# An investigation into relationships among neural, vascular and osseous factors in the diabetic foot

Submitted for the degree of Doctor of Philosophy

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## Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Alex Louise Barwick

# Statement of Authorship

I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications.

Alex Louise Barwick

## Acknowledgements

Firstly, I would like to thank my supervisors Vivienne Chuter, Xanne Janse de Jonge and John Tessier for their expertise and mentorship. Special thanks to Viv for her guidance and the opportunity to study such a fascinating topic.

Thank you to all those with whom I collaborated: Sean Lanting in Chapter Three, James Mirrow and James Ivers in Chapter Four and the team at Hunter New England Health Podiatry Service including Dominique Viola, Julie Zwartarveen, Bronwyn Hardy, Brittany Caracher, Bradley Moodie and Nicholas Miller, and the teams at Hunter Imaging and Angela Green Podiatry.

Thanks also to the Newcastle Star and the Newcastle Herald for promotion of the studies in Chapters Five and Six, to Jennifer Reeds for administrative support and the participants of the studies.

I would like to acknowledge the following financial support: Australian Postgraduate Award Scholarship, University of Newcastle Faculty of Health and Medicine Research Higher Degree Funding, University of Newcastle Faculty of Health departmental funding and Australian Diabetes Society/Merck Sharp & Dohme travel grant.

# Contents

Statement of Originality	ii
Statement of Authorship	iii
Acknowledgements	iv
Abstract	ix
List of Publications, Manuscripts, and Conference Abstracts	xi
List of Abbreviations	xiii
List of Appendices	xv

Chapter One: Introduction
Diabetes 1
Diabetic neuropathy
Epidemiology
Overview of the nervous system
Pathogenesis of diabetic neuropathy 4
Classification
Neuropathy and microvascular reactivity
Charcot foot9
Clinical presentation
Staging10
Epidemiology and impact10
Diagnosis
Treatment
The importance of early diagnosis and treatment

Pathogenesis	
Summary	
Chapter Two: Objectives	
Chapter Three: Literature Review	
Abstract	
Introduction	
Methods	
Results	
Study outcomes	
Quality assessment	
Meta-analysis and publication bias	
Discussion	
Conclusion	
References	
Chapter Four: Methodology Intra-tester and inter-tester reliability of post-occlusive	reactive hyperaemia measurement at the
hallux	
Abstract	
Introduction	
Material and methods	
Results	
Discussion	
References	

Reliability of computed tomography derived foot bone density measurements	in people with
diabetes	
Abstract	
Introduction	
Materials and Methods	
Results	
Discussion	
Conclusions	
References	
Results Discussion Conclusions References	

Abstract	59
Introduction	60
Research Design and Methods	61
Results	64
Conclusions	64
References	68

Cl	hapter Six: Foot bone density in diabetes may be unaffected by the presence of neuropathy	. 73
	Abstract	. 76
	Introduction	. 77
	Materials and Methods	. 78
	Results	. 82
	Discussion	. 83
	Conclusions	. 86

References	87
Chapter Seven: Conclusions	95
References	180

### Abstract

The social and financial cost of diabetes and associated lower limb complications is increasing markedly. Interaction between neurological and vascular dysfunction in diabetes are thought to influence bone in the periphery, predisposing to pathology such as Charcot foot, a rare but debilitating joint disease. However, there is a lack of conclusive evidence relating neurological and vascular function to peripheral bone health in people with diabetes. This thesis presents an investigation into relationships among neuropathy, vascular dysfunction and foot bone health in those with diabetes. Such information is useful in the prevention, diagnosis and management of lower limb complications of diabetes.

The research is designed to address two central hypotheses:

- That those with diabetic neuropathy have altered vascular reactivity in the feet
- That neuropathy induced vascular changes in those with diabetes, contribute to a reduction in bone mineral density in the feet

The research includes, firstly, a systematic review of current research related to foot bone strength in people with diabetic neuropathies with a meta-analysis of obtained data. Inconsistent findings were observed among the ten included studies and the meta-analysis was equivocal. Furthermore, the literature was limited by methodological quality and gaps within the literature were observed including the lack of data on foot bones other than the calcaneus prompting the need for further research.

Secondly, two studies developing methodologies required for the research were performed. A reliability study of techniques for assessing post-occlusive reactive hyperaemia at the hallux as a measure of microvascular function was performed. The study found that its measurement in the hallux, using laser Doppler with a probe heated to thermoneutral, is a reliable method of measuring microvascular function for use in research. The most reliable parameters were peak as a percentage of baseline and the index of the area under the curve post-occlusion to pre-occlusion. A reliability study of computed tomography derived densitometry of all tarsal and metatarsal bones was also performed.

The study found that foot bone density can be reliably measured in the tarsals and metatarsals using averaged regions of interest on computed tomography scans. Trabecular bone density was more reliably derived than that of cortical bone. These two methodologies, measurement of post-occlusive reactive hyperaemia at the hallux and bone density measurement in the foot, were used in the final two studies addressing the central hypotheses.

A cross-sectional study was performed to test the hypothesis that those with diabetic neuropathy have altered vascular reactivity in the feet. This approach was taken to examine the complex relationships among diabetic neuropathy types and vascular reactivity in a clinically relevant population, accounting for important confounders in the design and statistical analyses. The study found that the presence of sensory neuropathy was predictive of a slower time to peak perfusion following occlusion.

Finally, a cross-sectional case-control study was performed to test the hypothesis that neuropathy induced vascular changes in those with diabetes, contribute to a reduction in bone mineral density in the feet. The study compares the foot bone density of those with diabetic neuropathy with a diabetes control group. No clear association was demonstrated. Additional analyses were performed to observe potential relationships between subtype of neuropathy and foot bone density, and microvascular dysfunction and foot bone density. No relationships were observed.

These results, limited by the cross-sectional design of the studies, suggest that whilst peripheral neuropathy is associated with altered microvascular function, this may not have an impact on foot bone density in a manner that predisposes to pathology.

# List of Publications, Manuscripts, and Conference Abstracts

#### **Publications**

Barwick, A.L., Janse de Jonge, X. A. K., Tessier, J. W., Ho, A. & Chuter, V. H. *The effect of diabetic neuropathy on foot bones: a systematic review and meta-analysis.* Diabet Med, 2014. 31(2): p. 136-147

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(0): p. 67-71

In Press (see Appendix H)

Barwick, A. L., Tessier, J. W., Janse de Jonge, X., & Chuter, V. H. (2016). *Foot bone density in diabetes may be unaffected by the presence of neuropathy*. J Diabet Complications. In Press

#### Manuscripts under review

'Reliability of computed tomography derived foot bone density measurements in people with diabetes'

'Peripheral sensory neuropathy is associated with altered post-occlusive reactive hyperemia in the diabetic foot'

#### Conference Abstracts (see Appendix I)

Sydney Diabetic Foot Conference – May 2013 – The effect of diabetic neuropathy on peripheral bone: a systematic review and meta-analysis

NSW Australian Podiatry Association Conference – Sydney, April 2014 – The effect of diabetic neuropathy on peripheral bone: a systematic review and meta-analysis

Australasian Podiatry Council Conference – Dunedin, New Zealand, November 2014 – The effect of diabetic neuropathy on peripheral bone: a systematic review and meta-analysis

Australasian Podiatry Council Conference - Gold Coast, May 2015

Barwick, A., Lanting, S., & Chuter, V. Intra- and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux. Australasian Podiatry Conference. JFAR, 2015. 8(2): p. O1

European Association for the Study of Diabetes Conference - Stockholm, Sweden, September 2015

Barwick, A., Tessier, J., Janse de Jonge, X., & Chuter, V. Initial findings in the relationship between diabetic peripheral neuropathy and microvascular reactivity in the foot. European Association for the Study of Diabetes Annual Meeting. Diabetologia, 2015. 58(S1): p. S502

AGE	advanced glycated end-product
ANS	autonomic nervous system
BMD	bone mineral density
CAN	cardiac autonomic neuropathy
CGRP	calcitonin gene-related peptide
CN	neuropathic osteoarthropathy
HU	Hounsfield units
ICC	Intra-class correlation coefficient
LFN	large fibre neuropathy
LOA	limits of agreement
MRI	magnetic resonance imaging
OPG	osteoprotogerin
PORH	post-occlusive reactive hyperaemia
PRISMA	preferred reporting items for systematic reviews and meta-analyses
RAGE	receptor for advanced glycated end-product
RANK-L	receptor activator of nuclear factor kappa-B ligand
SEM	standard error of measurement
SFN	small fibre neuropathy

sRAGE soluble receptor for advanced glycated end-product

VPT vibration perception threshold

# List of Appendices

Appendix A: statements of authorship106
Appendix B: materials for the study in Chapter Four112
Appendix C: materials for the studies in Chapters Four, Five and Six121
Appendix D: computed tomography foot scan method for the studies in Chapters Four and Six153
Appendix E: intra-tester reliability of outcome measures used in Chapters Five and Six156
Appendix F: tables of correlations for the study in Chapter Five162
Appendix G: STROBE checklist for the case-control study in Chapter Six
Appendix H: publication of the study in Chapter Six167
Appendix I: conference abstracts

### Chapter One

### Introduction

### Diabetes

Diabetes mellitus is a metabolic condition in which the body fails to produce and/or utilise insulin resulting in sustained high blood glucose concentrations (hyperglycaemia) [1]. There are two main types of diabetes, type 1 and type 2 [2]. Type 1 diabetes is usually diagnosed in childhood or adolescence. It results from immune-mediated damage to the pancreatic cells that produce insulin resulting in an absolute inability for its endogenous production [1]. It must, therefore, be treated with insulin therapy. Type 2 diabetes usually develops in adulthood. It initiates as insulin resistance, where peripheral cells become unresponsive to insulin. This is compensated, at first, by an increase in insulin production but is followed by a reduction and eventually absolute loss of insulin production [3]. It can be managed with lifestyle changes, oral medication or insulin therapy, depending on its severity [1]. Diabetes can also occur secondary to conditions such as pancreatitis, and can occur during pregnancy (gestational diabetes) [1]. A diagnosis of diabetes is made through the testing of glycated haemoglobin and/or plasma glucose concentrations, often in the presence of symptoms (e.g. weight loss, frequent urination, thirst) although these may be less evident in cases of type 2 diabetes [2].

Diabetes has become a prominent worldwide health issue. In 2011, diabetes was estimated to affect 366 million people globally with prevalence expected to rise to 552 million by 2030 [4]. Nationally, between 45 000 and 100 000 Australians are diagnosed with diabetes each year. In 2004-2005, 700 000 people or 3.6% of the population had diagnosed diabetes, double the prevalence reported for 1989-1990 [5]. Continued increases are expected worldwide, particularly of type 2 diabetes, due to an increased the incidence of the condition and associated risk factors for it such as obesity [6]. In Australia, a longer life expectancy in those with diabetes, the aging population, and an increase in migration from countries with a high incidence also contribute to its prevalence [7]. Significantly, it is expected that by 2025, between 7.7 and 17% of the Australian population will have diabetes [7].

Diabetes is a recognised National Health Priority Area due to its increasing prevalence and its contribution to morbidity, mortality and poor quality of life in Australians [5]. In 2005, diabetes was responsible for 500 000 hospitalisations [5] and contributed to 12 000 deaths [8] having a direct health care burden of \$907 million [5]. Health complications associated with diabetes are significant, affecting multiple organ systems. Those with diabetes are twice as likely to suffer from a myocardial infarction, three times more likely to have a stroke and have a significantly increased risk of microvascular diseases such as retinopathy, nephropathy and neuropathy [5]. Diabetic neuropathy causes a range of complications, including silent myocardial ischaemia and sudden death [9]. In the lower extremity, neuropathy plays a major role in the development of foot complications including pressure ulceration, delayed wound healing and amputation [10]. Neuropathy has also been linked with increased bone fragility and Charcot neuropathic osteoarthropathy, a destructive joint disease, of the foot which is often referred to as Charcot foot [11].

### Diabetic neuropathy

#### Epidemiology

Neuropathies are extremely common in people with diabetes with up to 67% having at least one clinical sign of neuropathy [12]. Rates of neuropathy are higher in people with poorer glycaemic control [13], those with a longer duration of diabetes [13-15] and with increasing age [16]. Prevalence

estimates of diagnosed clinical neuropathy are between 13.1 [17] and 45% [18] in type 2 diabetes and between 22.7 [15] and 54% [18] in type 1 diabetes. Dysfunction can occur at multiple levels of the nervous system [10]. To gain a better understanding of diabetic neuropathies a brief overview of the nervous system is provided below.

#### Overview of the nervous system

The nervous system sends and receives information throughout the body via electrical signals conducted along nerve cells – neurons [19]. It is broadly divided into the central nervous system (brain and spinal cord) where information processing occurs, and the peripheral nervous system, which includes nerves functioning in the rest of the body [20]. The peripheral nervous system can be further subdivided into the autonomic (sympathetic and parasympathetic) and somatic (sensory and motor) components [19]. All components are interdependent and act with the endocrine system to control and regulate all body systems and respond to changes in the internal and external environments [20]. In addition to nerve cells themselves, support cells are a vital component of the nervous system. Notably, oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system provide an insulating sheath around the neurons, increasing the speed of conduction [21, 22]. Nerves can be classified based on the degree of myelination – small fibre vs large fibre neurons [23].

Peripheral nerves can be afferent (moving towards the central nervous system) or efferent (moving away from the central nervous system) [19]. Afferent nerves contain receptors in the periphery that detect changes in the internal (interoreceptors) or external (exteroreceptors) environment [20]. Exteroreceptors include: mechanoreceptors which sense touch and pressure; thermoreceptors which sense warmth and cold; nociceptors which sense painful stimuli; and proprioceptors which sense limb position. Interoreceptors include: baroreceptors in the aorta which detect blood pressure; and proprioreceptors in the cerebellum and somatosensory cortex [20]. When stimulated, these receptors trigger action potentials in the neurons that send the electrical signal along the nerve axon to the spinal cord and up to dedicated areas in the brain [20]. Efferent signals travel from the brain and spinal cord to the periphery. In the somatic nervous system this results in innervation of skeletal muscle and in the autonomic nervous system signals are sent to various organ systems such as the lungs, adrenal glands, eyes and heart [20].

The autonomic nervous system (ANS) has influence over cardiovascular function. Centrally, the sympathetic and parasympathetic systems control heart rate, conduction velocity, contraction and relaxation for rapid control of cardiac output and blood pressure in response to external stressors [24]. Both parasympathetic and sympathetic components of the ANS control heart rate and contractility and blood pressure. Stimulation of the sympathetic fibres causes an increase in heart rate and blood pressure, whilst stimulation of the parasympathetic fibres causes a slowing of heart rate and lowering of blood pressure [24].

Peripherally, the ANS influences blood vessel dilation and contraction [25]. Tonic constriction of the peripheral arteries, arterioles and veins is maintained by unmyelinated sympathetic fibres located in the adventitia media layers of these vessels [26]. Arterioles in the non-essential organs are most sensitive to this regulation – skin, muscle, kidneys and viscera [26]. When sympathetic tone is reduced the vessels dilate, increasing flow. This controls nutritional flow to peripheral tissues, functions to maintain body temperature and blood pressure within narrow ranges, and provides a blood flow response to injury [25]. Control of blood flow to skin is influenced by the presence of arteriovenous anastomoses. These are vessels that travel directly from artery to vein bypassing nutritive flow. In normal circumstances they remain closed due to sympathetic tone, allowing skin nutritive circulation. When sympathetic tone is relaxed, the vessels dilate and blood flow is redirected away from the skin [25]. Glabrous skin, such as on the plantar and palmar surfaces of the feet and hands respectively, has a higher density of arteriovenous anastomoses [27].

#### Pathogenesis of diabetic neuropathy

Diabetes causes dysfunction within the nervous system through nerve ischaemia [28, 29]. The structure and function of the microvasculature is affected by diabetes including endothelial cell hyperplasia, capillary basement membrane thickening, and a reduction in the secretion of vasodilators. This occurs in the endoneural blood vessels resulting in nerve hypoperfusion [28, 29]. This is coupled with hyperglycaemia induced disturbances to various biochemical pathways outlined below result in increased oxidative stress, causing further impairments to nerve functioning [30, 31].

Chronic hyperglycaemia causes an increase in glucose metabolism by the polyol pathway. This pathway reduces glucose to sorbitol. The resulting accumulation of sorbitol alters intracellular

osmolarity and reduces the synthesis of products essential to nerve functioning [30]. There is also an increase in AGE/RAGE (advanced glycated end-product/receptor for AGE) pathway activity that causes glycosylation of neural proteins which slows axonal transport [31]. Additionally, increased production and accumulation of reactive oxygen species further damages proteins and lipids involved in axonal transport and signalling [31]. These changes cause cell degeneration, slowed conduction and an inability to self-repair, eventually leading to cell death [31]. These processes can occur in most subsystems of the nervous system including the autonomic and somatic (sensory and motor) components resulting in multiple subtypes of diabetic neuropathy [10].

#### Classification

Diabetic neuropathy can be classified by function of the affected fibres (autonomic or somatic neuropathy) or by their degree of myelination (small fibre or large fibre) [32]. A clinically focused classification has been devised by the American Diabetes Association, which includes both focal and multifocal neuropathy as well as generalised symmetric polyneuropathies [10].

The widely accepted general definition of peripheral diabetic neuropathy is:

"the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" [33].

Peripheral neuropathies are length-dependent with the longer nerves being affected first, resulting in lower limb involvement before the upper limb and progression from distal to proximal [34]. Affected individuals may experience numbness, neuropathic pain, paraesthesia, muscle atrophy and weakness, foot deformity, and gait changes [34]. Clinical tests include psychophysical testing or nerve conduction studies [35].

Due to their commonness in association with diabetes and potential effects on vascular and osseous function, the three subtypes of neuropathy relevant to this work are: large fibre neuropathy (LFN), small fibre neuropathy (SFN), and autonomic neuropathy.

#### Large fibre neuropathy

Large fibre neuropathy affects both motor and sensory fibres of the somatic nervous system [10]. Diagnosis is usually made through a combination of self-reported symptoms and clinical assessment of primarily the sensory component [35]. Clinical psychophysical assessments include, touch/pressure perception which can be assessed with monofilaments calibrated to apply a specific pressure (usually 10g) [36]. The monofilament is applied to the foot, triggering pressure receptors (Meissner's corpuscles) in the superficial dermis which triggers a signal carried by large A-beta fibres to the central nervous system [37]. The distribution of these fibres can also be assessed by applying two points of pressure at varying distances and assessing the distance at which the two can be felt distinctly [38].

Large fibre integrity is also commonly assessed with vibration perception testing using a tuning fork, neurothesiometer or biothesiometer [36]. During this test, vibration is applied to a bony prominence on the foot, triggering receptors (Pacinian corpuscles) to send signals to the central nervous system also via A-beta fibres [39]. A vibration perception threshold (VPT) can be obtained by gradually increasing or decreasing the amount of vibration and observing the point at which the patient perceives or loses perception of its presence [36].

