-228.68)	0.893
Non-study Toe	**Diabetic Tissue Loss (n=6/6)**	**Resting Flux (flux)**	65.70 (36.40-112.70)	93.80 (24.40-183.50)	0.917
		Biological Zero (flux)	4.60 (3.10-10.20)	4.20 (2.90-4.50)	0.075
		Maximum Flux (flux)	138.90 (120.00-303.20)	213.90 (131.00-454.90)	0.173
		Maximum Flux/Biological Zero	18.02 (13.95-36.81)	41.48 (11.16-156.87)	0.046
		Maximum Flux/Resting Level	1.75 (1.32-3.30)	1.86 (1.73-6.03)	0.116
		Time to Recovery (s)	1.64 (1.00-8.18)	1.36 (1.33-1.40)	0.249
		Time to Maximum Flux (s)	38.98 (12.68-262.10)	15.53 (6.98-120.95)	0.345
		Time to Half Recovery (s)	53.20 (38.25-268.18)	26.44 (18.28-121.83)	0.173
	Percutaneous Angioplasty (n=4/5)	**Resting Flux (flux)**	41.50 (32.60-63.20)	26.30 (21.90-54.30)	0.273
		Biological Zero (flux)	4.40 (3.40-5.90)	4.80 (2.90-6.50)	0.715
		Maximum Flux (flux)	111.70 (83.80-132.20)	80.40 (77.00-208.80)	0.715
		Maximum Flux/Biological Zero	19.83 (16.59-35.01)	27.73 (22.59-43.50)	1.000
		Maximum Flux/Resting Level	2.39 (2.02-2.71)	2.93 (2.58-3.85)	0.068
		Time to Recovery (s)	2.03 (1.70-10.95)	2.15 (1.98-2.98)	1.000
		Time to Maximum Flux (s)	34.38 (8.66-62.28)	65.40 (30.03-69.55)	0.465

		Time to Half Recovery (s)	55.79 (11.19-98.08)	66.48 (34.68-126.33)	1.000
Non-study Dorsum	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	8.70 (8.10-10.90)	9.00 (8.60-17.50)	0.686
		Biological Zero (flux)	4.20 (3.10-4.70)	3.90 (3.70-4.00)	0.892
		Maximum Flux (flux)	24.50 (12.10-39.90)	27.10 (18.90-81.70)	**0.043**
		Maximum Flux/Biological Zero	5.31 (3.40-10.22)	7.31 (4.73-20.95)	0.138
		Maximum Flux/Resting Level	1.91 (1.70-2.80)	3.13 (2.92-3.94)	0.500
		Time to Recovery (s)	0.74 (0.68-0.85)	0.70 (0.58-0.93)	0.715
		Time to Maximum Flux (s)	10.20 (2.30-55.38)	18.70 (18.15-102.18)	0.500
		Time to Half Recovery (s)	17.53 (5.85-23.05)	22.35 (19.20-102.40)	0.068
	Percutaneous Angioplasty (n=4/5)	**Resting Flux (flux)**	15.10 (13.30-20.40)	13.70 (8.70-23.30)	0.465
		Biological Zero (flux)	5.30 (4.30-7.40)	5.40 (4.80-6.70)	0.713
		Maximum Flux (flux)	71.10 (39.10-87.70)	45.90 (30.80-59.00)	0.273
		Maximum Flux/Biological Zero	11.89 (7.30-13.51)	8.49 (6.56-11.56)	0.273
		Maximum Flux/Resting Level	3.88 (2.15-5.29)	4.30 (1.94-4.35)	0.715
		Time to Recovery (s)	1.74 (1.13-7.86)	1.50 (1.25-2.3)	0.715
		Time to Maximum Flux (s)	91.74 (58.41-125.70)	76.18 (65.08-243.90)	1.000
		Time to Half Recovery (s)	94.58 (64.60-126.45)	103.23 (78.40-245)	0.715

*Related Samples Wilcoxon Signed Rank Test

Table IX: Post occlusive reactive hyperaemia, baseline results compared to second visit. Unhealed only