#### Small fibre neuropathy

Dysfunction in the small diameter sensory fibres is less commonly tested for, though it may occur prior to or independent of LFN [32]. Small fibre sensory function includes temperature and pain perception [40]. Warmth can be assessed by applying stimuli with temperatures above 35°C and cold below 20°C [41] these signals are carried by small A-delta fibres [42]. Pain sensation is detected by free nerve endings in the dermis carried by both small A-delta and C fibres [43]. Both temperature and pain perception can be assessed with specialised devices [44]. Autonomic function is also controlled by small fibres.

#### Autonomic neuropathy

Autonomic neuropathy in diabetes is associated with small fibre sensory neuropathy but can occur in its absence [45]. Diabetic autonomic neuropathy can be divided into neuropathy affecting the cardiovascular system, the gastrointestinal system, the genitourinary system and the periphery [10]. Cardiac autonomic neuropathy (CAN) is the most commonly studied due to its prevalence and contribution to diabetes-related cardiovascular mortality [46]. Clinical manifestations of CAN include exercise intolerance due to an inability to adjust heart rate and blood pressure in response to demands of physical exertion, an increased risk of a cardiac event during exercise, intraoperative events and orthostatic hypotension [46]. Autonomic neuropathy is also related to hypoglycaemic unresponsiveness and silent myocardial infarction [46]. Consequently CAN screening is recommended at time of diagnosis of type 2 diabetes and within five years of diagnosis of type 1 diabetes [47]. It is diagnosed from analysis of heart rate and blood pressure variation in response to stressor tasks [10].

Peripheral autonomic neuropathy causes a loss of sympathetic vascular tone in the peripheral blood vessels. This results in vasodilation, which causes generalised hyperaemia in the hands and feet. This vasodilation occurs in arteriovenous shunt vessels that are prevalent in skin, especially volar skin, leading to increased shunting of blood flow from the arterial to venous circulation, bypassing capillaries leading to poor skin perfusion [27]. This is evidenced by increased venous oxygenation with increased temperature on the periphery [48], a loss of vessel vasomotion [49] and loss of peripheral sympathetic activity [50] in the lower limbs of those with neuropathy. Arterioles and small arteries undergo rhythmic contraction controlled by unmyelinated sympathetic C fibres that is believed to be lost following neuropathy in these branches [48]. The loss of sympathetic control also reduces postural vasoconstriction, increasing skin and capillary flow on dependence and altering pressure in the microcirculation in those with diabetic neuropathy [51]. Over time this causes capillary damage, basement membrane thickening and promotes oedema [51]. Such vascular dysfunction may be responsible for continued non-healing of wounds even following large vessel revascularisation [51].

Loss of sympathetic function due to peripheral autonomic neuropathy also affects sudomotor function resulting in reduced sweating [46]. This type of neuropathy results in warm, dry and cracked feet with poor skin integrity and a tendency to develop hyperkeratosis (callus) [46]. Similar to somatic neuropathy, peripheral autonomic neuropathy is likely to be length dependent. Diagnosis is also challenging, but can be indicated by sweat tests and tests of the vascular response to stress tasks, including the Valsalva manoeuvre and cold application [46].

Clinical testing of autonomic neuropathy is difficult due to invasiveness and availability of equipment and the many subsystems involved [52]. The goals of testing include assessment of severity, distribution and progression of autonomic dysfunction, assessment of orthostatic intolerance and monitoring of treatment outcomes [52]. The quantitative sudomotor axon reflex tests the postganglionic sympathetic sudomotor axon using iontophoresis of acetylcholine to test sweat function [52]. The thermoregulatory sweat test involves monitoring sweat response to heat to test sympathetic thermoregulatory pathways [52]. Tests of cardiovascular autonomic function involve monitoring the response of heart rate and blood pressure to stressor tasks such as the valsava maneuver and head tilt [52]. Most tests are established in their reliability and validity [53].

#### Neuropathy and microvascular reactivity

In addition to arteriovenous shunting and loss of rhythmic vasomotion, diabetic neuropathy is associated with other altered peripheral vascular states, including impaired microvascular vasodilation [54-57]. Central neural control of the microvascular system involves sympathetic cholinergic nerves and sympathetic adrenergic nerves that induce vasodilation and vasoconstriction, respectively. This occurs in arteriovenous shunt vessels and precapillary arterioles, thereby controlling tissue perfusion [51]. Whilst arteriovenous shunting is caused by a loss of vasoconstrictor nerve function, a loss of vasodilatory function from central or local neural reflexes may also be caused by a loss of nerve function.

A nerve axon reflex controls vasodilation in response to noxious stimuli such as intense heat, and is mediated by nociceptive C fibres [58]. Microvascular reactivity in this context is the ability to induce vasodilation through local endothelial and neurogenic responses to stressors such as heat and injury. The microvascular response to heat is characterised by an initial peak in vasodilation caused by c-fibre release of calcitonin gene-related peptide (CGRP) and substance P followed by a prolonged vasodilatory response mediated primarily by nitric oxide [59-61]. In the case of peripheral injury, vasoactive peptides and immune modulators are released, inducing the vasodilation [62]. Skin vasodilation can be induced by heat, occlusion and the iontophoresis of substances.

Diabetic neuropathy is associated with reductions in these vasodilatory responses including response to heating [54, 55, 57], reactive hyperaemia [54], and the iontophoresis of chemical substances [55-57]. These changes effectively prevent a normal vasodilatory response to injury, creating a functional ischaemia that contributes to the development and non-healing of neuropathy induced foot complications including ulceration [51, 63]. These changes in vascular states, along with sensation loss in neuropathy are also associated with the development of less frequent foot complications including Charcot neuroarthropathy which can result in severe foot deformity and increased risk of foot ulceration [64].

### Charcot foot

Charcot neuropathic osteoarthropathy is a rare joint condition that typically presents as a warm and swollen but painless joint of insidious onset [65]. The American Diabetes Association consensus report recommends the use of the terms Charcot neuropathic osteoarthropathy (abbreviated to CN) or Charcot foot to refer to its presence in the foot, so this terminology will be used throughout this thesis [66].

#### Clinical presentation

The condition causes the destruction of bone and soft tissue structures of primarily weight bearing joints [65]. It involves an initial active (acute) phase distinguished by marked inflammation, bone resorption, fracture, joint subluxation and dislocation. It presents with swelling and a localised temperature difference between limbs of between 2°C and 6°C in unilateral cases. This phase is followed by sclerosis, laying down of new bone and fusion of joints and an inactive (chronic) phase of deformity [65]. In this phase, the swelling and heat have resolved, leaving a permanent deformity.

CN can occur in any disease state that causes neuropathy. Whilst the earliest described cases were attributed to syphilis, diabetes has become the most common underlying condition today [67]. CN can occur in any neuropathic joint throughout the body including the shoulder [68], elbow [69], wrist [70], finger [71], spine [72], hip [73] and knee [74], however, in those with diabetes it is most common in the joints of the foot [67]. Charcot foot is classified by pattern referring to specific bones and joints involved. The most common patterns are pattern I involving the forefoot, pattern II involving the tarsometatarsal joints, and pattern III involving the midtarsal joints. Pattern IV affecting the ankle joint and pattern V affecting the calcaneus are less common presentations affecting approximately 10 and 2% of Charcot foot cases, respectively [75-77].

#### Staging

The active phase of the disease is commonly staged based on radiographic findings with accompanying clinical features [78]. These stages are described as follows:

- Stage 0: This stage presents as moderate to severe oedema, a temperature difference between limbs, possibly with presence of pain or history of trauma, with no deformity [78]. There are no discernible changes on radiographs, however, evidence can be seen on bone scans as evidence of bone stress or magnetic resonance imaging (MRI) as bone marrow oedema [78].
- Stage I (development): the foot has the cardinal signs of inflammation, is warm, red and swollen, there may be a deep pain. Radiographs may not show changes early in this phase, however, eventually changes to the joint capsule and articular cartilage, bone resorption, and fragmentation of subchondral bone with debris will be seen in this stage. There are local increases in vascular flow [78].
- Stage II (coalescence): the foot has reduced in inflammation. Radiographically it is characterised by absorption of the debris found in stage 1 and the fusion of large bone fragments to bones, sclerosis and joint fusion. There is a loss of vascularisation [78].
- Stage III (reconstruction): all inflammation has resolved, there is a reduction in sclerosis and return to normal bone density along with return to normal blood supply [78].

These stages are further evaluated with histopathologic examination of bone. In the early stages there is increased osteoclast functioning and lower strength of bone [79]. Normal trabecular bone is replaced with disorganised bone and there is a presence of inflammatory cells as well as abnormal marrow spaces during the latter stages [79].

#### Epidemiology and impact

Estimates of the prevalence of Charcot foot range from 0.08 to 13% [67] in those with diabetes depending on the population studied (general diabetic population vs those attending high risk clinics) and up to 29% in those with diabetic neuropathy [80]. Continued increases in prevalence are expected due to the increased incidence of diabetes and the improved life expectancy of those living with the disease. Charcot foot is associated with a longer duration of diabetes, poorer blood glucose control,

with renal-pancreas transplant [81], possibly with higher body weight [80]. It occurs equally in both genders [76] and typically occurs in the fifth or sixth decade of life [82, 83].

Charcot foot frequently results in permanent deformity that requires ongoing management in order to prevent further complications such as ulceration and amputation [66]. Deformity is linked to increased plantar pressures and is a major factor in the subsequent development of foot ulceration [84]. Incidence of ulceration after the occurrence of Charcot foot is 37% within 3 years [85], 49% within 3.8 years [86], and 67% within 8 years [87]. Ulceration is an important precursor to amputation which occurs in 2 [86] to 9.7% [85] of all cases of Charcot foot. A further episode of Charcot foot occurs in approximately 23% of cases and is associated with non-compliance and obesity [88].

Charcot foot is also associated with significant negative impact on quality of life [87, 89-91]. Those with diabetes and a history of Charcot foot report lower quality of life compared to both people with diabetes only and the general population [91], especially in domains of physical and social functioning and capacity to work [87, 91]. Similarly, it is associated with greater rates of mental health problems including anxiety and depression [89]. In a qualitative investigation, Lucas et al. [90] found those with Charcot foot experience feeling 'disabled', depression, guilt, loss of meaningful activity, and a negative impact on family relationships and on diabetes control. Mortality rates following Charcot foot are also high related primarily to cardiovascular disease and infection [92] with reported rates of up to 44.7% after 3.7 years [92], though other studies have produced more conservative figures of 29% after 8 years [87].

#### Diagnosis

There is an acknowledged difficulty in the diagnosis of CN especially in its early stages and by those who are not specialists [93]. Rates of misdiagnosis are as high as 79% [94], with CN frequently being mistaken for osteomyelitis, cellulitis, trauma, sprain, gout, deep vein thrombosis [95], infective arthritis and pseudogout [96]. The diagnosis of CN is made based on a combination of medical history, clinical signs and symptoms, blood work, and/or imaging studies [95]. Routine use of radiographs to diagnose the condition also delays early diagnosis and treatment. Very early stages of CN are not detectable on plain radiographs and their singular use in suspected cases has been shown to delay diagnosis and contribute to poor outcomes [97]. In addition, there is currently a lack of

awareness of Charcot foot amongst health professionals [98]. Accordingly, diagnostic delay can be as long as 29 months [99].

Greater use of more sensitive imaging in suspected cases of CN and greater awareness of the condition among primary care practitioners and appropriate patient education are expected to reduce such delays in diagnosis [95]. Clinical identification of Charcot foot at stage 0 may be possible with the use of MRI. Such imaging shows early signs of trauma including bone oedema, micro-fracture and joint effusion, which all occur early in Charcot foot [100].

#### Treatment

Treatment of Charcot foot remains a challenge. Little can be done to stop the disease process itself, though bisphosphonate therapy can be administered in an attempt to address bone metabolism and reduce bone resorption [66]. Evidence for the use of bisphosphonates is currently limited with several retrospective or pilot prospective studies demonstrating mixed findings. It appears in the acute phase, bone turnover and temperature are reduced [101, 102] but that the length of the disease process is not shortened [103] and long term outcomes including ulceration have not yet been assessed [102].

Standard treatment of active Charcot foot aims to prevent deformity and maintain foot structure with prompt offloading [66], the benefits of which have long been recognised [104]. The gold standard offloading technique is the total contact cast, which has proven effective [105-107]. Other options in the presence of contraindications to total contact casting include walker boots, which are used with varying degrees of success [108]. This should be followed by custom footwear, and/or accommodative orthoses in the chronic phase to prevent further complications such as ulceration [109]. Where treatment is delayed and significant deformity occurs, surgical intervention is required, with this occurring in up to 50% of cases [87]. Procedures are aimed at minimising deformity usually during chronic phases of the condition [66].

#### The importance of early diagnosis and treatment

Early identification and treatment of Charcot foot are crucial in the prevention of significant deformity and for achieving good long term health outcomes [87]. Multiple studies confirm better patient outcomes, reduced need for advanced interventions, and reduced health care expenditure when CN is diagnosed and treated early [86, 87, 94]. Early detection and offloading prior to joint degeneration reduces the extent of deformity and subsequent ulceration, infection and amputation [110]. Such treatment early in the disease course has been shown to reduce the need for surgical intervention [86] and have a positive impact on quality of life and functional outcomes such as walking capability [86, 87].

Identification of further risk factors, particularly those that are identifiable in a clinical setting, would also aid early diagnosis and reduce the expense of potentially unnecessary MRI. Currently, long term uncontrolled diabetes with advanced peripheral neuropathy, high body weight and renal transplant are the only consistently identified risk factors [67]. Furthermore, these have been identified from cross-sectional research and there is a lack of prospective research into the issue. Only a small proportion of this population develops Charcot foot and a greater understanding of why this is the case would aid not only diagnosis but would also help to target preventative patient education. Education to patients to protect their feet and to identify the signs and symptoms and appropriate behavioural reactions has been identified to improve outcomes [111]. A more specific understanding of who is at risk will guide the development of more effective diagnostic strategies and aid in both prevention and diagnostic strategies, improving treatment outcomes. This is currently hampered by a lack of understanding of the pathogenesis of the disease.

#### Pathogenesis

Two major theories have dominated the current understanding of the pathogenesis of Charcot foot. The first theory, put forth by Jean-Martin Charcot, was initially termed the neurotrophic theory [112]. In the mid-19<sup>th</sup> century Charcot observed the condition and theorised that the joint destruction was due to the damage to the ANS caused by the syphilitic disease process [11]. The neurotrophic theory posited the existence of trophic centres in the anterior horn of the spinal column that were responsible for bone nutrition [78]. This theory states that autonomic neuropathy damages these trophic centres resulting in a reduction in bone strength [78]. These trophic centres were found not to exist and the theory has subsequently been adapted into the neurovascular theory [78].

This version of the theory proposes that sympathetic denervation results in peripheral vasodilation and arteriovenous shunting, resulting in lower limb oedema and increased perfusion to bone and joints.

The increase in blood flow stimulates osteoclasts to demineralise bone [79, 113]. The resultant demineralisation of bone predisposes to fracturing which initiates the Charcot process [114]. In support of this theory, it has been demonstrated that arteriovenous shunting takes place in Charcot feet [115-117] and that there are increased osteoclast markers in active Charcot joints [118] along with reduced bone mineral density (BMD) in both limbs [119]. Furthermore, increased foot bone blood flow has been shown on bone scans in one small study in those with neuropathy, though this has never been reproduced [114].

The alternative traditional theory, the neurotraumatic theory, asserts that CN is a consequence of repetitive unrecognised microtrauma due to irregular mechanical stress on insensate bone and joints [78]. In the feet, neuropathy reduces strength of the intrinsic muscles resulting in overpowering of the long extensor muscles [120]. This, along with hyperglycaemia induced glycosylation of proteins and collagen cross-linking, is associated with increased Achilles and plantar fascia thickness [121], and a cavus deformity with limited joint mobility [98, 122]. Neuropathy is linked to trauma associated with repetition of the gait cycle on this cavus deformity causes abnormal loading and stress on bone and articular structures increasing the risk of injury such as fracture [98].

In support of this, it has been shown that those with Charcot foot have reduced elasticity in the Achilles tendon [123], limited ankle and first metatarsophalangeal joint motion [124] and abnormal tensile stresses in the foot [84]. In the absence of protective sensation, the injury may go unnoticed and untreated leading to a cycle of continued stress, which prevents normal healing. Under this theory the observed vascular and bone changes in Charcot foot are secondary to the inflammation of the active disease [112].

It is widely accepted that there is truth in both the neurovascular and neurotraumatic theories with neuropathy induced osteopaenia, altered pressure and unrecognised trauma all contributing to its pathogenesis [125-127]. Despite widespread support for the neurovascular theory, however, it remains untested whether there is localised osteopaenia prior to Charcot onset, or if the observed osseous changes occur in the active stage only, as a result of inflammatory processes. The extent to which sympathetic nervous system induced vascular changes contribute to osteopenia is controversial [112]. As it stands, this potential modifiable risk factor is under-researched. This is in part due to the complexity of the proposed relationship.

14

The relationship of neuropathy to bone is further complicated by the fact that diabetes itself affects bone. Bone remodelling on a cellular level is regulated by a dynamic mix of variables including local factors, systemic hormones and external influences [128]. The presence of diabetes can influence this balance, altering bone mineral density (BMD) and increasing the risk of fractures [129]. There have been a multitude of studies investigating the effect of diabetes on BMD [130-132] and fracture risk [131, 133, 134].

There is a demonstrated reduction in BMD at multiple sites in the presence of type 1 diabetes, as has been shown by multiple in vivo human studies [135-138]. Type 2 diabetes is less consistently associated with an increase in BMD at multiple sites in human studies [131, 134, 139-141]. Furthermore, diabetes has been shown to impair bone regeneration in animal models [142], lower fracture resistance, and not only affect bone mass but also the microstructure and matrix of bone [143]. Despite the association with an increased BMD in those with type 2 diabetes, there is an increase in fracture risk centrally and peripherally in both types of diabetes as shown in multiple human in vivo studies [131, 133, 134, 144-153] including in the foot [134, 152, 154, 155].

This increase in fracture risk is likely multifactorial and can partially be explained by an increased risk of falls in those with diabetes due to neuropathy, retinopathy and hypoglycaemic episodes as well as other comorbidities including nephropathy [152]. However, some human studies show maintenance of this increase in fracture risk after adjustment for falls and other risk factors suggesting that a direct diabetes-related reduction in bone strength is also likely to be involved [133, 134, 150].