			Baseline Value Median (IQR)	Second Value Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	50.10 (13.10-67.40)	104.8 (7.7-107.7)	**0.043**
		Biological Zero (flux)	4.70 (2.80-7.10)	3.3 (2.7-3.7)	0.686
		Maximum Flux (flux)	149.40 (105.90-204.50)	192.9 (75.4-277.8)	0.686
		Maximum Flux/Biological Zero	30.46 (18.60-56.96)	58.46 (34.28-97.1)	0.345
		Maximum Flux/Resting Level	4.16 (2.24-11.82)	2.58 (2.23-8.07)	0.080
		Time to Recovery (s)	0.85 (0.68-2.25)	1.03 (0.85-1.18)	0.715
		Time to Maximum Flux (s)	27.78 (3.44-83.36)	8.5 (4.28-11.08)	0.686
		Time to Half Recovery (s)	36.75 (13.68-92.81)	14.78 (13.83-36.13)	0.893
	Percutaneous Angioplasty (n=3/2)	**Resting Flux (flux)**	84.00 (74.50-106.60)	54.80 (16.40-93.10)	0.180
		Biological Zero (flux)	4.50 (2.90-4.70)	5.40 (4.40-6.40)	0.655
		Maximum Flux (flux)	137.40 (68.60-193.50)	76.90 (21.50-132.30)	0.180
		Maximum Flux/Biological Zero	41.18 (15.26-47.39)	16.73 (3.37-30.08)	0.180
		Maximum Flux/Resting Level	1.64 (0.92-1.82)	1.37 (1.31-1.42)	0.655
		Time to Recovery (s)	3.58 (2.93-117.55)	3.49 (3.33-3.65)	0.655
		Time to Maximum Flux (s)	288.78 (204.98-288.78)	120.65 (8.80-232.50)	0.655
		Time to Half Recovery (s)	290.63 (204.98-295.23)	124.30 (15.38-233.23)	0.655
Study Dorsum	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	16.10 (8.30-22.00)	16.2 (15.9-29.4)	0.500
		Biological Zero (flux)	3.80 (3.10-4.70)	3.3 (2.8-4.5)	1.000
		Maximum Flux (flux)	45.40 (21.90-63.50)	72.4 (48.8-87.3)	0.686
		Maximum Flux/Biological Zero	12.93 (6.07-23.75)	21.46 (9.56-26.45)	0.893
		Maximum Flux/Resting Level	2.06 (1.64-6.05)	2.6 (2.46-3.07)	0.138
		Time to Recovery (s)	1.44 (0.84-2.50)	1.03 (0.9-1.05)	0.225

		Time to Maximum Flux (s)	11.25 (3.83-21.35)	20.38 (9.78-209.2)	**0.043**
		Time to Half Recovery (s)	16.69 (11.93-27.03)	39.65 (12.4-210.93)	0.225
	Percutaneous Angioplasty (n=3/1)	**Resting Flux (flux)**	28.70 (22.60-37.40)	31.80	0.317
		Biological Zero (flux)	5.80 (4.00-10.70)	5.00	0.317
		Maximum Flux (flux)	49.00 (35.00-61.60)	68.50	0.317
		Maximum Flux/Biological Zero	8.76 (4.58-10.62)	13.71	0.317
		Maximum Flux/Resting Level	1.55 (1.31-2.15)	2.16	0.317
		Time to Recovery (s)	4.33 (3.08-5.93)	3.40	0.317
		Time to Maximum Flux (s)	220.28 (93.65-271.38)	55.05	0.317
		Time to Half Recovery (s)	222.18 (94.63-272.33)	60.43	0.317
Non-study Toe	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	46.30 (17.30-75.10)	36.70 (9.80-144.20)	0.225
		Biological Zero (flux)	3.60 (3.20-7.20)	5.00 (3.10-6.30)	0.893
		Maximum Flux (flux)	95.20 (32.30-168.20)	53.20 (33.00-269.60)	**0.043**
		Maximum Flux/Biological Zero	19.10 (5.05-28.47)	10.64 (8.44-50.76)	0.500
		Maximum Flux/Resting Level	2.33 (1.66-2.68)	1.87 (1.72-2.43)	0.686
		Time to Recovery (s)	1.31 (0.95-27.29)	1.48 (1.13-2.30)	0.893
		Time to Maximum Flux (s)	34.05 (10.68-123.23)	11.83 (11.68-105.38)	0.080
		Time to Half Recovery (s)	66.96 (14.99-128.04)	22.88 (16.63-108.43)	0.080
	Percutaneous Angioplasty (n=3/2)	**Resting Flux (flux)**	21.90 (8.10-78.50)	35.40 (20.60-50.10)	0.655
		Biological Zero (flux)	3.80 (3.10-5.50)	4.00 (3.10-4.80)	0.655
		Maximum Flux (flux)	38.70 (17.00-155.30)	90.00 (30.90-149.00)	0.655
		Maximum Flux/Biological Zero	7.04 (5.47-40.87)	27.25 (6.43-48.06)	0.180
		Maximum Flux/Resting Level	1.98 (1.77-2.09)	2.24 (1.50-2.97)	0.655
		Time to Recovery (s)	2.55 (2.45-3.75)	2.14 (1.90-2.38)	0.180
		Time to Maximum Flux (s)	127.43 (54.73-237.73)	10.85 (6.53-15.18)	0.180