Hyperglycaemia causes a wide range of sequelae that could potentially reduce bone integrity [156-159]. Hyperglycaemia encourages osteoclast action [159], inhibits osteoblast activity [156] and increases AGE/RAGE pathway activity [157]. Such activity causes chronic inflammation, increased osteoclast formation leading to bone resorption as well as non-enzymatic collagen cross-linking [160]. This reduces bone strength [158] by creating more fragile cross-linking in type 1 collagen [161-163]. Activation of the AGE/RAGE pathway found to be further increased in neuropathy and Charcot foot [160]. Further, the RAGE antagonist sRAGE (soluble RAGE) that blocks the inflammatory effects of the AGE/RAGE pathway is reduced in those with neuropathy and, particularly, those with CN. This disturbs the regulatory control of this inflammatory pathway, and is associated with poor bone quality [160]. Recent insights into the extent of bone innervation have provided an anatomical basis for the proposed changes to bone remodelling due to neuropathy [164-166]. Sensory fibres are present in the periosteum and in Haversian and Volkmann canals of cortical bone and there are sensory and sympathetic fibres in the epiphysis and metaphysis of long bones [164]. These nerves contain neuropeptides, such as CGRP and substance P [164], which have receptors on bone cells and are involved in mediating osteoblast and osteoclast function [164, 166]. There is growing biological evidence of significant neural control over bone remodelling suggesting bone should be affected by neuropathy [164-166].

There are two notable potential mechanisms for pathological changes in diabetic neuropathy one involving RANK-L/OPG (receptor activator of nuclear factor kappa-B ligand/ osteoprotogerin) system [167] and the other CGRP [165]. The sympathetic nervous system acts on the RANK-L/OPG signalling pathway that is involved in regulating bone remodelling. RANK-L is expressed on t-cells, monocytes and osteoblasts [167]. Increased expression of RANK-L causes the release of the glycoprotein OPG from osteoblasts and t-cells. This binds and inactivates the excessive RANK-L and is an essential regulatory process in bone remodelling [167]. Dysregulation of this system is shown Paget's disease, age-related osteoporosis as well as diabetic neuropathy [167]. This system is also interlinked with CGRP.

CGRP is a neuropeptide found all over the nervous system [165], but primarily in afferent sensory and sympathetic fibres [164] and extensively in bone nerve fibres [165]. It has widespread functions including modulating glucose metabolism, altering heart rate and force of contraction, gastric acid secretion in the stomach, and renin secretion by the kidneys [165]. It is particularly involved in vascular system function, acting as a vasodilator on smooth muscle and endothelial cells [165]. In bone, based on animal studies, it appears that most nerve fibres containing CGRP are located in the trabecular bone at the epiphysis and are either unmyelinated or thinly myelinated and act on blood vessels, bone marrow cells and osteoclasts [165].

CGRP acts to inhibit osteoclast precursors in bone marrow [168] and stimulate osteoblast growth and proliferation [165]. Fibres containing CGRP proliferate in the case of fracture and bone grafts during callus development and remodelling. CGRP also acts as an antagonist to RANK-L to down regulate osteoclasts and aid in fracture healing [125] and is a vasodilator thought to play a role in increasing

blood flow to areas of fracture. Evidence of a lack of innervation in non-uniting fractures also supports the role of CGRP in bone repair and remodelling [165]. Importantly, CGRP is deficient in the presence of neuropathy [167] and Charcot foot [125]. This provides a biological basis for the link between diabetic neuropathy, vascular and bone changes and for the potential excessive inflammatory response to injury [125]. CGRP is potentially the mechanism by which neuropathy induces vascular dysregulation in bone.

Blood supply to bone functions to provide nutrition, mineralisation and response to injury [169]. Vascular anatomy of bone is similar across bone types and includes a central nutrient artery and smaller arteries branching off in the epiphysis, metaphysis and periosteum. Capillary networks are located in cortical bone and the periosteum and sinusoids are located in the bone marrow [169].

The most flow typically occurs in the trabecular bone (20mL/min/100g) followed by cortical bone and the periosteum (5mL/min/100g), and bone marrow (1mL/min/100g) [169]. It is regulated by neural, humoral and metabolic factors. Blood vessels are associated with sympathetic nerve fibres including noradrenergic and peptidergic fibres that contain substance P and CGRP [164]. Together with the endothelium, they control skeletal homeostasis through vasomotor control of blood flow [169].

The investigation of bone blood flow in the presence of diabetic neuropathy is limited due the difficulty in ethically imaging such flow meaning that imaging in vivo of foot bone blood flow directly is limited to one small scale study which uses bone scanning [114]. Primarily, works investigating neuropathy and peripheral blood flow deregulation instead observe skin blood flow and vasomotion which may not be representative of the flow occurring in bone. Research investigating differences in microvascular function in those with neuropathy and Charcot foot have demonstrated reductions in the response to post-occlusive reactive hyperaemia (PORH) and iontophoresis of substances that provoke both endothelium dependent and independent responses [57]. Importantly, however, the response to heat has been found in multiple studies to be reduced in those with diabetic neuropathy but intact in those with Charcot foot [57].

In support of the neurovascular theory, autonomic neuropathy does appear to increase global foot blood flow including to bone [114] which may cause the proposed reduction in bone density prior to CN development. This may be in addition to, in select individuals, a resting state prone to an

17

exaggerated inflammatory response. That is, a lack of resting vascular tone coupled with the retained ability to vasodilate. The ability to mount such an inflammatory response is normally reduced in those with neuropathy at least at a skin level [63]. Neurogenic injury response has been shown to be intact, however, in those who have Charcot foot [114]. Therefore, there may be a particular neurovascular profile that leaves people vulnerable. This may be why Charcot foot is not more common in those with diabetic neuropathy [98]. If found to occur prior to Charcot foot onset, tests to identify those with the relevant neurovascular profile to place them at risk of CN would be useful to facilitate the early identification and treatment of the condition.

### Summary

The incidence of diabetes and its complications, including peripheral neuropathies and Charcot foot, is increasing markedly [7]. Favourable outcomes in such disease require early diagnosis and treatment. Currently, relationships among neurological, vascular and osseous dysfunction in the periphery are thought to contribute to high risk foot states in diabetes [165]. Such relationships have long been hypothesised, but are yet to be substantiated. More research is required to validate these relationships and aid in successful diagnosis and management of diabetic foot disease.

### Chapter Two

### Objectives

The aim of this thesis is to examine the relationships among diabetic neuropathies, vascular abnormalities and foot bone density. The two central hypotheses are

- that those with diabetic neuropathy have altered vascular reactivity in the feet, and
- that neuropathy induced vascular changes in those with diabetes, contributes to a reduction in bone mineral density in the feet.

The research addresses these by: evaluating the existing evidence base for neuropathy induced changes to bone density; developing feasible and reliable methods of assessing foot bone density and microvascular reactivity; investigating relationships between neurological and microvascular function that may be relevant in bone changes in diabetes and; ascertaining whether there are differences in foot bone density between those with diabetic neuropathy and those without. It aims overall to assess the validity of the assertion that deficits in particular components of the nervous system cause a change in vascular parameters that influence peripheral bone strength.

The following objectives are specified

- Review the existing literature related to differences in foot bone density in those with and without diabetic neuropathy – Addressed in Chapter Three.
- Develop a reliable measure of microvascular reactivity for clinical and research purposes Addressed in Chapter Four.
- Develop a feasible and reliable method of foot bone density measurement Addressed in Chapter Four.

- Explore relationships between clinical subtypes of diabetic neuropathy and microvascular characteristics in the diabetic foot – Addressed in Chapter Five.
- Explore relationships among diabetic neuropathy, microvascular reactivity and foot bone density in those with diabetes – Addressed in Chapter Six.

# Chapter Three

### Literature Review

The research in this chapter relates to the following objective

1. Review the existing literature related to differences in foot bone density in those with and without diabetic neuropathy.
# Preface

Diabetic neuropathy is proposed to affect bone density, increasing in the risk of foot bone pathology such as Charcot foot in this population. The objective of this paper was to systematically review the current literature on foot bone density in the presence of diabetic neuropathies with reference to diabetic control populations and present summary statistics of all available data. This provides a starting context for the research and identifies gaps and limitations within the literature that will be used to guide the subsequent research. The review found 10 relevant studies, of which seven were included in a meta-analysis, which was inconclusive. Furthermore, the review identified several limitations within the literature including a lack of data on foot bones other than the calcaneus. This will be addressed in subsequent methodology.

This manuscript was published in *Diabetic Medicine* which has an impact factor of 3.241 and an ISI ranking of 49/122 in Endocrinology and Metabolism.

Citation: Barwick, A.L., et al., *The effect of diabetic neuropathy on foot bones: a systematic review and meta-analysis.* Diabet Med, 2014. 31(2): p. 136-147

Appendix A contains an author contribution statement for the publication.

This research was presented at the Sydney Diabetic Foot Conference in May 2013, the NSW Australian Podiatry Association conference in Sydney in April 2014 and the Australasian Podiatry Council conference in Dunedin, New Zealand in November 2014. Abstracts for these conferences are found in Appendix I.

### **Systematic Review or Meta-analysis**

# The effect of diabetic neuropathy on foot bones: a systematic review and meta-analysis

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Accepted 18 October 2013

#### Abstract

**Aims** It is proposed that diabetic neuropathy may affect peripheral bone. Direct innervation of bone as well as neural control over its vascular supply and muscular influences may be affected by diabetes-induced peripheral neuropathies. Associated changes to bone may contribute to the occurrence of foot bone pathology in this population. This systematic review aims to examine the literature related to the effect of diabetic neuropathy on foot bones.

**Methods** Studies examining relationships between neuropathy and indicators of bone health (e.g. bone mineral density) in populations with diabetes were sought. Relevant publications were obtained from searches in MEDLINE, CINAHL and Embase in the period up to March 2013. Meta-analysis was performed using a random effects model in the statistical package Stata version 12.1.

**Results** Ten studies met the inclusion criteria and were included in the narrative synthesis. All studies were cross-sectional or case–control in design. Four of the 10 included studies found results indicating poorer bone health in those with diabetes and neuropathy compared with those with diabetes without neuropathy. Seven of the 10 studies were able to be included in a meta-analysis. The mean pooled effect was -0.36 (95% CI -0.76 to 0.04; P = 0.08), indicating a non-significant trend towards poorer bone health in those with diabetic neuropathy.

**Conclusions** We did not find a significant relationship between presence of neuropathy in those with diabetes and poorer peripheral bone health. However, methodological limitations of the included studies mean further research is required to investigate this theoretical relationship.

Diabet. Med. 31, 136-147 (2014)

#### Introduction

Diabetic peripheral neuropathy, defined as distal nerve damage in the presence of diabetes mellitus that cannot be attributed to other causes [1], occurs in up to 67% of people with diabetes and affects both the somatic (sensory and motor functioning) and autonomic components of the nervous system [2]. In this population, both peripheral sensorimotor and autonomic neuropathy have been linked to a range of pathologies in the feet [3], including causing detrimental changes to peripheral bone, creating an increased risk of bone pathology [4].

Afferent sensory fibres are known to directly innervate bone [5]. Histological studies have shown the presence of these fibres in both cortical and trabecular bone and demonstrated contact between these fibres and bone cells [6]. This, in addition to the presence of receptors for

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neuromediators including neuropeptides and catecholamines, on bone cells suggests an important role for the nervous system in bone maintenance [6]. However, the exact functions of this innervation and the potential effect of pathology in these nerves on bone health is currently unknown [5,7].

Autonomic nerve fibres, specifically sympathetic fibres, are also abundant in bone tissue [5]. These fibres are associated with osseous blood vessels and are likely to regulate bone blood flow [6]. Neuropathy in these fibres may cause a reduction in sympathetic tone causing vasodilation leading to an increased blood flow to bone that is proposed to cause bone to resorb [8,9] via an increase in osteoclast activity [10].

It is known that a major factor involved in maintenance of bone mass is mechanical load, which is in part made up of muscular force applied by actively contracting muscles [11]. Diabetic peripheral neuropathy frequently involves the intrinsic foot muscles [2]. This provides another mechanism by which neuropathies may influence bone health, as a decrease in muscular activity associated with motor neuropathy would reduce the muscular force applied to bone and may result in bone loss. However, the majority of current research investigating the effect of diabetic neuropathy on peripheral bone health has evaluated a proposed link with sensory and autonomic dysfunction and is the focus of this review.

Any detrimental effects to bone resulting from these mechanisms may predispose individuals to foot injury, including fractures and Charcot neuroarthropathy [8]. It is not currently known whether any or all of these systems are affected by neuropathy in a way that causes negative changes to bone. The objective of this review is to assess and synthesize the literature investigating differences in the health of foot bones (bone mass, bone mineral density, morphological characteristics etc.) related to presence of diabetic neuropathies. We hypothesize that diabetic neuropathies will be related to poorer measures of bone health in foot bones of people with diabetes.

#### **Methods**

#### Search strategy

Original studies investigating the effects of diabetic neuropathy on aspects of peripheral bone health (bone mass, bone mass density, morphological characteristics, etc.) were sought for inclusion in the review. The review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [12]. Database searches of MEDLINE (1946–March 2013), CINAHL (1982–March 2013) and Embase (1947–March 2013) were performed using the following search query: ('bone density' OR 'bone strength' OR 'bone mineral density' OR 'BMD' OR 'quantitative ultrasound' OR 'QUS') AND ('peripheral' OR 'foot' OR 'tars\*' OR 'calcane\*' OR 'metatarsal') AND 'diabet\*' AND 'neuropath\*'. No language restrictions were applied.

Titles and abstracts of retrieved publications were searched for relevancy by two investigators independently (ALB and VHC). Full-text versions of potentially relevant publications were obtained and reviewed for inclusion. In cases of dispute, a third reviewer (XAKJdJ) was consulted. All studies of human participants of any study type were considered. Review articles, case studies, theses and commentaries were excluded. Further relevant articles were identified by hand searching the reference lists of included articles and relevant reviews. In cases of publication in a foreign language, article translation was performed with 'Google Translate'.

#### Study selection

Studies were considered relevant if they met the following criteria: (1) participant group(s) included humans with either Type 1 or Type 2 diabetes; (2) participant groups were

without current foot conditions known to affect bone, e.g. Charcot neuroarthropathy, ulceration or infection; (3) any type of diabetic neuropathy was measured; (4) any quantitative parameter (using any imaging technique) of bone health (e.g. bone mass, bone mass density or morphological characteristics) of one or more foot bones was measured; (5) bone parameters and neuropathy were statistically analysed against each other.

Authors were contacted for further information when seemingly appropriate data were collected (criteria 1–4 satisfied), but relevant statistical analyses were not performed and, when relevant, participants were grouped together with excluded participant groups. Where criteria 1–5 could not be satisfied after authors were contacted, studies were excluded. Authors were also contacted in cases where criteria were met, but numerical outcome values were not provided.

#### Data extraction and study analysis

Data extraction was performed using a standard pro forma including author, year, publication, location, participant characteristics (age, sex and diabetes type), sample size, test used to establish neuropathy, technique and location of bone testing, statistical analysis performed and outcomes.

The quality of each study was assessed systematically with a modified Critical Appraisal Skills Program tool [13]. The tool included 13 items related to definition of study population, the likelihood of bias, adequate blinding, accuracy of outcome measures and appropriateness of statistical analysis. Four response options were given: yes, no, unsure and insufficient information provided. No minimum quality standard was required for inclusion in the review.

Meta-analysis was performed to assess the strength of the relationship between bone parameters and any type of diabetic neuropathy. Differences in bone parameters were measured in terms of standardized mean differences in g/cm<sup>2</sup>, *t*-score and rate of osteoporosis/osteopaenia. The rate of osteoporosis/osteopaenia was approximated as a mean difference using Hasselblad and Hedges method [14]. All standardized mean differences were also adjusted using Hedges' g to counteract the lower sample sizes present in each group. A random effects model was used for the meta-analysis to account for the differing conditions of each study. Heterogeneity was examined using  $I^2$  statistic and publication bias was assessed through the use of a funnel plot and Egger's test of asymmetry [15].

#### Results

#### Search results

The results of the search can be found in Fig. 1. The database search identified 99 unique publications. A reference search yielded an additional two publications. Of these, 70 were



FIGURE 1 PRISMA (preferred reporting items for systematic reviews and meta-analyses) flow chart of study identification and selection process [12].

excluded based on title and abstract because of being letters, case studies, commentaries, review articles, or otherwise clearly not meeting inclusion criteria. Thirty-one full-text publications were assessed. Sixteen of these were excluded after full-text review: seven because of participant groups of the study not meeting the inclusion criteria of the review; two because of an absence of quantitative measurement of bone; and seven because bone parameters were measured in areas other than the foot. A further five studies were excluded after attempt to contact authors as data could not be supplied [16–20].

The remaining 10 studies met all five inclusion criteria and were included in the narrative synthesis. Seven of these studies examined differences in bone health between groups (with and without some type of neuropathy) with Student's *t*-test [21,22] or a non-parametric alternative [23], or analysis of variance (ANOVA) [24–26], with the statistical test used in one study unclear [27]. The remaining three studies tested associations between measures of bone health and measures of some type of neuropathy with  $\chi^2$  [28] or linear regression [29], with the statistical test used in the remaining study unclear. Two studies did not provide numerical outcomes required to be included in the meta-analysis [29,30]. One study was deemed too heterogenous to be included in meta-analysis because of measurement technique and location [22]. The remaining seven studies were included in the meta-analysis. One study required translation from Polish [27]. The characteristics of the included studies are presented in Table 1.

#### Study outcomes

Four of the included studies found statistically significant outcomes suggesting that those with diabetic neuropathy (peripheral sensory peripheral sensorimotor and/or autonomic neuropathy) have poorer bone health, with one study finding an association between poorer bone health and neuropathy [30] and three studies finding poorer bone health in those with neuropathy compared with those without neuropathy [22,26,27]. Sieradzki et al. [27] used quantitative ultrasound to measure calcaneal bone mineral density and classify participants as having normal bone density, osteopaenia or osteoporosis. They found a greater percentage of people with calcaneal osteoporosis in participants with peripheral sensory neuropathy (determined with an undefined neurological assessment) (60%) compared with those without (17.7%, P < 0.05). Additionally, a greater percentage of people with calcaneal osteoporosis was found among participants with autonomic neuropathy (determined with heart rate variation analysis with undefined thresholds for abnormality) (66.7%) compared with those without (20%, P < 0.05).

Outcomes	Neuropathic group: mean $t$ -score -0.54, sE $\pm$ 0.26. Non-neuropathic group: -0.75, $\pm$ 0.4, $P$ = NS	Neuropathic group: 25% had a normal <i>t</i> -score, 33.33% osteopaenia, 41.67% had osteoporosis. Non-neuropathic group: 1.5.5% had normal <i>t</i> -score, 2.6.67% osteoporosis, <i>P</i> = NS	Neuropathic group: mean <i>t</i> -score foot one $0.84 \pm sc$ $0.06;$ foot two $0.82 \pm 0.06$ Non-neuropathic group $0.75 \pm 0.04$ , $P = 0.47$ ; $0.74 \pm 0.03$ , $P = 0.45$	Speed of sound was significantly correlated with vibration perception threshold measures, deep breathing and lying-to- standing test. In men with Type 1 diabetes a correlation quantitative ultrasound index and speed of sound and vibration perception threshold, deep breathing, lying-to-standing and couch test	Volume of cortical bone was lower in neuropathic group: mean $0.75 \pm st 0.01$ compared with the non-neuropathic group: 0.84, 0.01, P < 0.001	Neuropathy: speed of sound mean $1551 \pm 5D$ 30, broadband ultrasound attenuation $66.1 \pm 15.2$ , quantitative
Method of bone parameter measurement	Quantitative ultrasound-derived bone density expressed as a <i>t</i> -score	Quantitative ultrasound-derived broadband ultrasound attenuation expressed as adjusted <i>t</i> -score and classified as normal, osteopaenia or osteoporosis	Dual-energy X-ray absorptiometry-derived bone mineral density and expressed as <i>t</i> -score	Quantitative ultrasound- derived speed of sound, broadband ultrasound attenuation and quantitative ultrasound index	Standard anterior- posterior radiographs used to determine volume of cortical bone	Quantitative ultrasound- derived speed of sound (m/s), broadband ultrasound attenuation (db/MHz), quantitative
Location of bone parameter measurement	Calcaneus	Calcaneus	Calcaneus	Calcaneus	Second metatarsal at the mid-point	Calcaneus
Neuropathy measurement	Vibration perception threshold. Motor and sensory nerve conduction velocity. Deep breathing and lying-to-standing tests of heart rare variation	Nerve conduction velocity	Biothesiometry	Biothesiometry: vibration perception threshold of the hallux and medial malleolus. Beat-to-beat heart rate variation, deep breathing, expiration-to-inspiration ratio, heart rate and blood pressure response to standing and cough test	History of neuropathic ulceration. Severe stocking sensory disturbance. Absence of ankle reflexes. Deep breathing heart rate variation (< 10 b min <sup>-1</sup> )	Michigan Neuropathy Screening Instrument score > 7 and vibration perception threshold > 25 V
Participants	Diabetes (type not specified) Male (23):female (15) Age [mean (years), sp]: neuropathy (64.11, 9.86), no neuropathy (61.10, 6.94)	Type 1 diabetes (32): Type 2 diabetes (106) Male and female—ratio unspecified Age range 11–70 years mean 41.96 years, sp 1.74	Type 1 diabetes (4): Type 2 diabetes (16) Male (17):female (3) Age [mean (years), sp]: neuropathy 61.7, 4.1, no neuropathy: 63.2, 17.9	Type 1 diabetes (51): Type 2 diabetes (214) Male (145):female (120)	Insulin-dependent diabetes mellitus (40): non-insulin-dependent diabetes mellitus (1) Male (21):female (20) Age range 19–61 years	Type 1 diabetes (8): Type 2 diabetes (19) Sex unspecified Age (years): neuropathy
Number of participants (neuropathy/ no neuropathy)	38 (28/10)	138 (48/90)	20 (9/11)	265	41 (19/22)	27 (14/13)
Location	Unknown	Kolkata, India	Copenhagen, Denmark	Unknown	Unknown	Tuscany, Italy
Author (year)	Barbaro <i>et al.</i> (2008) [21]	Chakrabarty <i>et al.</i> (2004) [28]	Christensen <i>et al.</i> (2010) [24]	Conti et al. (2010) [30]	Cundy <i>et al.</i> (1985) [22]	Piagessi <i>et al.</i> (2002) [25]

Author (year)	Location	participants (neuropathy/ no neuropathy)	Participants	Neuropathy measurement	of bone parameter measurement	Method of bone parameter measurement	Outcomes
			(mean 63.5, sp 12), no neuropathy (61.5, 7.5)			ultrasound index and bone mineral density (g/cm <sup>2</sup> )	ultrasound index 90.7 $\pm$ 17.5, bone mineral density 0.5 $\pm$ 0.1. No neuropathy: 1555 $\pm$ 32, 67.5 $\pm$ 20.6, 93.8 $\pm$ 20.1, 0.5 $\pm$ 0.1 respectively, p = NC
Rix <i>et al.</i> (1999) [26]	Unknown	42 (21/21)	Type 1 diabetes Male Age (years): neuropathy (mean 57, sE 6), no neuropathy (56, 5)	Severe neuropathy: absence of peripheral reflexes, symptoms and vibration perception threshold > 36 V. No/mild neuropathy: vibration perception threshold of 25 V and preserved reflexes	Calcaneus	Quantitative ultrasound-derived broadband ultrasound attenuation (db/MHz)	Significantly lower broadband ultrasound attenuation in severe neuropathy group: mean $108 \pm \text{st}$ 2.6 compared with the no/mild neuropathy group: 116 $\pm$ 2.4, $P < 0.05$
Sieradzki <i>et al.</i> (1995) [27]	Unknown	<ul><li>34 (Peripheral sensory neuropathy 10 Autonomic neuropathy 9)</li></ul>	Insulin-dependent diabetes mellitus Male (13):female (21) Age range 21–68 years mean 38	Peripheral sensory neuropathy: unspecified neuropathy: unspecified neurological examination. Autonomic neuropathy: abnormal deep breathing, Valsava manoeuvre and laying-to-standing heart rate variation	Calcaneus	Quantitative ultrasound-derived bone mineral density expressed as <i>t</i> -score and classified as osteopaenia or osteoporosis	Significantly greater percentage of those with osteoporosis of the heel in the neuropathy group (60%) than in the group without neuropathy (17.7%), P < 0.05; and autonomic neuropathy: 66.7% than no autonomic neuropathy 20% D < 0.05
Singh <i>et al.</i> (2011) [23]	Hertfordshire, UK	65	Type 2 diabetes Male (44):female (21) Age (years): mean 62, sp 11	Absence of 10-g monofilament at > 3/4 sites on the foot or vibration perception threshold > 25 V with neurothesiometer	Calcaneus	Dual-energy X-ray absorptiometry-determined bone mineral density expressed as $t$ -score and reported as low heel bone mineral density where t-score is $< -1$	58% of those with low bone density had neuropathy compared with $72%$ of those without low bone density, $P = 0.29$ . Neuropathy was not an independent predictor of low heel bone mineral
Strotmeyer <i>et al.</i> [ (2006) [29]	Pittsburgh, PA, USA	67	Type 1 diabetes Female Age (years): mean 43.1, \$D 4.3	Physician diagnosed neuropathy. Michigan Neuropathy Screening Instrument. 10-g monfalament testing (absence of detection 8/10 applications). Vibration perception threshold (scored from 0 to 20)	Calcaneus	Quantitative ultrasound-derived broadband ultrasound attenuation	density Physician diagnosed neuropathy, Michigan Neuropathy Screening Instrument score and reduced vibration sensation were nor related to calcaneal broadband ultrasound attenuation

Table 1 (Continued)

Rix *et al.* [26] compared quantitative ultrasound-derived broadband ultrasound attenuation (dB/MHz) of the calcaneus in two groups with Type 1 diabetes, one group categorized as having severe peripheral sensory neuropathy (classified as those with a vibration perception threshold higher than 36 V, self-reported symptoms and an absence of patella and Achilles reflexes) and the other categorized as having mild/no peripheral sensory neuropathy (those with a vibration perception threshold of less than 25 V, no self-reported symptoms and normal reflexes). A statistically significant lower broadband ultrasound attenuation in the group with severe peripheral sensory neuropathy (108  $\pm$  2.6) was found in comparison with the group with mild/no peripheral sensory neuropathy (116  $\pm$  2.4, P < 0.05).

Conti et al. [30] measured quantitative ultrasound parameters of the calcaneus and found significant correlations between poorer quantitative ultrasound parameters and higher vibration perception threshold at the hallux and medial malleolus and worse outcomes of cardiac autonomic neuropathy testing (heart rate variation analysis of deep breathing and lying to stand), particularly in men with Type 1 diabetes. Finally, Cundy et al. [22] found a reduced volume of cortical bone (measured as a proportion of the cross-sectional area obtained from X-ray) in the second metatarsal of participants with both cardiac autonomic neuropathy (abnormal heart rate variation response to deep breathing, thresholds not provided) and peripheral sensory neuropathy (determined by a history of neuropathic ulceration or severe stocking sensory loss with absence of Achilles reflexes)  $(0.7 \pm 0.01)$  compared with participants without neuropathy (0.84  $\pm$  0.01; P < 0.001).

The outcomes of the remaining six studies did not indicate a difference in bone health in those with diabetic neuropathies [21,23-25] or an association between diabetic neuropathy and bone health parameters [28,29]. Piagessi et al. [25] obtained quantitative ultrasound parameters of the calcaneus and demonstrated no differences in these measures between those with diabetic peripheral sensory neuropathy (defined as a Michigan Neuropathy Screening Instrument score of greater than 7 and a vibration perception threshold of greater than 25 V) and those without. Christensen et al. [24] obtained dual-energy X-ray absorptiometry-derived bone mineral density of the calcaneus and found no differences in this measure between those with diabetic peripheral sensory neuropathy determined with biothesiometry (threshold unknown) and those without. Similarly, Barbaro et al. [21] did not find any differences between calcaneal quantitative ultrasound parameters between those with diabetic sensory neuropathy (measured with vibration perception threshold and motor and sensory nerve conduction velocity, thresholds not provided) and autonomic neuropathy (determined with abnormal heart rate response to deep breathing and lying-to-standing test, thresholds not provided) and those without.

sensory neuropathy (Michigan Neuropathy Screening Instrument score, absence of 10-g monofilament detection for 8/10 applications and vibration perception threshold scored between 0 and 20) not to be related to calcaneal broadband ultrasound attenuation. Singh et al. [23] used dual-energy X-ray absorptiometry to categorize participants as having healthy bone density, osteopaenia or osteoporosis of the calcaneus and found no difference between these groups in the percentage of participants with peripheral sensory neuropathy (determined as 10-g monofilament detection absent at three or more sites or vibration perception threshold of greater 25 V) and without peripheral sensory neuropathy. Furthermore, in this study, regression analysis found the presence of peripheral sensory neuropathy not to be an independent predictor of low heel bone mineral density. Finally, Chakrabarty et al. [28] found no differences in the proportion of those with osteoporosis or osteopaenia of the calcaneus in those with and without peripheral sensory neuropathy (determined with nerve conduction studies, thresholds not provided).

Strotmeyer et al. [29] found measures of peripheral

#### **Quality assessment**

Answers to the modified Critical Appraisal Skills Program form for all studies are provided in Table 2. After selecting relevant subsamples from the studies, sample sizes ranged from 20 to 265, with power analysis reported in only one study [23]. Six of the studies reported on the recruitment of participants [23,25,26,28–30], which included recruitment from local health services and diabetes clinics, and their databases.

The method used to diagnose diabetes was reported in only one study, which used the World Health Organization and National Diabetes Data Group criteria [28]. Measurement of neuropathies varied widely among the studies. Most studies used a combination of tests, although many failed to provide adequate information to determine appropriate use of tests [21,22,24,27,28]. All studies investigated peripheral sensory neuropathy, with four studies also investigating autonomic neuropathy [21,22,27,30]. Five studies included a measure of motor involvement, with one study examining motor nerve conduction velocity [21] and four measuring deep tendon reflexes [22,25,26,29]. The impact of motor neuropathy on bone was not, however, isolated in any study. Several studies required placing participants into two groups according to neuropathy status (present/severe and absent/mild); of these, some failed to provide thresholds used to determine these groups [21,24,27,28]. Reliability of neuropathy measurements was not described in any of the studies.

Precision estimates of bone measurements were included in four studies with scores of  $\leq 5\%$  [22,23,25,26]. Two studies reported blinding of assessors with respect to the outcomes of interest to this review [22,25]. Studies controlled for extraneous variables (such as weight and systemic health condi-

Barbaro <i>et al.</i> (2008)	Chakrabarty et al. (2004)	Christensen et al. (2010)	Conti <i>et al.</i> (2010)	Cundy et al. (1985)	Piagessi et al. (2002)	Rix et al. (1999)	Sieradzki <i>et al.</i> (1995)	Singh et al. (2011)	Strotmeyer et al. (2006)
Yes Yes, case- control Insufficient information	Yes Yes, case- control Yes	Yes Yes, case- control Yes, the participant groups we were interested in were selected in an attempt to match the acute Charcot group on age, duration, sex and diabetes	Yes, cross- sectional Yes	Yes Yes, case- control Insufficient information	Yes Yes, case-control Yes	Yes, case- control Yes	Yes Yes, case- control Insufficient information	Yes Yes, cross- sectional Yes	Yes Yes, case- control Yes
Insufficient	Yes	type Yes, see above	N/A	Insufficient information	Yes	Yes	Yes	N/A	Yes
Unsure . Cut-off values not provided	Unsure. Cut-off values not provided	Unsure. Cut-off values not provided	Yes	Unsure. Cut-off values not provided	Yes	Yes	Unsure. Cut-off values not provided	Yes	Yes
Presence of contralateral foot ulceration	Exclusions: history of drugs such as	Participants were matched for age, sex	Anthropometric and serological measures were	Participants were matched for sex	Exclusions: active foot ulcer; recent	Exclusions: immobilization, amputation,	Found no relationship between	Exclusions: recent urinary tract infection,	All participant were pre-
in the neuronathy	glucocorticoids or anticonvulsants:	and diabetes	taken, including	distribution, mean age and	history of trainma: hone	severe cardiovascular	duration of diabetes and	foot ulceration, immino-	menopaus. none had
group. Age, duration of diabetes,	history of diseases associated with osteopaenia.	246	HbA <sub>lc</sub> . Sex and type of diabetes were	mean duration of diabetes. Menstrual	fracture' clinical osteoporosis; clinical infection;	disease, or treatment with medications	presence of osteoporosis. Those with	suppressant therapy, pregnancy,	Coeliac's disease or a history
HbA <sub>1c</sub> and BMI were measured and compared	HbA <sub>1c</sub> , presence of nephropathy and retinopathy		accounted for. Quantitative ultrasound parameters	history of women participants was obtained.	serum creatinine > 177 µmol/l; malnutrition; malignancies;	known to affect bone metabolism (hormone replacement	higher HbA <sub>1c</sub> had more osteoporosis	hormone replacement therapy, malignancy.	of dialysis of dialysis Exclusions: kidney transplant
between the two groups. Significant differences	were measured. None correlated with bone density		were correlated with age, BMI, waist circumference,	Amputation and history of ulceration may be confounding	chronic pathologies affecting bone metabolism;	therapy, glucocorticoids, anticoagulants). Participants were		Renal function, duration of diabetes, gender, age,	Clinical fracture history, current
were reported between the groups in HbA1e and duration of diabetes. All participants were receiving or all treatment for diabetes			% body tat, cholesterol and HbA <sub>ic</sub>	factors	drugs such as steroids, bisphosphonates, calcium, and/or vitamin D; hyper- parathyroidism; Paget's disease. There were no differences	matched for sex, age, duration of diabetes and weight. There were no differences between groups in HbA <sub>1c</sub> , renal function,		werght, smoking, smoking, blood pressure, blood and urine measures, HbA <sub>1c</sub> were also accounted for through measurement	and past smoking and alcoh consumpti history, exercise and total activity, menstrual

Strotmeyer et al. (2006)	use, education, income category, self-reported health and limitations, and any history of physician diagnosis of diabetes- related complications were determined by	questionnaire No relationship found	Insufficient information N/A N/A N/A Yes No blinding described
Singh et al. (2011)	Those without low heel bone density had a higher weight	No relationship found	Insufficient information N/A N/A N/A Yes Precision values are provided No blinding described
Sieradzki <i>et al.</i> (1995)		Greater proportion of osteoporosis in those with sensory and autonomic neuropathy	Insufficient information N/A N/A N/A Unsure. Unsure. Used Used dentived bone mineral density and compared with World Health Organization osteoporosis guidelines No blinding
Rix et al. (1999)		Significantly reduced bone mineral density in those with neuropathy	Insufficient information N/A N/A Yes Precision values are provided No blinding described
Piagessi et al. (2002)	groups in calcium consumption, HbA <sub>1s</sub> , age, diabetes duration and BMI	No relationship found	Insufficient information N/A N/A N/A Yes Precision values are provided Assessors were blinded Operators were the same
Cundy <i>et al.</i> (1985)		Lower volume of corrical bone in those with diabetic neuropathy	Insufficient information N/A N/A N/A Messurements taken from radiographs. Multiple measurements and precision estimates provided. Validity of this method to detect reduced bone strength is questionable
Conti <i>et al.</i> (2010)		Correlation between quantitative ultrasound measures and sensory and autonomic neuropathy	measures information N/A N/A N/A Yes No blinding described
Christensen <i>et al.</i> (2010)		No relationship found	Insufficient information N/A N/A Yes No blinding described
Chakrabarty et al. (2004)		No relationship found	Insufficient information N/A N/A N/A N/A Unsure. Broadband ultrasound attenuation compared with a modification of the World Health Organization criteria for osteoporosis. The authors cite studies
Barbaro <i>et al.</i> (2008)		No relationship found	Insufficient information N/A N/A Insufficient information No blinding described

Barbaro <i>et al.</i> (2008)	Chakrabarty et al. (2004)	Christensen et al. (2010)	Conti <i>et al.</i> (2010)	Cundy et al. (1985)	Piagessi et al. (2002)	Rix et al. (1999)	Sieradzki <i>et al.</i> (1995)	Singh et al. (2011)	Strotmeyer et al. (2006)
13 Insufficient information	83% specificity for bone mineral density measurements. No blinding described Insufficient information	Insufficient information	Insufficient information	Insufficient information	Insufficient information	Insufficient information	Insufficient information	Yes, power analysis related to a different aspect of the study	Insufficient information
<ol> <li>Did the study ad selected in an accer potential confoundii results? 10. Can the assessors of bone qf N/A, not applicable</li> </ol>	dress a clearly focused table way? 5. Was thu ing factors in the desig results be applied to t uality blind to the neu	l issue? 2. Did the a e exposure accuratel n and/or in their and the local population? tropathy status of th	uthors use an appre y measured to mini ulysis? 7. What are 11. Do the results e participant? 13. V	opriate method to i imize bias? 6. Wha the results of this si of this study fit wi Was power analysi	answer their questic tt confounding facto tudy? 8. How precis ch other available ev s performed?	m? 3. Were the cases 1 ors have the authors ac e are the results? How idence? 12. Was the o	recruited in an acce counted for? Have r precise is the estin utcome measured a	eptable way? 4. W e the authors taker nate of risk? 9. Dc iccurately and to a	ere the controls a account of the you believe the void bias? Were

**DIABETIC**Medicine

tions) by either participant matching, excluding participants with these factors, by measuring them and establishing differences between groups and/or adjusting for them in the statistical analyses.

Most studies accounted for age [21-26,29] and sex [21-26,29,30] with some also accounting for body weight [21,25,26,29]. Duration of diabetes was accounted for in six studies [22-24,26,27,29] and severity of diabetes, as measured by HbA<sub>1c</sub>, in four [23,26,28,29]. Renal function was accounted for in five studies [23,25-28].Some studies excluded a range of conditions and medication use that could affect bone density [23,25,26,28]. Factors such as cholesterol, diet, menopause status, smoking status, physical activity level and ethnicity were taken into consideration only rarely. Statistical analyses were heterogeneous across the studies.