		Time to Half Recovery (s)	133.98 (128.93-240.75)	16.75 (15.53-17.98)	0.180
Non-study Dorsum	**Diabetic Tissue Loss (n=8/5)**	**Resting Flux (flux)**	9.30 (7.10-12.40)	6.80 (5.40-12.60)	0.715
		Biological Zero (flux)	4.80 (2.90-7.00)	3.50 (3.30-4.00)	0.715
		Maximum Flux (flux)	23.60 (15.30-34.60)	21.30 (14.40-32.30)	0.345
		Maximum Flux/Biological Zero	4.01 (3.76-5.74)	6.09 (6.00-8.07)	0.500
		Maximum Flux/Resting Level	2.27 (1.99-3.27)	3.11 (2.65-3.67)	0.345
		Time to Recovery (s)	1.21 (0.75-1.51)	0.33 (0.30-1.10)	0.068
		Time to Maximum Flux (s)	15.56 (5.91-117.88)	29.60 (12.88-234.58)	0.138
		Time to Half Recovery (s)	31.31 (14.40-141.03)	34.90 (29.6-234.88)	0.225
	Percutaneous Angioplasty (n=3/1)	**Resting Flux (flux)**	47.50 (11.30-86.60)	20.10	0.317
		Biological Zero (flux)	6.40 (4.20-9.60)	4.80	0.317
		Maximum Flux (flux)	73.20 (22.30-192.90)	29.50	0.317
		Maximum Flux/Biological Zero	7.63 (5.30-30.15)	6.14	0.317
		Maximum Flux/Resting Level	1.97 (1.54-2.23)	1.47	0.317
		Time to Recovery (s)	20 (1.53-2.50)	2.25	0.317
		Time to Maximum Flux (s)	31.10 (13.23-273.9)	60.08	0.317
		Time to Half Recovery (s)	120.08 (34.78-282.78)	63.10	0.317

*Related Samples Wilcoxon Signed Rank Test

Table X: Post occlusive reactive hyperaemia, healed compared to unhealed. Baseline Visit

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=6/8)**	**Resting Flux (flux)**	83.50 (48.10-151.50)	50.10 (13.10-67.40)	0.142
		Biological Zero (flux)	5.00 (3.10-7.20)	4.70 (2.80-7.10)	0.662
		Maximum Flux (flux)	171.50 (14.00-254.60)	149.40 (105.90-204.50)	0.491
		Maximum Flux/Biological Zero	41.37 (21.21-58.72)	30.46 (18.60-56.96)	0.755
		Maximum Flux/Resting Level	1.87 (1.68-2.91)	4.16 (2.24-11.82)	0.142
		Time to Recovery (s)	1.50 (1.25-1.73)	0.85 (0.68-2.25)	0.228
		Time to Maximum Flux (s)	13.40 (6.33-73.85)	27.78 (3.44-83.36)	0.662
		Time to Half Recovery (s)	20.58 (13.45-78.28)	36.75 (13.68-92.81)	0.95
	Percutaneous Angioplasty (n=6/3)	**Resting Flux (flux)**	66.40 (15.30-172.80)	84.00 (74.50-106.60)	0.548
		Biological Zero (flux)	3.80 (3.20-4.20)	4.50 (2.90-4.70)	0.714
		Maximum Flux (flux)	81.40 (74.50-168.30)	137.40 (68.60-193.50)	0.905
		Maximum Flux/Biological Zero	20.01 (7.01-27.89)	41.18 (15.26-47.39)	0.584
		Maximum Flux/Resting Level	1.25 (1.13-1.74)	1.64 (0.92-1.82)	1.000
		Time to Recovery (s)	3.23 (2.25-45.13)	3.58 (2.93-117.55)	0.548
		Time to Maximum Flux (s)	210.50 (72.18-231.00)	288.78 (204.98-288.78)	0.381
		Time to Half Recovery (s)	200.20 (80.03-222.50)	290.63 (204.98-295.23)	0.143
Study Dorsum	**Diabetic Tissue Loss (n=6/8)**	**Resting Flux (flux)**	18.90 (11.80-22.90)	16.10 (8.30-22.00)	0.573
		Biological Zero (flux)	4.90 (4.60-5.00)	3.80 (3.10-4.70)	0.142
		Maximum Flux (flux)	45.80 (32.10-63.20)	45.40 (21.90-63.50)	0.852
		Maximum Flux/Biological Zero	9.43 (6.98-9.66)	12.93 (6.07-23.75)	0.662
		Maximum Flux/Resting Level	2.79 (2.49-2.92)	2.06 (1.64-6.05)	0.852