#### Meta-analysis and publication bias

Results of the meta-analysis are found in Fig. 2. The seven studies included in the meta-analysis had a total sample size of 364. The meta-analysis generated a non-statistically significant mean effect size of -0.36 (95% CI -0.76 to 0.04; P = 0.08). Significant heterogeneity was present ( $I^2 = 71.2\%$ ; P < 0.05). The funnel plot (Fig. 3) demonstrated no apparent asymmetry; however, Egger's test was significant (P = 0.022).

#### Discussion

Ten studies met the criteria for inclusion in this review. Meta-analysis of seven of the studies (n = 364) demonstrated an effect size of -0.36 (95% CI -0.76 to 0.04; P = 0.08) indicating no significant difference in calcaneal bone parameters between those with diabetes and neuropathy and those with diabetes without neuropathy. This result is limited by the high  $I^2$  (71.2%) indicating poor consistency among the included studies. One factor contributing to this may be a lack of equivalence of the neuropathy diagnosis across the studies. All studies used a different method to quantify and classify neuropathy. Although each test can be used in isolation to measure the degree of neuropathy, equivalency of these tests cannot be assumed. Furthermore, test thresholds used to place participants into groups were different across studies or, in some cases, were not provided. To ensure sufficient data for the meta-analysis, studies using different criteria for diagnosis of neuropathy were included, which may have contributed to the high  $I^2$ .

Another limitation of the meta-analysis is that potentially relevant data from three studies could not be included [22,29,30]. Of these studies, one study did not find a significant association between peripheral sensory neuropathy and broadband ultrasound attenuation of the calcaneus [29]. However, another found a correlation between poorer calcaneal quantitative ultrasound measures and poorer



FIGURE 2 Forest plot showing cumulative effect size of seven studies.



**FIGURE 3** Funnel plot with 95% confidence intervals indicating no significant asymmetry.

measures of sensory nerve and autonomic functioning [30]. The final study found a reduced metatarsal cortical volume in those with diabetic peripheral sensory neuropathy and autonomic neuropathy compared with those without neuropathy [22].

A potential source of bias in this systematic review is failing to isolate the influence of neuropathy from other factors that may independently affect bone including: age, sex, diabetes type, duration of diabetes, severity of diabetes, ethnicity, body weight, renal function, cholesterol, menopausal status, smoking status, physical activity level, co-morbid conditions and their treatment. Among the included studies, these were generally handled with participant matching, exclusions and statistical analysis. Few of the studies accounted for weight, physical activity level, co-morbid conditions or medications. Of the six studies that did not find a significant relationship between neuropathy and bone, only two clearly accounted for weight [21,25]. Weight may

© 2013 The Authors. Diabetic Medicine © 2013 Diabetes UK mask any effect of neuropathy inducing a loss of bone as higher weight is associated with both a higher bone mineral density [31] and the presence of neuropathy [32]. Three of the four studies that did find a significant relationship, whilst accounting for age, sex, diabetes type and duration of diabetes, inadequately controlled for factors such as ethnicity, cholesterol, diabetes severity, physical activity level, weight and renal function [22,27,30].

Type 1 and Type 2 diabetes have different effects on central bone mineral density. Type 1 diabetes is associated with a reduced bone mineral density, while Type 2 diabetes is associated with a higher bone mineral density [33], and research has demonstrated a much more consistent link between neuropathy and low bone mineral density in Type 1 diabetes [34-36]. In agreement with this, three of the four studies in this review that found evidence of a relationship between neuropathy and poorer indicators foot bone health studied Type 1 diabetes or, primarily, insulin-dependent diabetes [22,26,27]. In the seven studies included in the meta-analysis. the definitions and terminology of diabetes diagnosis were heterogenous and four of the studies used mixed populations of Type 1 and Type 2 diabetes [21, 25, 28], so it was not possible to perform subgroup analysis by diabetes type. The effects of neuropathy in Type 1 diabetes may be more likely to manifest because of an existing propensity for osteoporosis; this may have been masked by the inclusion of participants with Type 2 diabetes.

Limitations in imaging techniques may impair a full understanding of the effect of neuropathy on foot bones. Techniques included primarily quantitative ultrasound measures or dual-energy X-ray absorptiometry measurement of bone mineral density. Quantitative ultrasound only measures a small area of the bone that is difficult to reliably reproduce between individuals and may not reflect whole bone density [24]. Dual-energy X-ray absorptiometry allows assessment of the whole bone; however, there is no universally accepted method of dual-energy X-ray absorptiometry screening for the feet. Additionally, because of practical limitations, neither quantitative ultrasound nor dual-energy X-ray absorptiometry can be used in foot bones other than the calcaneus. This limitation in selecting foot bones is problematic, because, if neuropathy does cause changes to bone, this is likely to begin distally because of the distal origins of neuropathy [37]. Currently, there is a lack of data into foot bones more distal than the calcaneus. In our review, only one study examined a bone more distal than the calcaneus (second metatarsal) showing a reduced cortical volume in participants with both peripheral sensory and autonomic neuropathy compared with those without neuropathy [22]. This study, however, used plain radiographs and the validity and reliability of this imaging technique for this purpose is questionable.

Bone has complex innervation involving the autonomic, sensory and motor nervous systems. The effects of deficits in these different fibres are possibly distinct, affecting bone in different ways. Direct afferent sensory innervation of bone may be affected by diabetic neuropathy impacting bone metabolism [5]. Autonomic neuropathy is proposed to reduce bone strength via a reduction in sympathetic vascular tone and bone hyperaemia [9]. Motor neuropathy may reduce the muscular load on bone that influences remodelling [11].

Only two of the studies included in the meta-analysis investigated participants with autonomic neuropathy, with the others assessing peripheral sensory neuropathy. Also, although three studies in the meta-analysis included a measure of motor neuropathy in their diagnosis of neuropathy, this aspect was not isolated in any study. The results of our meta-analysis therefore only reflect 'general' diabetic neuropathy status and do not allow for interpretation of the effects of the different types of neuropathy on foot bones. Techniques for the measurement of peripheral sensory neuropathy varied between the studies, often being poorly defined. It is therefore unclear how sensitive these measurement techniques were and what affect this may have had on the results. Additionally, the studies that assessed autonomic neuropathy all examined cardiac autonomic neuropathy. This may or may not occur with reduced sympathetic tone, which is proposed to cause blood flow-related changes to bone.

#### Conclusion

Whether diabetic neuropathies induce a reduction in bone health remains to be seen. Most of the studies included in this review did not find significant differences in bone health between those with and without diabetic neuropathies or an association between bone health and diabetic neuropathy. Furthermore, the meta-analysis did not demonstrate a significant difference in parameters of bone health in people with diabetes and neuropathy compared with those with diabetes without neuropathy. Limited methodological quality and heterogeneity between the trials, however, means further research into the potential relationship between diabetic neuropathy and foot bone health is warranted. Studies examining the mid-foot with reliable imaging techniques, homogenous populations, rigid control of potential confounders, and distinguishing between types of neuropathy with fully defined and sensitive measurement techniques are required.

#### **Funding sources**

This study was supported by a Faculty of Heath Research Grant from the University of Newcastle (Australia).

#### **Competing interests**

None declared.

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# Chapter Four

# Methodology

The research in this chapter relates to the following objectives

- 2. Develop a reliable measure of microvascular reactivity for clinical and research purposes.
- 3. Develop a feasible and reliable method of foot bone density measurement.

# Preface

The studies in Chapters Five and Six of this thesis required a quantifiable measure of microvascular reactivity in order to assess its relationship to diabetic neuropathy and foot bones. Post-occlusive reactive hyperaemia is a non-invasive measure of the cutaneous blood flow reaction to a period of ischaemia that is indicative of microvascular dysfunction that may be of relevance to pathology such as wounds and Charcot foot. The reliability of post-occlusive reactive hyperaemia measurement at the hallux has not been previously assessed. The objective of this section was to investigate the intra and inter-tester reliability of post-occlusive reactive hyperaemia and charcot for clinical and research purposes. The study demonstrated that post-occlusive reactive hyperaemia can reliably be measured at the hallux and identified the most reliable parameters for use in research. It also showed that quantification of the response with a blood pressure response is not reliable.

The experiments performed in this section were approved by the University of Newcastle human research ethics committee: approval number H-2010-1230. Appendix A contains an author contribution statement for the publication. Appendix B contains ethics approval, recruitment materials, participant information statement, consent form and data collection forms used in the study.

This manuscript was published in Microvascular Research which has an impact factor of 2.432.

Citation: Barwick, A., S. Lanting, and V. Chuter, *Intra-tester and inter-tester reliability of post*occlusive reactive hyperaemia measurement at the hallux. Microvasc Res, 2015. 99(0): p. 67-71

The research was presented at the Australasian Podiatry Conference on the Gold Coast in May 2015. The abstract was published in the Journal of Foot and Ankle Research and is found in Appendix I.

Citation: Barwick, A., Lanting, S., & Chuter, V. *Intra- and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux. Australasian Podiatry Conference.* JFAR, 2015. 8(2): p. O1

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# Intra-tester and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux



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#### ARTICLE INFO

Article history: Accepted 7 March 2015 Available online 14 March 2015

Keywords: Endothelial function Microvascular Peripheral arterial disease Laser Doppler Reliability Post-occlusive reactive hyperemia

#### ABSTRACT

*Background:* Post-occlusive reactive hyperaemia (PORH) is a measurement of the vasodilatory capacity of the microvasculature that is associated with cardiovascular disease, peripheral arterial disease and foot ulceration. The reliability of its measurement in the hallux (great toe) for clinical and research purposes has not been adequately assessed. This study assesses both the intra-tester reliability and inter-tester reliability of four methods of assessing PORH in the hallux.

*Methods and results:* A within-subject repeated measures design was used. Forty-two participants underwent PORH testing using four methods: pressure measurement with photoplethysmography; an automated laser Doppler technique with local heating; an automated laser Doppler technique without local heating; and a manual laser Doppler technique. Participants underwent testing on two occasions with a three to 14 day interval. Laser Doppler measurement with a heating probe was found to be the most reliable method of PORH measurement. The index of the area under the curve pre- and post-occlusion and peak perfusion as a percentage of baseline were the most reliable variables.

*Conclusions:* PORH can be reliably measured using laser Doppler when combined with a heating probe. Further research is required to determine the clinical utility of photoplethysmography in the measurement of PORH. © 2015 Elsevier Inc. All rights reserved.

#### Introduction

Post-occlusive reactive hyperaemia (PORH) is a measure of microvascular function characterised by the occurrence of a rapid rise in skin and muscle blood flow (in excess of baseline flow) following a period of proximal arterial occlusion (Cracowski et al., 2006). Typically, arterial occlusion causes shear stress and the release of vasodilators such as nitric oxide from the endothelium, lowering myogenic vascular tone and vessel pressure (de Mul et al., 2005). Once the occlusion is released, the lowered vessel pressure allows for a rapid and excessive increase in skin blood flow until the tone is restored and the flow returns to its resting state (de Mul et al., 2005). Impairment in this reaction is indicative of microvascular dysfunction.

Microvascular dysfunction is associated with atherosclerosis (Sitia et al., 2010), diabetes and diabetic foot disease (Chao and Cheing, 2009), peripheral arterial disease (Brevetti et al., 2008) and kidney disease (Long et al., 2012). An impaired post-occlusive reactive hyperaemia response, specifically, is associated with coronary artery

disease (Tibirica et al., 2015), peripheral arterial disease (Morales et al., 2005; Nukada et al., 1998) and diabetes (Gomes et al., 2008; Jorneskog et al., 1995), especially those with poor blood glucose control (Jorneskog et al., 1998). Importantly, it has been shown to precede clinically apparent microvascular dysfunction and atherosclerosis as well as late diabetes complications (Yamamoto-Suganuma and Aso, 2009). Consequently, a valid and reliable measure of assessing PORH is needed for both clinical and research purposes.

Several methods can be used to quantify PORH. Cutaneous microcirculation in the periphery can be measured continuously during the task with laser Doppler technology. This allows for quantification of the response through comparison of baseline flux with post-occlusion flux as well as a selection of variables such as the peak flux during hyperaemia and time to peak. This can be performed with or without local heating. Reliability data is available for PORH measurement with laser Doppler in the upper limb in non-pathological populations (Agarwal et al., 2010; Binggeli et al., 2003; Boignard et al., 2005; Roustit et al., 2010; Tew et al., 2011; Yvonne-Tee et al., 2005); however, data for the lower limb in at-risk populations is lacking.

Laser Doppler flowmetry requires expensive equipment that is not widely available to primary care clinicians. As an alternative technique, blood pressure in the small vessels can be obtained by using a digital cuff and sphygmomanometer along with a photoplethysmograph (PPG) probe (Bergstrand et al., 2009). A pressure reading pre- and post-occlusion can be used to measure hyperaemia indirectly through

Abbreviations: AUC, area under the curve; BL, baseline; BZ, biological zero; CVC, cutaneous vascular conductance; ICC, intra-class correlation coefficient; LOA, limits of agreement; PORH, post-occlusive reactive hyperaemia; PPG, photoplethysmography; PU, perfusion units.

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vessel pressure comparison before and after occlusion. The reliability of this technique is yet to be determined. This study will investigate the reliability of a PPG method as well as three methods of laser Doppler flowmetry for the measurement of PORH.

#### Materials and methods

#### Participants

Participants were recruited from podiatry clinics on a volunteer basis. All participants met the current guidelines for regular screening for peripheral arterial disease i.e. over the age of 65 years or those who are over 50 years of age who have other risk factors for peripheral arterial disease (Rooke et al., 2011). None were confirmed as having peripheral arterial disease. Exclusion criteria included: the presence of ulceration, injury or infection of the hallux or foot that prevented measurements being taken, amputation of both halluces, severe lymphoedema, connective tissue diseases, vasospastic conditions, and any condition precluding supine lying. The study was approved by the University of Newcastle Human Research Ethics Committee and all participants gave their informed consent to participate.

#### Equipment and measurement

Participants were asked to refrain from nicotine, caffeine and exercise for 2 h before testing. Room temperature was maintained at 23–24 °C for the duration of testing. Participants were placed in a supine lying position with feet at heart level for 10 min prior to testing and asked to avoid coughing, talking, yawning and moving for the duration of the tests.

Laser Doppler measurements were made with a moorVMS-LDF2 laser Doppler module and a VP1T combined optic and temperature skin probe for the non-heated measurements and a VHP2 digit skin heater probe and needle probe for the heated measurements (Moor Instruments Ltd, Axminster, United Kingdom). Probes were calibrated according to manufacturer instructions.

The laser probe was fixed to the plantar surface of the participant's right hallux using a probe holder and adhesive pad. A 2.5 cm pneumatic cuff (Moor Instruments Ltd) was placed proximal to the probe. The following automated settings were utilised with the moorVMS-PRES pressure module (Moor Instruments Ltd): 3 min of baseline flux recording, inflation of the cuff to 220 mm Hg for 3 min, cuff deflation at maximum speed, and post-occlusive flux recording for a further 4 min. This process was performed with (heated automated method) and without (non-heated automated method) local heating to 33 °C and was repeated using a hand-held a blood pressure gauge (ERKA, Bad Tölz, Germany) and an inflatable digital cuff (Hadeco, Kawasaki, Japan) (manual method). All data were processed with moorVMS recording and analysis software Version 3.1 (Moor Instruments Ltd). All measurements obtained were in arbitrary perfusion units (PU).

Variables obtained manually include: mean (pre-occlusion) flux during 60 s (baseline; BL); mean flux during 60 s of occlusion (biological zero; BZ); highest flux in the 60 s following occlusion (Peak); peak as a percentage of baseline flux (Peak%BL); baseline flux subtracted from the peak (Peak — BL); time from release of occlusion to the peak (TtPeak); and area under the curve (AUC) of 1 min from release of occlusion relative to the AUC of 1 min of baseline flux (Index). Variables obtained automatically by VMS software for the automated method include: mean (pre-occlusion) flux during 180 s (baseline; BL); mean flux during the second half of occlusion (biological zero; BZ); highest flux in the 240 s following occlusion (Peak); peak as a percentage of baseline flux (Peak%BL); baseline flux subtracted from the peak (Peak — BL); time from release of occlusion to the peak (TtPeak); and AUC of 1 min from release of occlusion relative to the area under the curve of 1 min of baseline flux (Index). Photoplethysmography measurements were made with a Biodop ES-100V3 hand-held Doppler (Hadeco, Kawasaki, Japan), a blood pressure gauge (ERKA, Bad Tölz, Germany) and an inflatable digital cuff (Hadeco, Kawasaki, Japan). A baseline toe pressure was measured by observing the PPG output on a Doppler monitor whilst inflating the digital cuff until the signal fell flat. The cuff was then gradually deflated until the signal reappeared. This was recorded as the systolic toe pressure. After a two minute rest period, the cuff was then inflated to 220 mm Hg for 3 min then rapidly deflated. After 15 s, the toe pressure was taken again. Data were expressed as a ratio of pre-occlusion to postocclusion systolic pressure.

All measurements were performed by two testers – both podiatrists – trained in the methods described above.

The order of techniques and of the tester was randomised for each participant with a computer generated random allocation function. For each participant, this order and the pre-testing and testing protocol were identical in both sessions taking place between three and 14 days apart at the same time of day. Laser Doppler measurements with heating took place at a separate testing session and repeated three to 14 days later. Testers were blinded to each-other's results and the results of the previous session. Skin temperature was monitored for the duration of testing using a Dermatemp DT-1001RS infrared thermographic scanner (Exergen, Watertown).

#### Statistics

Statistical analysis was performed in SPSS Version 22 for Windows (SPSS Inc., Chicago, USA). Intra-tester reliability between sessions 1 and 2 and inter-tester reliability between testers 1 and 2 in session 1 were determined with intra-class correlation coefficients (ICCs) and 95% confidence intervals for all four methods. Interpretation of ICCs was in accordance with Portney and Watkins (2000): >0.75 = good, 0.50 to 0.75 = moderate, and <0.50 = poor. T-tests with 95% limits of agreement (LOA) with significance set at P < 0.05 (two-tailed test) were also calculated for all four methods to assess agreement.

#### Results

Forty-two participants at risk of peripheral arterial disease were recruited for the non-heated measurements and thirty-two participants for the heated measurements. Nineteen of the participants took part in both the non-heated and heated measurements. Participant characteristics are shown in Table 1.

ICCs with 95% confidence intervals for intra-rater testers 1 and 2 and intra-rater for session 1 are found in Table 2. Means, standard deviations and 95% LOA for testers 1 and 2 are presented in Table 3. Means, standard deviations and 95% LOA for session 1 are presented in Table 4.

Variables from the non-heated automated method with moderate reliability for both inter-tester and intra-tester were: BL, Peak and Peak — BZ. These were the same for the manual method. Using the automated method with heating, all variables had moderate or good inter-tester reliability. Intra-tester reliability was good for Index and Peak%BL for both testers. Though ICCs were acceptable, LOA for these variables were wide, indicating that a large difference in outcomes would be required to confirm that the change was not due to error.

Table 1	
Participant characteristics	5.

	Heating $(n = 32)$	No heating $(n = 42)$
Age (years) Sex distribution (male/female) Diabetes present (yes/no) Smoker (yes/no)	$\begin{array}{l} 72\pm7.4\\ 17~(53\%)/15~(47\%)\\ 20~(63\%)/12~(37\%)\\ 5~(16\%)/27~(84\%) \end{array}$	$\begin{array}{l} 71.5 \pm 7.8 \\ 18 \; (43\%)/24 \; (57\%) \\ 17 \; (40\%)/25 \; (40\%) \\ 6 \; (14\%)/36 \; (86\%) \end{array}$

Data are presented as mean  $\pm$  standard deviation.

#### Table 2

ICCs with 95% confidence intervals for testers 1 and 2 and intra-rater for session 1.

	Variable	Tester 1		Tester 2		Inter-tester	
		ICC	95% CI	ICC	95% CI	ICC	95% CI
Automated							
No heat	BL	0.70 <sup>a</sup>	0.50, 0.83	0.61 <sup>a</sup>	0.37, 0.77	0.74 <sup>a</sup>	0.56, 0.85
	BZ	0.06	- 0.25, 0.36	0.61 <sup>a</sup>	0.38, 0.77	0.26	-0.04, 0.52
	Peak	0.52 <sup>a</sup>	0.25, 0.71	0.61 <sup>a</sup>	0.37, 0.77	0.57 <sup>a</sup>	0.33, 0.74
	tPeak	0.22	- 0.09, 0.49	0.00	-0.30, 0.31	0.16	-0.14, 0.44
	Index	0.43	0.15, 0.65	0.22	-0.09, 0.49	0.39	0.10, 0.62
	Peak — BL	0.09	- 0.22, 0.39	0.43	0.14, 0.65	-0.05	-0.35, 0.26
	Peak%BL	0.64 <sup>a</sup>	0.41, 0.79	0.43	0.15, 0.65	0.53 <sup>a</sup>	0.27, 0.71
	Peak — BZ	0.52 <sup>a</sup>	0.26, 0.71	0.61 <sup>a</sup>	0.37, 0.77	0.57 <sup>a</sup>	0.33, 0.75
Heat	BL	0.46	0.14, 0.69	0.46	0.14, 0.70	0.91 <sup>b</sup>	0.83, 0.96
	BZ	0.48	0.17, 0.71	0.61 <sup>a</sup>	0.33, 0.79	0.92 <sup>b</sup>	0.85, 0.96
	Peak	0.48	0.17, 0.71	0.56 <sup>a</sup>	0.26, 0.76	0.94 <sup>b</sup>	0.88, 0.97
	tPeak	0.54 <sup>a</sup>	0.23, 0.74	0.39	0.06, 0.65	0.52 <sup>a</sup>	0.21, 0.73
	Index	0.78 <sup>b</sup>	0.60, 0.89	0.80 <sup>b</sup>	0.63, 0.90	0.86 <sup>b</sup>	0.73, 0.93
	Peak — BL	0.36	0.01, 0.62	0.46	0.14, 0.69	0.87 <sup>b</sup>	0.76, 0.94
	Peak%BL	0.77 <sup>b</sup>	0.57, 0.88	0.81 <sup>b</sup>	0.65, 0.90	0.85 <sup>b</sup>	0.72, 0.93
	Peak — BZ	0.49	0.17, 0.71	0.56 <sup>a</sup>	0.26, 0.76	0.94 <sup>b</sup>	0.88, 0.97
Manual	BL	0.75 <sup>b</sup>	0.57, 0.86	0.58 <sup>a</sup>	0.34, 0.75	0.74 <sup>a</sup>	0.56, 0.85
	BZ	0.92 <sup>b</sup>	0.85, 0.96	0.43	0.15, 0.65	0.26	-0.41, 0.52
	Peak	0.63 <sup>a</sup>	0.40, 0.78	0.66 <sup>a</sup>	0.45, 0.81	0.57 <sup>a</sup>	0.33, 0.74
	tPeak	0.14	-0.17, 0.43	0.61 <sup>a</sup>	0.37, 0.77	0.16	-0.14, 0.44
	Index	0.40	0.11, 0.63	0.40	0.11, 0.63	0.39	0.10, 0.62
	Peak — BL	0.25	- 0.06, 0.52	0.32	0.02, 0.57	-0.05	-0.35, 0.26
	Peak%BL	0.63 <sup>a</sup>	0.40, 0.78	0.44	0.16, 0.66	0.53ª	0.27, 0.71
	Peak — BZ	0.63 <sup>a</sup>	0.40, 0.78	0.66 <sup>a</sup>	0.45, 0.80	0.57ª	0.33, 0.75
PPG		0.37	0.08, 0.61	0.28	-0.03, 0.54	-0.14	-0.42, 0.17

<sup>a</sup> Variable has moderate reliability.

<sup>b</sup> Variable has good reliability.

The PPG index did not have acceptable intra-tester reliability for either tester or acceptable inter-tester reliability in either session.

#### Discussion

Post-occlusive reactive hyperaemia is a measure of microvascular reactivity that may be useful in research and clinical settings including the investigation of the at-risk foot, if found to be reliable. Previous evidence for its reliability is mixed and the literature currently focuses on the upper limb in healthy populations. This study is the first to assess the inter- and intra-tester reliability of PORH in the hallux in those at risk of peripheral arterial disease. The findings support the use of PORH as measured by laser Doppler using automatically calculated variables and using a heating probe. They do not support using laser Doppler with manually calculated variables or without a heating probe nor using a handheld pressure technique with PPG.

#### Table 3

Means (SD) and 95% limits of agreement for intra-tester reliability for testers 1 and 2.

Method	Variable	Tester 1			Tester 2		
		Mean (SD)		95% LOA	Mean (SD)		95% LOA
		Session 1	Session 2		Session 1	Session 2	
Automated, no heat	BL	80.79 (77.36)	111.33 (106.03)	- 30.54, 72.3	94.54 (100.28)	79 (67.31)	15.53, 75.71
	BZ	3.01 (0.75)	3.57 (4.27)	0.55, 4.22	3.01 (1.07)	2.87 (0.97)	0.14, 0.9
	Peak	214.29 (96.66)	231.68 (126.86)	- 17.39, 110.71	240.67 (142.89)	233.25 (124.49)	7.42, 119.05
	tPeak	16.62 (40.01)	19.96 (49.38)	- 3.34, 56.1	30.67 (62.57)	19.6 (49.06)	11.07, 79.49
	Index	1.91 (1.35)	1.59 (1.07)	0.32, 1.3	2.27 (1.64)	2.42 (1.62)	-0.15, 2.03
	Peak — BZ	211.27 (96.42)	228.11 (126.28)	13.14, 89.85	237.67 (142.7)	230.39 (124.13)	-8.1, 93.42
	Peak%BL	324.53 (317.01)	279.45 (291.78)	45.07, 259.72	358.72 (291.59)	408.37 (401.36)	- 50.01, 375.56
	Peak — BL	133.5 (57.37)	120.35 (74.78)	- 16.84, 110.15	146.13 (94.04)	154.24 (79.94)	7.28, 18.89
Automated, heat	BL	89.82 (76.15)	77.6 (55.1)	- 123.25, 147.67	92.3 (75.63)	82.26 (72.87)	- 140.87, 160.93
	BZ	1.97 (0.61)	1.95 (0.66)	- 1.25, 1.29	2.03 (0.68)	2.02 (0.78)	- 1.25, 1.29
	Peak	247.88 (104.96)	254.19 (113.05)	-217.27, 216.01	260.35 (105.8)	252.5 (115.74)	- 197, 212.72
	tPeak	37.46 (48.49)	48.15 (44.64)	- 98.79, 77.41	29.49 (39.45)	24.63 (24.79)	-66.33, 76.05
	Index	2.97 (2.17)	3.22 (2.26)	- 3.13, 2.63	3.23 (2.33)	3.17 (2.1)	-2.69, 2.83
	Peak — BZ	245.9 (104.72)	252.24 (112.84)	- 192.36, 155.3	258.32 (105.5)	250.48 (115.32)	- 148.81, 144.45
	Peak%BL	302.67 (269.22)	338.8 (275.35)	- 400.55, 328.29	333.73 (322.6)	356.96 (310.1)	-403.25, 356.79
	Peak — BL	158.06 (72.31)	176.59 (83.5)	-44.64, 31.96	168.06 (66.61)	170.23 (76.77)	- 196.31, 211.99
Manual	BL	84.97 (79.21)	110.12 (109.57)	25.16, 68.26	83.96 (92.55)	77.83 (72.52)	6.12, 76.1
	BZ	3.88 (2.54)	4.07 (3.23)	0.19, 1.18	3.8 (1.15)	3.63 (1.67)	0.17, 1.53
	Peak	284.64 (98.44)	312.74 (139.98)	28.1, 104.42	282.33 (137.61)	288.86 (127.53)	-6.53, 108.9
	tPeak	8.9 (20.6)	3.63 (4.79)	- 5.35, 19.62	7.19 (10.47)	6.61 (12.55)	0.57, 10.24
	Index	0.68 (0.33)	0.75 (0.58)	0.07, 0.52	0.61 (0.5)	0.47 (0.26)	0.14, 0.44
	Peak — BZ	280.77 (97.99)	308.68 (139.83)	2.94, 88.82	278.52 (137.03)	285.23 (126.94)	-12.65, 98.82
	Peak%BL	500.53 (512.52)	536.2 (622.37)	35.67, 491.9	595.49 (467.77)	726.17 (785.75)	-130.68, 685.2
	Peak — BL	199.68 (69.52)	202.62 (75.32)	27.91, 104.31	198.37 (89.59)	211.02 (80.04)	- 6.7, 108.63
PPG	Index	1.02 (0.11)	1.01 (0.1)	-0.22, 0.26	0.96 (0.13)	0.94 (0.09)	-0.24, 0.26

 Table 4

 Means (SD) and 95% limits of agreement for the inter-tester reliability for session 1.

Method	Variable	Mean (SD)		95% LOA
		Tester 1	Tester 2	
Automated, no heat	BL BZ	79.19 (77.12) 3 (0.75)	92.6 (99.84) 3 (1.06)	-13.41, 64.56 -0.00, 1.11
	tPeak	17.16 (39.68)	30.11 (69.91)	-26.74, 112.28 -12.96, 67.25
	Index	2.04 (1.58)	2.33 (1.67)	-0.3, 1.79
	Peak — BZ	209.04 (96.33)	235.78 (141.48)	- 13.33, 111.53
	Peak%BL	335.29 (320.80)	376.66 (310.58)	-41.37, 307.2
	Peak — BL	132.85 (56.82)	146.18 (92.89)	-26.73, 111.87
Automated,	BL	89.82 (76.15)	92.3 (75.63)	-63.81, 58.85
heat	BZ	1.97 (0.61)	2.03 (0.68)	-0.55, 0.43
	Peak	247.88 (104.96)	260.35 (105.8)	-84.41, 59.45
	tPeak	37.46 (48.49)	29.49 (39.45)	-77.21, 93.15
	Index	2.97 (2.17)	3.23 (2.33)	-2.62, 2.08
	Peak —	245.9 (104.72)	258.32 (105.5)	- 78.42, 58.42
	BZ Deak%BI	302 67 (260 22)	222 72 (222 6)	- 346 40 284 28
	Peak -	158.06 (72.31)	168.06 (66.61)	- 94 18 50 34
	BL	156.00 (72.51)	108.00 (00.01)	-04.10, 55.54
Manual	BL	83.64 (78.72)	82.29 (92.05)	1.35, 67.53
	BZ	3.84 (2.52)	3.78 (1.14)	0.06, 2.47
	Peak	280.25 (101.31)	280.6 (136.38)	-0.35, 100.42
	tPeak	8.78 (20.39)	7.18 (10.35)	1.6, 18.35
	Index	0.68 (0.33)	0.6 (0.5)	0.08, 0.48
	Peak — BZ	276.41 (100.82)	276.82 (135.8)	- 1.7, 95.21
	Peak%BL	494.46 (507.76)	614.65 (478.42)	- 120.18, 581.21
	Peak — BI	196.62 (71.47)	198.31 (88.49)	-0.41, 100.1
PPG	Index	0.92(1.02)	0.85 (0.96)	-0.290.41

This study found that assessing PORH by comparing pre- and post-occlusion pressures using a PPG and sphygmomanometer had insufficient reliability to be used as a clinical measurement. The reliability of the PORH measurement at the hallux using laser Doppler when variables were calculated both manually and with automatic software calculation was poor. These findings suggest that these techniques are not sufficiently accurate for clinical or research purposes. The addition of a heating probe to the automatic measurements greatly improved the reliability such that it is a valuable measurement. This result supports a previous study that found adequate same-day repeatability of PORH in the hallux (Jorneskog et al., 1995).

The majority of evidence for the reliability of PORH has been performed in the upper limb with mixed results. Some research has indicated that the reliability is poor (Roustit et al., 2010; Tew et al., 2011; Tibiriçá et al., 2011) with others indicating that it is good (Boignard et al., 2005; Yvonne-Tee et al., 2005). It is unclear why there is such variation within the literature; however, it is evident that the measurement is sensitive to environmental factors such as temperature that must be controlled and that some variables are more reliable than others.

Ideally, the variables used to quantify the response should be both reliable and of physiological significance. There is no consensus on which variables should be used to quantify PORH output (Roustit et al., 2010). The response can be quantified as: the peak flux which can be raw, subtracted with biological zero or relative to the baseline flux; temporal variables such as time to peak and time to return to resting flux; or a measure of the AUC relative to pre-occlusion, among others. The most commonly used parameters are peak blood flow and time to peak blood flow as they are traditionally linked with microvascular dysfunction (Cracowski et al., 2006). However, AUC and time to resting flux may be more physiologically interesting variables that are reflective of disease states (Morales et al., 2005; Yamamoto-Suganuma and Aso, 2009). Our results indicate that for reliability purposes the best variables as measured by laser Doppler with a heating probe are peak as a percentage of baseline and the index of 1 min pre-occlusion to 1 min post-occlusion. However, the wide LOA relative to the means of these variables indicate that any difference or change found would need to be large to ensure that it is a true change, which may limit their application.

Our findings support previous recommendations for expressing variables as a percentage of baseline whilst controlling temperature (Cracowski et al., 2006). A previous investigation, Agarwal et al. (2010), found poor reproducibility in those with variables expressed as a percentage of baseline as a small difference observed in the baseline flux could have a large effect on the outcome variable. However, it is recommended that to account for this, temperature can be strictly controlled (Cracowski et al., 2006). Our study demonstrated that percentage of baseline measurements had higher reliability when taken in conjunction with controlled local heating to standardised skin temperature as opposed to simply controlling environmental temperature. As the skin blood supply plays a role in thermoregulation, changes in temperature cause a change in blood flow to the skin, particularly where arteriovenous anastomoses are most dense such as in the plantar surface of the feet (Cracowski et al., 2006). Our results suggest that controlling room temperature may not be sufficient to minimise variation in flow due to temperature. One disadvantage of doing this, however, is that it is a less physiological measurement (Cracowski et al., 2006).

The results of this study need to be considered in light of several limitations. Limitations of the use of laser Doppler include that laser Doppler cannot measure absolute perfusion in ml/min relative to volume or weight of tissue (flow), but rather uses arbitrary perfusion units or raw amplitude in mV (Cracowski et al., 2006) thereby making the measure less physiological. It is recommended that data is expressed both as arbitrary perfusion units (PU/mV) as well as cutaneous vascular conductance (CVC) which takes into account variations in blood pressure (Roustit et al., 2010). A limitation of this study is that we did not obtain reliability for variables expressed as CVC. Another limitation of this study is that we did not examine time to resting flux, which, as stated earlier may be a useful measurement physiologically.

There is no consensus in occlusion times used to measure PORH with various occlusion times (usually between three and 10 min) within the literature for both the upper and lower limbs (Cracowski et al., 2006; Yvonne-Tee et al., 2005). We used 3 min at the hallux because shorter occlusion times may not elicit a maximal response and longer occlusion times may cause participant discomfort (Tee et al., 2004). Occlusion time was kept consistent throughout the current study at 3 min. The reliability of other occlusion times at the hallux remains to be studied.

Another potential pitfall in examining PORH in at-risk populations is that medial wall calcinosis may prevent the arteries from being truly occluded (Brooks et al., 2001). A large proportion of our participants (63% during the heated measurements and 40% for the non-heated measurements) had diabetes, meaning it is likely that they had some degree of medial wall calcinosis (Young et al., 1993). However, since calcification is rare in the toe arteries (Pareira et al., 1953), and we were able to achieve occlusion in all cases as evidenced by a fall in PU to <3 it is not likely that medial wall calcinosis affected our measurements. Nevertheless, this is an important consideration in examining PORH in diabetic populations.

To use PORH in a clinical setting outside of research settings, and assess its value as a screening tool, the test must be reliable, accessible and easy to perform. The PPG pressure method is easily accessible and easy to perform but unfortunately we did not find it to be reliable. It is possible that waiting 15 s between taking the first and second measurements is too short a time frame. Hoffman et al. (1991) found that pressure returned to normal at 2 min post-occlusion so it is possible that the optimal time period to take the second measurement is between these two times. This method warrants further investigation.

#### Conclusions

A reliable and standardised approach to PORH at the hallux is a promising measurement for research into peripheral arterial disease and the at-risk foot and diabetic foot disease. It may have utility in investigating the pathogenesis of disease and as an intervention end-point. We found the measurement of PORH at the hallux as measured with laser Doppler flowmetry to have good intra- and inter-tester reliability for use in research only when used with local heating. A method of measuring PORH with the reliability and practicality to be clinically useful requires further investigation.

#### **Funding sources**

This project was supported by a Faculty of Health and Medicine grant from the University of Newcastle Australia (188269789) held by Vivienne Chuter.

#### Acknowledgments

No acknowledgements.

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# Preface

The study in Chapter Six requires a valid and reliable way of testing foot bone density in order to assess the influence of neuropathy and vascular parameters on density of bone that may be of relevance to pathology such as Charcot foot. Such measurement is plagued by ethical, practical and methodological restrictions. Accordingly, a standardised method of measurement has not been previously devised. Currently, densitometry of foot bones other than the calcaneus is restricted to three-dimensional segmentation, which is cost and time inefficient. In this study, a simplified computed tomography method for quantifying foot bone density was devised and assessed for intratester reliability. The reliability of bone densitometry including trabecular and cortical bone in multiple foot bones, which may be more relevant for pathology such as Charcot foot, using a novel approach is established is this study. This technique is used for the study presented in Chapter Six.