		Time to Recovery (s)	0.83 (0.48-1.15)	1.44 (0.84-2.50)	0.181
		Time to Maximum Flux (s)	5.23 (2.85-9.28)	11.25 (3.83-21.35)	0.282
		Time to Half Recovery (s)	8.60 (5.13-14.48)	16.69 (11.93-27.03)	0.108
	Percutaneous Angioplasty (n=6/3)	**Resting Flux (flux)**	28.80 (21.90-43.30)	28.70 (22.60-37.40)	1.000
		Biological Zero (flux)	5.50 (4.10-10.20)	5.80 (4.00-10.70)	1.000
		Maximum Flux (flux)	65.60 (37.80-94.30)	49.00 (35.00-61.60)	0.714
		Maximum Flux/Biological Zero	12.35 (8.04-15.21)	8.76 (4.58-10.62)	0.548
		Maximum Flux/Resting Level	1.88 (1.59-2.22)	1.55 (1.31-2.15)	0.548
		Time to Recovery (s)	2.08 (1.60-2.68)	4.33 (3.08-5.93)	0.167
		Time to Maximum Flux (s)	198.79 (69.18-287.33)	220.28 (93.65-271.38)	1.000
		Time to Half Recovery (s)	119.83 (70.00-291.73)	222.18 (94.63-272.33)	1.000
Non-study Toe	**Diabetic Tissue Loss (n=6/8)**	**Resting Flux (flux)**	65.70 (36.40-112.70)	46.30 (17.30-75.10)	0.282
		Biological Zero (flux)	4.60 (3.10-10.20)	3.60 (3.20-7.20)	0.573
		Maximum Flux (flux)	138.90 (12.00-303.20)	95.20 (32.30-168.20)	0.282
		Maximum Flux/Biological Zero	18.02 (13.95-36.81)	19.10 (5.05-28.47)	0.755
		Maximum Flux/Resting Level	1.75 (1.32-3.30)	2.33 (1.66-2.68)	1.000
		Time to Recovery (s)	1.64 (1.00-8.18)	1.31 (0.95-27.29)	1.000
		Time to Maximum Flux (s)	38.98 (12.68-262.10)	34.05 (10.68-123.23)	0.662
		Time to Half Recovery (s)	53.20 (38.25-268.18)	66.96 (14.99-128.04)	0.345
	Percutaneous Angioplasty (n=4/3)	**Resting Flux (flux)**	41.50 (32.60-63.20)	21.90 (8.10-78.50)	0.400
		Biological Zero (flux)	4.40 (3.40-5.90)	3.80 (3.10-5.50)	1.000
		Maximum Flux (flux)	111.70 (83.80-132.20)	38.70 (17.00-155.30)	0.629
		Maximum Flux/Biological Zero	19.83 (16.59-35.01)	7.04 (5.47-40.87)	0.400
		Maximum Flux/Resting Level	2.39 (2.02-2.71)	1.98 (1.77-2.09)	0.229
		Time to Recovery (s)	2.03 (1.70-10.95)	2.55 (2.45-3.75)	0.400

		Time to Maximum Flux (s)	34.38 (8.66-62.28)	127.43 (54.73-237.73)	0.229
		Time to Half Recovery (s)	55.79 (11.19-98.08)	133.98 (128.93-240.75)	0.057
Non-study Dorsum	**Diabetic Tissue Loss (n=6/8)**	**Resting Flux (flux)**	8.70 (8.10-10.90)	9.30 (7.10-12.40)	1.000
		Biological Zero (flux)	4.20 (3.10-4.70)	4.80 (2.90-7.00)	0.573
		Maximum Flux (flux)	24.50 (12.10-39.90)	23.60 (15.30-34.60)	1.000
		Maximum Flux/Biological Zero	5.31 (3.40-10.22)	4.01 (3.76-5.74)	0.852
		Maximum Flux/Resting Level	1.91 (1.70-2.80)	2.27 (1.99-3.27)	0.573
		Time to Recovery (s)	0.74 (0.68-0.85)	1.21 (0.75-1.51)	0.228
		Time to Maximum Flux (s)	10.20 (2.30-55.38)	15.56 (5.91-117.88)	0.491
		Time to Half Recovery (s)	17.53 (5.85-23.05)	31.31 (14.40-141.03)	0.284
	Percutaneous Angioplasty (n=4/3)	**Resting Flux (flux)**	15.10 (13.30-20.40)	47.50 (11.30-86.60)	0.629
		Biological Zero (flux)	5.30 (4.30-7.40)	6.40 (4.20-9.60)	0.629
		Maximum Flux (flux)	71.10 (39.10-87.70)	73.20 (22.30-192.90)	0.857
		Maximum Flux/Biological Zero	11.89 (7.30-13.51)	7.63 (5.30-30.15)	1.000
		Maximum Flux/Resting Level	3.88 (2.15-5.29)	1.97 (1.54-2.23)	0.400
		Time to Recovery (s)	1.74 (1.13-7.86)	2.00 (1.53-2.50)	0.857
		Time to Maximum Flux (s)	91.74 (58.41-125.70)	31.10 (13.23-273.90)	0.629
		Time to Half Recovery (s)	94.58 (64.60-126.45)	120.08 (34.78-282.78)	0.857

*Independent-Samples Mann-Whitney U Test

Table XI: Post occlusive reactive hyperaemia, healed compared to unhealed. Second Visit

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	77.00 (33.40-104.20)	104.80 (7.70-107.70)	1.000
		Biological Zero (flux)	3.40 (2.70-7.80)	3.30 (2.70-3.70)	1.000
		Maximum Flux (flux)	184.00 (168.20-197.60)	192.90 (75.40-277.80)	0.792
		Maximum Flux/Biological Zero	40.95 (25.33-65.56)	58.46 (34.28-97.10)	0.537
		Maximum Flux/Resting Level	2.66 (1.81-4.32)	2.58 (2.23-8.07)	0.429
		Time to Recovery (s)	1.25 (1.05-4.60)	1.03 (0.85-1.18)	0.177
		Time to Maximum Flux (s)	19.19 (6.83-27.08)	8.50 (4.28-11.08)	0.429
		Time to Half Recovery (s)	22.59 (12.55-29.20)	14.78 (13.83-36.13)	1.000
	Percutaneous Angioplasty (n=6/1)	**Resting Flux (flux)**	38.40 (29.70-59.00)	54.80 (16.40-93.10)	1.000
		Biological Zero (flux)	4.30 (4.10-10.90)	5.40 (4.40-6.40)	0.643
		Maximum Flux (flux)	102.90 (80.80-152.40)	76.90 (21.50-132.30)	0.643
		Maximum Flux/Biological Zero	22.18 (13.99-27.92)	16.73 (3.37-30.08)	1.000
		Maximum Flux/Resting Level	2.55 (2.08-4.76)	1.37 (1.31-1.42)	0.286
		Time to Recovery (s)	1.80 (0.58-2.10)	3.49 (3.33-3.65)	0.286
		Time to Maximum Flux (s)	53.79 (27.38-177.83)	120.65 (8.80-232.50)	1.000
		Time to Half Recovery (s)	74.01 (29.00-178.90)	124.30 (15.38-233.23)	1.000
Study Dorsum	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	18.50 (11.60-23.10)	16.20 (15.90-29.40)	1.000
		Biological Zero (flux)	4.00 (3.40-4.70)	3.30 (2.80-4.50)	0.429
		Maximum Flux (flux)	37.40 (35.70-46.10)	72.40 (48.80-87.30)	0.429
		Maximum Flux/Biological Zero	9.97 (6.89-14.88)	21.46 (9.56-26.45)	0.662
		Maximum Flux/Resting Level	2.50 (2.15-3.22)	2.60 (2.46-3.07)	0.792
		Time to Recovery (s)	0.89 (0.75-0.95)	1.03 (0.90-1.05)	0.247