The experiments performed in this section were approved by the University of Newcastle Human Research Ethics Committee (reference number H-2013-0404). Appendix A contains an author contribution statement for the manuscript. Appendix C contains ethics approval, recruitment materials, participant information statement, consent form, authority to release information, general practitioner information statement and request form, demographic information form and data collection materials used in the study. Appendix D contains examples of participant set up for computed tomography scans and examples of region of interest selection from the scans. Reliability of computed tomography derived foot bone density measurements in people with diabetes

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### Abstract

Introduction: Accurate and reliable methods of assessing bone quality are essential for the investigation of foot disease, such as Charcot neuroarthropathy and fractures in those with diabetes. There is currently no gold standard for the assessment of bone mineral density in the feet. Computed tomography is a promising tool for such assessment. This study investigated the reliability of a novel method of assessing trabecular and cortical foot bone density with computed tomography in people with diabetes.

Methodology: We scanned 10 feet with computed tomography twice with repositioning and assessed bone density (in Hounsfield units) in the trabecular and cortical bone in all tarsals and metatarsals. We assessed reliability with intra-class correlation coefficients (ICC; 95% confidence intervals) and standard errors of measurement (SEM).

Results: The reliability of the trabecular density of most bones was excellent with ICC values ranging from 0.69 to 0.91. Additionally, cortical bone density showed fair to good reliability at the talus, calcaneus, navicular, cuboid, intermediate cuneiform and first metatarsal (ICC range 0.46 to 0.70).

Conclusions: We established the reliability of a practical method of assessing foot bone density. This methodology is useful in the investigation of foot bone disease occurring in diabetes and its early diagnosis, intervention and assessment of treatment efficacy.

#### Keywords:

Bone

Foot

Computed tomography

BMD

Reliability

### Introduction

People with diabetes have an increased risk of bone fracture both centrally and peripherally (1, 2). Diabetes has been shown to affect bone mass and its microstructure (3), reduce fracture resistance, and impair bone regeneration (4). This is partially due to the disruption of regulatory pathways involving hypercalcuria, increased reactive oxygen species, increased polyol pathway activity and non-enzymatic glycosylation of bone (5).

Increased risk of foot fracture in diabetic cohorts (1, 6) may be associated with disease-related complications, which manifest in the periphery including peripheral neuropathy and vascular disease. These disease states cause changes to bone remodelling via deterioration in direct innervation by sensory and sympathetic nerve fibres, as well as systemic alterations to bone metabolism via hormone pathways controlled by the autonomic nervous system (7). Additionally, peripheral muscle wasting and changes to pressure and loading in the foot may contribute to bone changes.

Significant bone changes in the periphery of people with diabetes are characteristic of specific complications, such as Charcot neuroarthropathy, which involves extreme alterations to bone density throughout its natural history (8). Due to the potentially destructive nature of the disease process and the lack of conclusive evidence regarding the specific cause, the reliable assessment of foot bone integrity is essential for the investigation of such disease processes and may assist in early diagnosis, intervention and accurate monitoring of management.

The intricate nature of foot bone morphology results in many traditional bone density measurement techniques lacking the accuracy to establish bone density of individual foot bones, particularly in the mid-foot. For example, dual-energy x-ray absorptiometry is widely used in the assessment of bone density centrally, but does not have the capability to distinguish between the small bones within the foot that sit closely together (9). Similarly, ultrasound has been used to assess the integrity of the calcaneus, but is impractical to use on the rest of the bones of the foot, which are also prone to fractures in people with diabetes (10). Computed tomography (CT) is a promising tool to achieve accurate and reliable information on foot bone integrity (11). Computed tomography is a non-projection technique that can not only distinguish between individual bones in the foot, but also

between trabecular and cortical bone. This is useful as trabecular bone is more metabolically active and therefore more affected by disease processes (12).

Computed tomography can be used to assess the bone mineral density (BMD) through segmentation, as well as through analysis of single slices. Peripheral quantitative computed tomography (pQCT) provides volumetric analysis of individual bone slices, but is complicated by the need to replicate scan location based on bony landmarks. As such, it has been restricted to the radius and tibia (12), though it can also reliably be used to assess the second metatarsal (13). Excellent precision of three-dimensional segmentation of the tarsals and metatarsals has been established (9), although the bone registration and segmentation process is very time consuming and requires specialised software. This may be avoided by averaging several slices from the obtained three-dimensional images. This may prove a simpler, but sufficient, means of assessing foot bone quality in at risk populations on a larger scale. To the authors' knowledge there has been no assessment of the reliability of bone density analysis of single slices of foot bones obtained from three-dimensional acquisition techniques.

The objective of this study was to investigate the reliability of a novel method of assessment of cortical and trabecular bone density of the tarsals and metatarsals of the feet in those with diabetes using CT.

### Materials and Methods

#### Participants

Adults with type 1 or 2 diabetes were recruited from a podiatry clinic in New South Wales, Australia. Participants were excluded if they were pregnant, took corticosteroids, or hormone replacement therapy, had osteoporosis, chronic renal failure, current bilateral foot ulceration, Charcot neuroarthropathy, malignancy, endocrine disorders (other than diabetes), a recent history of foot trauma or had participated in research involving ionising radiation in the previous 12 months. Ethics was obtained from the University of Newcastle Human Research Ethics Committee and informed consent was obtained from all participants.

#### Equipment and Procedure

An Aquilion One 320 slice CT scanner (Toshiba Medical Systems, Japan) was used for all examinations. One radiographer performed all aspects of the examinations, including participant positioning, scanning and acquisition of measurements.

A pre-planned program was utilized for each examination. No adjustments to the pre-planned program were made for any participant. Volume acquisition was utilised with the following settings applied: CTDIvol 7.2mGy; dose-length product 115.9 (mGy x cm); 120kV; 150mA; rotation time 0.5s; range 16cm; display field of view medium or large (depending on foot size).

The right foot of all participants was scanned, except where prohibited by injury or amputation in which case the left foot was scanned. Each participant was placed in a recumbent position on the CT table, offset to the participants' left side in order to allow a more midline position for the right lower extremity of the participant. The left knee was flexed to prevent scanning of the left foot. The degree of angulation of the left leg was determined by the comfort of the patient to assist in maintaining the desired position throughout examination in an effort to prevent any movement artefact and the need for repeat scanning.

The right foot was placed against a wooden box with the ankle in a neutral position as close to 90° to the table surface as possible. The foot was scanned using the pre-planned program and resultant images were assessed by the radiographer for any movement artefact and to ensure that all anatomical areas were covered. Once the radiographer ratified the imaging data the participant was removed from the CT table. This whole process was then repeated for each individual participant.

All seven tarsals and the five metatarsals were assessed in the axial plane of reconstruction. Axial images were viewed using a bone algorithm so that clear differentiation was possible between trabecular and cortical bone. All images were viewed with a window level of 350 and a window width of 2700. Images were reconstructed 0.5mm thick at intervals of 0.25mm.

Three random slices were obtained from the body of each of the 12 bones. The radiographer selected appropriate regions from the slices of each participant and Hounsfield units (HU) measurements were obtained. Three slices were randomly selected from the mid-portion of the bone (without proximal or

distal cortical bone included). The largest region of interest possible was traced in the trabecular bone and three regions of interest were taken from the cortical bone from each slice image yielding a total of three trabecular readings and nine cortical readings for each bone.

#### Statistics

Statistical analysis was performed in SPSS Version 22 for Windows (SPSS Inc, Chicago, USA). Testretest reliability between session 1 and 2 was determined with intra-class correlation coefficients (ICC) and 95% confidence intervals (CI) for all twelve bones for both cortical and trabecular bone. Interpretation of ICC values was in accordance with Fleiss (14): > 0.75 considered excellent reliability, 0.40 to 0.75 considered fair to good reliability and, < 0.40 considered poor reliability. The standard error of the measurement (SEM) presented in the units of the scale (HU) was calculated to estimate the precision of each measurement to give an indication of test to test variability in cortical and trabecular densitometry.

### Results

Ten participants with type 2 diabetes, were included in the study. Demographic characteristics are shown in Table 1.

ICC values with 95% CI, means of HU measurements for each bone assessed and SEM are included in Table 2. All trabecular measurements displayed excellent reliability with ICC values ranging from 0.81 to 0.91, except for the navicular, cuboid and fourth metatarsal which displayed fair to good reliability. Cortical measurements at the talus, calcaneus, intermediate cuneiform, navicular cuboid and first metatarsal displayed fair to good reliability, with the remaining bones displaying poor reliability. Measurement precision as measured by SEM was much poorer in less reliable measures. SEM ranged from 2 to 12% of the mean of the HU measurement for the trabecular measurements, while for the cortical bone SEM ranged from 5 to 19% of the mean for the HU measurement indicating poorer precision than for the trabecular bone.

### Discussion

We assessed the reliability of a novel method of assessing foot bone density, finding excellent reliability for its use in the trabecular bone in most tarsals and metatarsals, as well as fair to good reliability for the cortical bone of multiple foot bones. Precision of three-dimensional analyses of foot bones previously has shown the method to have very little error. Commean et al. (9) examined the precision of three-dimensional whole BMD of the tarsals and metatarsals after segmentation and obtained coefficients of variance ranging from 0.2% for the talus to 1.6% for the fifth metatarsal. Additionally, repeatability of pQCT BMD measurement has been found to be excellent in the second metatarsal in cadavers obtaining an ICC of 0.98 for both cortical and trabecular BMD (mg.cm3) (13). To the author's knowledge we are the first to assess reliability of foot bone measurements from multiple CT slices obtained from full foot scans in vivo.

We found the trabecular measurements to be more reliable than cortical estimates. This is probably due the inability to sample the whole cortical bone on the slices in our methodology, resulting in the use of smaller sample regions of interest. It is possible that sampling more regions of interest (more than the nine used in this study) may yield more reliable cortical BMD estimates using this method. The separation of cortical and trabecular bone measures is useful as the two are metabolically distinct and may be affected differently by disease processes. All of the bones were found to have acceptable reliability in the trabecular bone, which is thought to be more susceptible to change during disease processes due to its higher turnover (9). However, cortical density has been found to be more indicative of fracture risk (15). Though generally reliability of the cortical bones was lower, we found fair to good reliability for cortical bone measurement in the talus, intermediate cuneiform, calcaneus, cuboid, navicular and first metatarsal. In these six bones, therefore, our novel method could be used to assess the relative effect of disease processes and treatments on these two compartments of bone.

We did not use BMD calculated in mg.cm3, but rather retained the values in HU. HU are quantitative units of the radiodensity of objects as obtained from CT scanning where water is calibrated to zero (12). HU are relatively simple to attain and have been associated with bone strength and fracture risk, making them a useful measure (16). The disadvantage of conversion to mg.cm3 is that it requires phantoms that are not readily available in the range of bone densities encountered in the foot (9). In the interest of developing a practical method of examining foot bone density in the presence of disease values were therefore left as HU.

Furthermore, in an effort to develop an efficient method to assess fracture risk in the periphery we chose to assess average densities (HU) across multiple CT slices rather than perform a time consuming registration and segmentation process. Our assessment of the accuracy of our densitometry method is limited to comparison with values in existing literature in a similar population. Commean et al. (9) obtained combined cortical and trabecular BMD (HU) for all tarsals and metatarsals in those with diabetes, peripheral neuropathy and history of ulceration. For example the average density at the calcaneus was 333 HU, the navicular was 481 HU and the first metatarsal was 427 HU. Our trabecular values were almost universally lower than those found by Commean et al. (9), whilst our cortical values were considerably higher. However, since Commean et al. (9) reported the density of the bone inclusive of both trabecular and cortical bone, our values may be consistent with theirs when considering the relative contribution of trabecular and cortical bone to the overall volume of each bone. It should be noted in the current study, like Commean et al. (9), we found significant variation in BMD among the bones of the foot. We therefore recommend that more than one bone is used in assessment, as one foot bone is unlikely to be representative of all foot bones. In particular, we would recommend measurement of those bones that were shown to have fair to good reliability for bone cortical and trabecular bone, i.e. the talus, intermediate cuneiform, calcaneus, cuboid, navicular and first metatarsal.

We acknowledge several limitations to this study. We performed repeat scans on the same day meaning that the results represent only same day reliability, although scans were read on different days. The radiographer assessing the scans was blinded to a limited extent. Scans were taken on the same day, but assessed at least a week apart with the assessor unable to review previous results. Data were not, however, de-identified which is a weakness in the study design that may have introduced bias. Smith et al. (17) examined the effect of varying technical and biological parameters on foot BMD estimates from CT to find potential sources of variation. They showed that the impact of simulated soft tissue also resulted in a small amount of variation with an inverse relationship between the amount of soft tissue and resulting HU. Finally, due to ethical concerns our sample size was small,

possibly our ICC values may have been closer to that found in reliability investigations of previous studies if the sample size was greater.

## Conclusions

We demonstrated that foot bone density can be reliably measured in those with diabetes by assessing averaged densities from slices of full foot CT scans. Trabecular bone densities of the tarsals and metatarsals showed good to excellent reliability and cortical bone density measurement is most reliable in the navicular, cuboid and first metatarsal. These findings offer a relatively simple, quick and reliable method of quantifying foot bone density which can be used as an indicator of risk of foot disease and its progression, to predict treatment outcomes, assess treatment effectiveness and investigate underlying causes of disease states.

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Table 1: Participant demographic information

M/F	8/2
Age (SD)	72.90 (4.56)
BMI (SD)	31.30 (5.01)
Diabetes duration (SD)	12.15 (11.81)
SD - standard deviation	

	ICC	95% CI	Mean (HU) Session 1	Mean (HU) Session 2	SEM
rtical					
Talus	0.52*	-0.12, 0.85	2987.09	3111.51	271.73
Calcaneus	0.59*	-0.21, 0.88	2764.06	2693.04	199.35
Navicular	0.70*	0.18, 0.92	2823.12	2779.88	129.64
Cuboid	0.69*	0.16, 0.91	2573.46	2862.21	161.96
Medial cuneiform	0.17	-0.48, 0.70	2728.22	2672.93	382.99
Intermediate cuneiform	$0.46^{*}$	-0.20, 0.83	2699.67	2735.92	191.93
Lateral cuneiform	$-0.18^{a}$	-0.71, 0.47	2668.16	2717.88	507.69
First metatarsal	0.61*	0.01, 0.89	2897.07	2929.04	183.62
Second metatarsal	$-0.17^{\mathrm{a}}$	-0.70, 0.48	2872.36	2894.9	470.19
Third metatarsal	0.22	-0.44, 0.72	2719.87	2892.68	330.67
Fourth metatarsal	$-0.03^{a}$	-0.62, 0.58	2728.13	2791.24	525.02
Fifth metatarsal	0.37	-0.30, 0.80	2704.72	2716.83	272.25
becular					
Talus	$0.91^{**}$	0.67, 0.98	457.88	491.11	10.17
Calcaneus	$0.90^{**}$	0.64, 0.97	231.85	246.37	9.10
Navicular	0.70*	0.17, 0.92	367.88	386.10	34.25
Cuboid	$0.68^{*}$	0.47, 0.91	227.12	223.40	24.01
Medial cuneiform	$0.83^{**}$	0.46, 0.96	371.71	378.28	24.12
Intermediate cuneiform	$0.88^{**}$	0.58, 0.97	498.84	493.50	19.23
Lateral cuneiform	$0.86^{**}$	0.54, 0.96	373.96	355.08	17.34
First metatarsal	$0.90^{**}$	0.66, 0.98	248.28	237.65	9.99
Second metatarsal	$0.81^{**}$	0.40, 0.95	309.19	317.91	28.21
Third metatarsal	$0.82^{**}$	0.44, 0.95	280.10	281.72	19.18
Fourth metatarsal	0.69*	0.15, 0.91	268.50	271.72	32.45
Fifth metatarsal	0.85 **	0.50, 0.96	252.35	275.59	16.72

## Chapter Five

Peripheral sensory neuropathy is associated with altered post-occlusive reactive hyperemia in the diabetic foot

The research in this chapter relates to the following objective

4. Explore relationships between clinical subtypes of diabetic neuropathy and vascular characteristics in the diabetic foot.

It tests the hypothesis that those with diabetic neuropathy have altered vascular reactivity in the feet.

# Preface

Diabetic neuropathy is thought to influence blood flow distribution in the feet, which has implications for the development of foot complications such as ulceration and Charcot foot. This study aimed to examine whether the presence of diabetic neuropathy or and cardiac autonomic neuropathy are predictive of the post-occlusive reactive hyperaemia response in a diabetic population. The study showed diabetic sensory neuropathy to be associated with a delayed post-occlusive reactive hyperaemia response.

The experiments performed in this study were approved by the University of Newcastle Human Research Ethics Committee (reference number H-2013-0404). Appendix A contains an author contribution statement for the manuscript. Appendix C contains ethics approval, recruitment materials, participant information statement, consent form, authority to release information, general practitioner information statement and request form, demographic information form and data collection materials used in the study. Appendix E contains intra-tester reliability information for outcomes measures used in the study. Appendix F contains correlation tables of the independent variables used in the regression analyses.

This research was presented in a poster presentation at the European Association for the Study of Diabetes Conference in Stockholm, Sweden in September 2015 for which an Australian Diabetes Society travel grant was awarded. The abstract was published in *Diabetologia* and is found in Appendix I.

Citation: Barwick, A., Tessier, J., Janse de Jonge, X., & Chuter, V. *Initial findings in the relationship* between diabetic peripheral neuropathy and microvascular reactivity in the foot. European Association for the Study of Diabetes Annual Meeting. Diabetologia, 2015. 58(S1): p. S502

This manuscript is currently under review at a peer reviewed journal.
Peripheral sensory neuropathy is associated with altered post-occlusive reactive hyperemia in the diabetic foot

RUNNING TITLE: Diabetic neuropathy and PORH

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# Abstract

Objective: This study examined whether the presence of peripheral sensory neuropathy or cardiac autonomic deficits are predictive of variance in post-occlusive reactive hyperemia (reflective of microvascular function) in the diabetic foot.

Research Design and Methods: Ninety-nine participants with type 2 diabetes were recruited into this cross-sectional study. Presence of peripheral sensory neuropathy was determined with standard clinical tests and cardiac autonomic function was assessed with heart rate variation tests. Post-occlusive reactive hyperemia was measured with laser Doppler in the hallux. Multiple hierarchical regression was performed to examine relationships between neuropathy and the peak perfusion following occlusion and the time to reach this peak.

Results: Peripheral sensory neuropathy predicted 22% of the variance in time to peak following occlusion (p<0.05), being associated with a slower time to peak but was not associated with the magnitude of the peak. Heart rate variation was not associated with the post-occlusive reactive hyperemia response.

Conclusion: These results show an association between the presence of peripheral sensory neuropathy in people with diabetes with altered microvascular function in the lower limb.

# Introduction

Microvascular dysfunction is common in diabetes and acts as a major contributor to cardiovascular disease [1] as well as lower limb complications [2]. Regulation of the microvasculature is complex and relies on endothelial function and myogenic input but also has neural influences [3]. Neuropathy in diabetes, whilst being a microvascular complication, may in turn affect microvascular functioning [3].