		Time to Maximum Flux (s)	3.09 (2.15-5.28)	20.38 (9.78-209.20)	**0.030**
		Time to Half Recovery (s)	5.79 (3.53-15.33)	39.65 (12.40-210.93)	0.052
	Percutaneous Angioplasty (n=6/1)	**Resting Flux (flux)**	13.40 (10.60-26.80)	31.80	0.286
		Biological Zero (flux)	4.40 (3.10-5.40)	5.00	0.857
		Maximum Flux (flux)	29.10 (22.40-53.80)	68.50	0.571
		Maximum Flux/Biological Zero	9.88 (4.72-11.46)	13.71	0.571
		Maximum Flux/Resting Level	1.94 (1.33-3.12)	2.16	0.857
		Time to Recovery (s)	1.54 (0.93-4.08)	3.40	0.857
		Time to Maximum Flux (s)	142.33 (80.13-227.13)	55.05	0.571
		Time to Half Recovery (s)	143.8 (80.35-228.68)	60.43	0.571
Non-study Toe	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	93.80 (24.40-183.50)	36.70 (9.80-144.20)	0.537
		Biological Zero (flux)	4.20 (2.90-4.50)	5.00 (3.10-6.30)	0.662
		Maximum Flux (flux)	213.90 (131.00-454.90)	53.20 (33.00-269.60)	0.329
		Maximum Flux/Biological Zero	41.48 (11.16-156.87)	10.64 (8.44-50.76)	0.329
		Maximum Flux/Resting Level	1.86 (1.73-6.03)	1.87 (1.72-2.43)	0.792
		Time to Recovery (s)	1.36 (1.33-1.40)	1.48 (1.13-2.30)	0.931
		Time to Maximum Flux (s)	15.53 (6.98-120.95)	11.83 (11.68-105.38)	0.931
		Time to Half Recovery (s)	26.44 (18.28-121.83)	22.88 (16.63-108.43)	1.000
	Percutaneous Angioplasty (n=5/2)	**Resting Flux (flux)**	26.30 (21.90-54.30)	35.40 (20.60-50.10)	1.000
		Biological Zero (flux)	4.80 (2.90-6.50)	4.00 (3.10-4.80)	1.000
		Maximum Flux (flux)	80.40 (77.00-208.80)	90.00 (30.90-149.00)	0.571
		Maximum Flux/Biological Zero	27.73 (22.59-43.50)	27.25 (6.43-48.06)	1.000
		Maximum Flux/Resting Level	2.93 (2.58-3.85)	2.24 (1.50-2.97)	1.000
		Time to Recovery (s)	2.15 (1.98-2.98)	2.14 (1.90-2.38)	1.000
		Time to Maximum Flux (s)	65.40 (30.03-69.55)	10.85 (6.53-15.18)	0.095

		Time to Half Recovery (s)	66.48 (34.68-126.33)	16.75 (15.53-17.98)	0.095
Non-study Dorsum	**Diabetic Tissue Loss (n=5/5)**	**Resting Flux (flux)**	9.00 (8.60-17.50)	6.80 (5.40-12.60)	0.310
		Biological Zero (flux)	3.90 (3.70-4.00)	3.50 (3.30-4.00)	0.548
		Maximum Flux (flux)	27.10 (18.90-81.70)	21.30 (14.40-32.30)	0.548
		Maximum Flux/Biological Zero	7.31 (4.73-20.95)	6.09 (6.00-8.07)	0.841
		Maximum Flux/Resting Level	3.13 (2.92-3.94)	3.11 (2.65-3.67)	0.841
		Time to Recovery (s)	0.70 (0.58-0.93)	0.33 (0.30-1.10)	0.548
		Time to Maximum Flux (s)	18.70 (18.15-102.18)	29.60 (12.88-234.58)	0.690
		Time to Half Recovery (s)	22.35 (19.20-102.40)	34.90 (29.60-234.88)	0.421
	Percutaneous Angioplasty (n=5/1)	**Resting Flux (flux)**	13.70 (8.70-23.30)	20.10	1.000
		Biological Zero (flux)	5.40 (4.80-6.70)	4.80	0.667
		Maximum Flux (flux)	45.90 (30.80-59.00)	29.50	0.667
		Maximum Flux/Biological Zero	8.49 (6.56-11.56)	6.14	0.667
		Maximum Flux/Resting Level	4.30 (1.94-4.35)	1.47	0.667
		Time to Recovery (s)	1.50 (1.25-2.30)	2.25	1.000
		Time to Maximum Flux (s)	76.18 (65.08-243.9)	60.08	0.667
		Time to Half Recovery (s)	103.23 (78.40-245.00)	63.10	0.333

*Independent-Samples Mann-Whitney U Test

Table XII: Post occlusive reactive hyperaemia, healed compared to unhealed. Last Visit

			Healed Median (IQR)	Unhealed Median (IQR)	p-value*
Study Toe	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	59.80 (32.30-122.70)	107.70 (104.80-138.90)	0.537
		Biological Zero (flux)	5.20 (2.60-7.30)	3.40 (3.30-3.70)	0.792
		Maximum Flux (flux)	167.00 (128.00-190.90)	192.90 (138.30-277.80)	0.329
		Maximum Flux/Biological Zero	35.19 (23.27-49.22)	58.46 (37.69-97.10)	0.329
		Maximum Flux/Resting Level	3.00 (1.39-3.97)	2.23 (1.84-2.58)	0.537
		Time to Recovery (s)	1.50 (0.80-6.35)	1.18 (1.03-1.38)	1.000
		Time to Maximum Flux (s)	64.43 (22.05-114.20)	9.68 (8.50-154.38)	0.792
		Time to Half Recovery (s)	69.06 (28.53-121.43)	36.13 (15.18-154.38)	1.000
	Percutaneous Angioplasty (n=6/2)	**Resting Flux (flux)**	33.80 (29.70-56.00)	69.30 (16.40-122.10)	1.000
		Biological Zero (flux)	5.30 (3.30-9.10)	5.80 (5.10-6.40)	1.000
		Maximum Flux (flux)	80.40 (55.70-125.60)	86.10 (21.50-150.60)	1.000
		Maximum Flux/Biological Zero	22.21 (6.12-25.12)	16.45 (3.37-29.52)	1.000
		Maximum Flux/Resting Level	2.38 (1.56-2.79)	1.27 (1.23-1.31)	0.286
		Time to Recovery (s)	2.26 (1.68-4.88)	8.35 (3.65-13.05)	0.286
		Time to Maximum Flux (s)	50.71 (27.38-105.18)	141.08 (49.65-232.50)	0.429
		Time to Half Recovery (s)	67.24 (29.15-106.15)	142.73 (52.23-233.23)	0.429
Study Dorsum	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	11.20 (10.20-19.90)	23.60 (16.20-29.40)	0.177
		Biological Zero (flux)	4.00 (3.40-4.60)	3.30 (3.10-4.50)	0.329
		Maximum Flux (flux)	29.20 (20.10-37.40)	72.40 (24.80-87.30)	0.429
		Maximum Flux/Biological Zero	6.61 (5.90-8.92)	21.46 (6.82-26.45)	0.247
		Maximum Flux/Resting Level	2.19 (1.78-3.01)	1.84 (1.68-2.46)	0.792
		Time to Recovery (s)	0.86 (0.58-1.28)	1.05 (0.90-1.58)	0.247

		Time to Maximum Flux (s)	13.15 (5.68-13.60)	20.38 (4.95-192.98)	0.429
		Time to Half Recovery (s)	15.80 (9.60-18.48)	39.65 (12.40-193.23)	0.247
	Percutaneous Angioplasty (n=6/1)	**Resting Flux (flux)**	24.80 (15.00-30.40)	22.80	1.000
		Biological Zero (flux)	5.70 (4.60-6.50)	4.60	0.571
		Maximum Flux (flux)	45.90 (32.50-60.40)	30.50	0.571
		Maximum Flux/Biological Zero	8.18 (4.72-9.95)	6.63	1.000
		Maximum Flux/Resting Level	1.88 (1.20-3.34)	1.34	1.000
		Time to Recovery (s)	2.13 (0.78-4.53)	2.40	0.857
		Time to Maximum Flux (s)	175.76 (105.20-227.13)	30.08	0.571
		Time to Half Recovery (s)	176.43 (106.03-228.68)	43.38	0.571
Non-study Toe	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	122.10 (51.00-184.50)	109.30 (21.00-144.20)	0.662
		Biological Zero (flux)	5.60 (3.20-11.50)	3.10 (2.60-6.90)	0.537
		Maximum Flux (flux)	221.50 (173.90-273.40)	220.00 (84.80-269.60)	0.662
		Maximum Flux/Biological Zero	32.11 (22.57-85.43)	42.39 (10.64-50.76)	0.792
		Maximum Flux/Resting Level	1.98 (1.21-9.29)	2.01 (1.87-3.35)	0.792
		Time to Recovery (s)	2.06 (1.40-6.48)	1.48 (1.20-2.03)	0.429
		Time to Maximum Flux (s)	87.20 (18.83-291.13)	11.83 (11.68-15.53)	0.082
		Time to Half Recovery (s)	92.98 (19.68-292.73)	22.88 (16.63-23.70)	0.247
	Percutaneous Angioplasty (n=5/2)	**Resting Flux (flux)**	50.30 (34.60-54.30)	26.40 (20.60-32.10)	0.190
		Biological Zero (flux)	4.80 (3.20-6.50)	4.20 (3.50-4.80)	1.000
		Maximum Flux (flux)	145.30 (83.90-208.80)	54.70 (30.90-78.50)	0.190
		Maximum Flux/Biological Zero	36.49 (21.37-43.50)	14.43 (6.43-22.43)	0.381
		Maximum Flux/Resting Level	2.27 (1.67-3.85)	1.97 (1.50-2.44)	1.000
		Time to Recovery (s)	2.38 (2.15-3.93)	2.06 (1.75-2.38)	0.571
		Time to Maximum Flux (s)	69.55 (35.33-146.28)	15.51 (15.18-15.85)	0.095

		Time to Half Recovery (s)	126.33 (76.13-163.45)	19.04 (17.98-20.10)	0.095
Non-study Dorsum	**Diabetic Tissue Loss (n=6/5)**	**Resting Flux (flux)**	17.00 (9.60-20.70)	8.00 (6.80-15.20)	0.082
		Biological Zero (flux)	4.90 (3.90-5.50)	3.50 (2.50-3.80)	**0.030**
		Maximum Flux (flux)	34.00 (20.10-54.00)	21.30 (18.60-32.30)	0.662
		Maximum Flux/Biological Zero	5.50 (4.26-10.81)	7.42 (6.09-8.07)	0.247
		Maximum Flux/Resting Level	2.38 (1.22-3.35)	3.11 (1.90-3.67)	0.537
		Time to Recovery (s)	0.95 (0.55-1.43)	1.10 (0.30-1.43)	1.000
		Time to Maximum Flux (s)	17.05 (12.13-142.95)	13.90 (12.88-215.65)	1.000
		Time to Half Recovery (s)	24.73 (15.60-143.58)	29.60 (17.85-216.83)	1.000
	Percutaneous Angioplasty (n=5/1)	**Resting Flux (flux)**	15.90 (13.70-20.00)	15.30	1.000
		Biological Zero (flux)	4.80 (4.70-5.90)	3.30	0.333
		Maximum Flux (flux)	30.80 (29.20-55.60)	17.90	0.333
		Maximum Flux/Biological Zero	5.39 (4.96-6.56)	5.42	1.000
		Maximum Flux/Resting Level	2.01 (1.47-4.30)	1.17	0.333
		Time to Recovery (s)	3.23 (1.50-3.58)	2.58	1.000
		Time to Maximum Flux (s)	161.88 (76.18-265.28)	17.23	0.333
		Time to Half Recovery (s)	162.98 (78.40-266.98)	21.45	0.333

*Independent-Samples Mann-Whitney U Test

APPENDIX VIII: COMPARISON OF DEMOGRAPHICS OBSERVED IN THE MATCHED COHORTS TO THE EXPECTED PROPORTIONS BASED ON THE RAW DATASET

Table XIII: Comparison of demographics observed in the matched cohorts to the expected proportions based on the raw dataset

		Diabetes Mellitus					No Diabetes Mellitus				
		Observed	Expected	Residual	X^2 (df)	p-value*	Observed	Expected	Residual	X^2 (df)	p-value*
Sex	**Female**	43	48.7	-5.7	0.963 (1)	0.326	42	56.8	-14.8	6.104 (1)	0.013
	Male	110	104.3	5.7			111	96.2	14.8		
Ethnicity	**White**	149	128.2	20.8	20.843 (2)	<0.001	149	144.8	4.2	2.407 (2)	0.300
	Asian	2	15.1	-13.1			2	3	-1		
	Black	2	9.7	-7.7			2	5.2	-3.2		
Smoking	**Never**	17	34.7	-17.7	11.907 (2)	0.003	17	26.3	-9.3	14.196 (2)	0.001
	Ex-smoker	104	88.5	15.5			103	79.8	23.2		
	Still Smoking	32	29.8	2.2			33	47	-14		
Hypertension	**No**	25	29.5	-4.5	27.776 (1)	0.356	25	56.5	-31.5	0.708 (1)	<0.001
	Yes	128	123.5	4.5			128	96.5	31.5		
High Cholesterol	**No**	60	55.1	4.9	0.687 (1)	0.407	59	67.2	-8.2	1.789 (1)	0.181
	Yes	93	97.9	-4.9			94	85.8	8.2		
Renal	**Normal**	148	122.4	25.6	26.727 (1)	<0.001	148	142.5	5.5	3.059 (1)	0.080
	Failure	5	30.6	-25.6			5	10.5	-5.5		
Timing	**Elective**	108	98.5	9.5	2.561 (1)	0.110	137	119.6	17.4	11.598 (1)	0.001
	Emergency	45	54.5	-9.5			16	33.4	-17.4		

*Chi^2 Goodness-of Fit Test, df-degrees of freedom