In the foot, autonomic neuropathy results in a loss of sympathetic activity in peripheral blood vessels causing vasodilation and increased arterial flow [4]. However, the increase in blood flow bypasses the cutaneous structures due to the opening of arteriovenous shunts, resulting in local ischemia [4]. Neuropathy has also been associated with reductions in microvascular reactivity (ability to vasodilate in response to stressors) that likely to contribute to the development of ulceration, impaired healing and difficulty fighting infection [5, 6].

Capacity for vasodilation can be assessed by measuring local skin blood flow whilst introducing stressors such as heat and iontophoresis of chemical substances. This approximates the capacity in that individual to mount blood flow and inflammatory responses to injury [5] and can also be indicative of early cardiovascular disease [7].

Whilst reduced vasodilation in response to heat and iontophoresis of acetylcholine (ACh) has been observed in the presence of diabetic neuropathy [8-10], the effect of neuropathy on post-occlusive reactive hyperemia (PORH) is less explored.

Post-occlusive reactive hyperemia is the increase in blood flow that occurs in response to a period of arterial occlusion. Impaired PORH has been associated with diabetes [11, 12] and with poor blood glucose control [13] and notably has been shown to precede late diabetes complications [7]. The underlying mechanisms for the response are not fully understood but it is thought that prostaglandins [14] and other metabolic and endothelial dilators [15], as well as sensory nerves [16] play a role. As a relatively simple, reliable and non-invasive measure, the PORH response represents the sum of both endothelial dependent and independent functions [15]. If nerves are involved in the response,

neuropathy may affect the PORH response. This study aimed to determine whether neuropathy in diabetes is associated with altered microvascular reactivity in the foot.

# **Research Design and Methods**

#### Participants and Procedure

A volunteer convenience sample was recruited from patients attending public and private podiatry clinics for general foot care in New South Wales, Australia as well as those responding to poster and newspaper editorial advertising. Recruitment took place between March 2014 and January 2015. All participants were adults with type 2 diabetes mellitus and were excluded if they were pregnant, took corticosteroids, or hormone replacement therapy, had osteoporosis, chronic renal failure, current bilateral foot ulceration, neuropathic osteoarthropathy, malignancy, endocrine disorders (other than diabetes), or a recent history of foot trauma. Ethics was obtained from the University of Newcastle Human Research Ethics Committee and written informed consent was obtained from all participants. Details of HbA1c, presence of retinopathy and other medical history, date of diabetes diagnosis and medication use were obtained from medical history supplied by the participant's general practitioner.

Participants refrained from nicotine, caffeine and exercise for two hours and lay supine for at least 10 min prior to testing whilst room temperature was controlled at 23-24°C. Tests were performed in the following order: monofilament detection, vibration perception, sharp/blunt detection and temperature detection followed by PORH and heart rate monitoring. All tests, as described below, were performed by a single podiatrist.

#### Sensory neuropathy assessment

Presence of large fibre sensory neuropathy was assessed with the four point monofilament test and vibration perception threshold (VPT) [17] and small fibre sensory neuropathy was assessed with sharp/blunt perception and temperature perception [18].

A Bailey Instruments (Chorlton, Manchester, United Kingdom) 5.07 monofilament was utilised for the four site monofilament test. The test was performed three times and an average of the three was taken. A score of three or less out of four sites correctly identified is indicative of large fibre sensory loss in that foot [17]. Vibration perception threshold was assessed with a Horwell neurothesiometer (Scientific Laboratory Supplies ltd., Nottingham, United Kingdom) placed on the dorsal hallux. The amplitude of the instrument was gradually increased until the participant indicated they could feel vibration. This voltage was recorded as the VPT. The mean of three readings was taken. A value of over 25V was considered abnormal [17]. Where a participant failed both tests, they were classified as having sensory neuropathy.

A Neurotip (Owen Mumford, Oxford, United Kingdom) installed in a calibrated Neuropen (Owen Mumford) was utilised to assess sharp/blunt perception. After demonstration of the instrument on the patient's hand, the sharp or blunt end of the instrument was placed randomly on the plantar surface of the hallux three times and the participant was asked to identify which end they perceived. This was performed three times with an average of the three being taken. A score of one or less out of three was considered abnormal. Temperature perception with a Tiptherm device (AXON Gmbh Dusseldorf, Germany). The cold or warm end of the instrument was places randomly on the dorsum of the foot and the participant was asked to identify which end they perceived. A score of one or less out of three was considered abnormal. Where participants failed both these tests, they were also classified as having sensory neuropathy.

#### Post-occlusive reactive hyperemia

Post-occlusive reactive hyperemia was measured as described in Barwick et al. [19]. Briefly, measurements were made with a moorVMS-LDF2 laser Doppler module and a VHP2 digit skin heater probe and needle probe (Moor Instruments Ltd, Axminster, United Kingdom). The laser probe was fixed to the plantar surface of the participant's hallux and heated to a thermoneutral 33°C. Where access to the right hallux was precluded by injury or amputation, the left hallux was utilised. A 2.5cm pneumatic cuff (Moor Instruments Ltd) was placed proximal to the probe. The following automated settings were utilised with the moorVMS-PRES pressure module (Moor Instruments Ltd): three minutes of baseline flux recording, inflation of the cuff to 220mmHg for three minutes, cuff deflation at maximum speed, and post-occlusive flux recording for a further four minutes. All data were processed with moorVMS recording and analysis software Version 3.1 (Moor Instruments Ltd). The variables of peak expressed as a percentage of baseline (P%BL) and time to peak (TtP) were chosen due to their representation of the magnitude and temporal representation of the response and their established reliability [19].

#### Cardiac autonomic function assessment

A Polar RS800cx heart monitor (Polar Electro Oy, Kempele, Finland) was utilised to assess heart rate variability (HRV) as a measure of cardiac autonomic function. Participants completed a supine five minute rest recording. The R-R interval tachogram was analysed with Kubios heart rate variability software (2.1, Kuopio, 2012) with ectopic beats removed using linear interpolation of previous and subsequent beats. Both time and frequency domain parameters were assessed. Time domain measured included the standard deviation of the N-N interval (SDNN) and the root mean square of the R-R intervals (RMS-SD). Frequency domain measures were divided by spectral power analysis into high (0.15-0.40 Hz), low (0.04-0.15Hz) and very low frequency (0.00-0.04 Hz) powers with total power calculated as the sum of all powers [20]. Variables were left continuous due to a lack of cut-off values established to indicate pathology.

#### Statistical Analysis

Statistical analysis was performed in Statistical Packages for the Social Sciences Version 22 for Windows (SPSS Inc, Chicago, USA). Reliability of sensory neuropathy diagnosis was assessed with duplicate tests on a subset of 31 participants seven to 14 days apart. Kappa statistics were calculated and interpreted as per Landis and Koch:  $\geq 0.75 =$  excellent agreement, 0.4-0.75 = fair to good agreement and <0.40 = poor agreement [21]. Reliability of continuous measures (HRV) was assessed with duplicate tests on a subset of 29 participants seven to 14 days apart. Intra-class correlation coefficients were calculated and interpreted in accordance with Portney and Watkins: > 0.75 = good, 0.50 to 0.75 = moderate, < 0.50 = poor [22].

Several hierarchical regression models were performed to determine how much the presence of sensory neuropathy and HRV predicted the variance in PORH variables (P%BL and TtP). One model for each of the four neurological variables (presence of sensory neuropathy, RMS-SD, SDNN and total power) was assessed. In each of the models, demographic variables identified as confounds (diabetes duration, gender and age) were entered in level one and the neurological variable as the

independent predictor variable in level two. Prior to the regression models being conducted, the assumptions of adequate sample size, considering the five variables in the analysis [23], singularity (through assessment of correlations between independent variables), normality, linearity and homoscedasticity (through examination of residual and scatter plots) were checked [24]. Non-normally distributed data were log transformed.

### Results

Ninety-nine participants were recruited – participant characteristics are found in Table 1. Heart rate variability data for three participants was unavailable leaving 96 participants for analysis of this data. Time to peak and P%BL were log transformed due to their non-normal distribution.

The intra-tester reliability of the diagnosis of sensory neuropathy was excellent (large fibre tests: left foot 1.00; right foot 0.93, small fibre tests: left foot 0.82; right foot 0.92). The reliability of HRV was moderate for the time domains (SDNN 0.70; RMS-SD 0.66) and good for the frequency domain (total power 0.94).

Results of the hierarchical regression are found in Tables 2 and 3. Presence of sensory neuropathy predicted 22% of the variance in TtP (p = 0.03) with presence of neuropathy indicating a longer latency to peak flux following release of occlusion. None of the HRV variables were predictive of the response.

### Conclusions

The relationship between diabetic neuropathy and microvascular reactivity is poorly understood. Such information is useful in the early diagnosis and management of diabetic foot complications. This study aimed to investigate relationships between clinically detectable peripheral sensory neuropathy, cardiac autonomic deficits and the PORH response in the periphery. Presence of sensory neuropathy predicted 22% of the variance in the timing of the response but did not predict its magnitude. Heart rate variation did not predict temporal or magnitudinal aspects of the response.

These findings are in keeping with previous studies that have shown other microvascular reactivity parameters to be affected by the presence of neuropathy. The role of nerves in the blood flow

response to heating and ACh iontophoresis and the effect of neuropathy on those responses is more established than in the PORH response [15, 25]. Numerous studies have demonstrated that in the presence of diabetic neuropathy there is a reduction in blood flow response to heating [9, 10] and iontophoresis of ACh [8-10].

Post-occlusive reactive hyperemia is thought to be mediated by a mix of metabolic dilators, endothelial dilators, myogenic relaxation and sensory nerve activity [16]. The work of Larkin and Williams [26] and Lorenzo and Minson [16] demonstrated the role of sensory nerves in PORH by showing a reduction in the response following anaesthetisation. This suggests that the presence of neuropathy should also reduce the response. In support of this, Yamomoto et al. [7] showed that a reduction in magnitude of the response is associated with slower sensory nerve conduction speed.

The diagnosis of sensory neuropathy in the current study was based on unsophisticated clinical testing which may explain the small size of the relationship found with time to peak perfusion and the fact that there was no association with the peak itself. These measures were chosen to investigate whether identification of neuropathy with non-invasive clinical tests can give information on the microvascular status of individuals. The observed relationship on the timing of the response and not the magnitude of the response is in contrast to previous findings [7]. The PORH response is characterised by a sharp initial peak followed by a delayed prolonged hyperemia. A previous study showed that at first a loss of neural responsiveness may be compensated by an increase in myogenic activity which may have resulted in maintenance of the peak [27]. A large proportion of the participants in this study had clinically detectable neuropathy suggesting a more advanced state of the condition. It is unknown if increases in myogenic activity remain in the presence of advanced neuropathy, and therefore such a relationship cannot be assumed in this instance. Furthermore, the lack of association with peak may be due to the inability of clinical testing methods for neuropathy to detect early stages of the pathology. Furthermore, the physiological significance of a delayed response and whether it is indicative of pathology is unknown. This warrants additional research including with nerve conduction studies.

Other parameters of the PORH response such as curve morphology [28] or an index of the area under the curve post-occlusion to pre-occlusion [7] may be more useful indicators of disease states. Another

65

limitation of this work is that those with peripheral arterial disease were not excluded and presence of macrovascular disease may affect microvascular and PORH function. However, resting toe pressures were less than 50mmHg in only three of the 99 participants, so severe peripheral arterial disease is not likely to have influenced the results. Similarly, evidence of previous microvascular disease was obtained from medical history and strict classification was not applied to diagnosis. Given the high proportion of neuropathy in this study, it is likely that the incidence of other microvascular diseases was higher than reported in this study. In addition to these concerns, other factors to consider in future research include the influence of edema and factors affecting blood viscosity on the PORH response.

A major consideration in this work is that it is unclear to what extent the PORH response is reflective of the microvascular disease that causes neuropathy. Although both diabetes-induced alterations in vascular and metabolic pathways are implicated in the pathogenesis of neuropathy, the disease is considered a microvascular complication of diabetes due to the predominance of the ischemic pathway [3]. Endoneural blood vessels display cell hyperplasia, capillary basement membrane thickening [29] causing hypoperfusion and ischemia to the nerves, predominating in the lower limbs [30]. These changes also occur to the cutaneous microvasculature [30]. Nevertheless, as the current study was cross-sectional in nature it cannot determine whether the neuropathy caused the observed changes to PORH or whether microvascular disease caused both the PORH changes and neuropathy.

The literature, however, is suggestive of a contribution of nerve function to reduced microvascular reactivity, independent of microvascular disease. Arora et al. found that the reductions in the response to heating and iontophoresis of ACh seen in those with neuropathy were not concurrent with changes to sodium nitroprusside (which does not stimulate nerve fibres) [8]. This was confirmed by Caselli et al. [31]. Furthermore, the temporal relationships between microvascular disease and diabetic neuropathy have recently been under dispute [32]. There is likely to be a cycle present whereby microvascular disease contributes to neuropathy which contributes to further microvascular dysfunction [3].

Microvascular and neural complications of diabetes are major contributors to lower limb pathology in diabetes. The impact of diabetic neuropathy on microvascular function is complex and underresearched. This study aimed to investigate whether clinically detectable peripheral sensory neuropathy or cardiac autonomic neuropathy was indicative of a reduction in the capability for vasodilation that may be relevant in cases of ulceration and non-healing. The study found that presence of peripheral sensory neuropathy in diabetes was associated with slower time to peak dilatory response to ischemia. Future research should investigate whether this change in the PORH response is relevant for pathology as well as the causal link between neuropathy and microvascular dysfunction.

#### Author contributions

A. B. contributed to the design of the study, acquisition of data, analysis of data and writing of the manuscript. J. T. contributed to the design of the study and writing of the manuscript. X. J. D .J contributed to the design of the study and writing of the manuscript. J. I. contributed to the acquisition of data and writing of the manuscript V. C. oversaw the project and contributed to the design of the study, analysis of data and writing of the manuscript. All authors agree on the final manuscript.

#### Acknowledgements

We acknowledge the statistical support of the Hunter Medical Research Institute. A subset of the data presented in this manuscript was published in an abstract in Supplement of the 58th volume of Diabetologia. This study was supported by departmental funding of the Faculty of Health and Medicine from the University of Newcastle (Australia). The authors have no conflicts of interest to declare.

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Table 1: participant characteristics $n = 99$	
Age (mean, SD)	68.6 (9.56)
Gender (Male/Female)	64/35
Toe pressure (mean, SD)	99.39 (31.45)
$HbA_{1c}$ (%) (mean, SD)	7.26 (1.47)
HcA <sub>1c</sub> (mmol/mol) (mean, SD)	55.8 (16.15)
BMI (mean, SD)	34 (7.42)
Retinopathy (present/absent)	7/92
Sensory neuropathy (present/absent)	41/58
Diabetes Duration	11.72 (9.73)

Table 1: participant characteristics n = 99

Table 1: regression analyses of sensory neuropathy with post-occlusive reactive hyperemia

	R <sup>2</sup> Change	β	р
TtP			
Step 1*	0.18		< 0.01
Step 2*	0.22		0.03
Duration		-0.05	0.60
Age		0.05	0.58
Gender*		-0.44	0.00
Sensory neuropathy*		0.21	0.03
P%BL			
Step 1	0.03		0.12
Step 2	0.02		0.68
Duration		-0.16	0.12
Age		-0.03	0.76
Gender		0.21	2.06
Sensory neuropathy		0.04	0.41

P%BL, peak as a percentage of baseline; TtP, time to peak \*significant at p < 0.05

¥	R <sup>2</sup> Change	β	р
TtP			
Step 1*	0.18		< 0.01
Step 2	0.02		0.15
Duration		-0.05	0.60
Age *		-0.41	< 0.01
Gender		0.002	0.98
RMS-SD		-0.14	0.15
P%BL			
Step 1	0.06		0.12
Step 2	0.006		0.45
Duration		-0.16	0.14
Age*		0.22	0.04
Gender		-0.03	0.75
RMS-SD		0.08	0.45
TtP			
Step 1*	0.18		< 0.01
Step 2	0.02		0.13
Duration		-0.06	0.56
Age *		-0.41	< 0.01
Gender		0.01	0.90
SDNN		-0.15	0.13
P%BL			
Step 1	0.06		0.12
Step 2	0.005		0.48
Duration		-0.15	0.15
Age*		0.22	0.04
Gender		-0.04	0.70
SDNN		0.07	0.48
TtP			
Step 1*	0.18		< 0.01
Step 2	0.001		0.76
Duration		-0.04	0.67
Age*		-0.41	< 0.01
Gender		0.01	0.89
Total Power		-0.03	0.76
P%BL			
Step 1	0.06		0.12
Step 2	0.001		0.83
Duration		-0.16	0.13
Age*		0.22	0.04
Gender		-0.04	0.70
Total Power		0.02	0.83

Table 3: regression analyses of autonomic neuropathy variables with postocclusive reactive hyperemia

P%BL, peak as a percentage of baseline; RMS-SD, root mean square of the R-R interval; SDNN, standard deviation of the N-N interval; TtP, time to peak

\*significant at p < 0.05

# Foot bone density in diabetes may be unaffected by the presence of neuropathy

The research in this chapter relates to the following objective

 Explore relationships among diabetic neuropathy, microvascular reactivity and foot bone density in those with diabetes.

It tests the hypothesis that neuropathy induced vascular changes in those with diabetes, contribute to a reduction in bone mineral density in the feet.

# Preface

The neurovascular theory of Charcot joint development describes a neuropathy induced osteopaenia that leaves individuals prone to development of the condition. As demonstrated by the literature review in Chapter Three empirical data for a reduction in bone density in those with diabetic neuropathy is currently lacking. The objective of this study was to address this by comparing bone density between those with diabetes only and those with diabetes and neuropathy whilst controlling for potentially confounding factors. A subset of the participants in Chapter five were used to form the majority of the participants (96%) in this case control study. The study found that contrary to commonly cited theory, the presence of diabetic neuropathy is not associated with reductions in bone strength. The study was the first to investigate the relationship in foot bones other than the calcaneus and represents a valuable contribution to the literature in its null finding.

This study was approved by the University of Newcastle Human Research Ethics Committee (reference number H-2013-0404). Appendix A contains an author contribution statement for the manuscript. Appendix C contains ethics approval, recruitment materials, participant information statement, consent form, authority to release information, general practitioner information statement and request form, demographic information form, data collection materials used in the study. Appendix E contains intra-tester reliability information for outcomes measures used in the study. Appendix G contains a STROBE statement for the manuscript.

A version of this manuscript is in press in the Journal Diabetes and Its Complications (Appendix H), which has an impact factor of 3.005.

Citation: Barwick, A. L., Tessier, J. W., Janse de Jonge, X., & Chuter, V. H. (2016). *Foot bone density in diabetes may be unaffected by the presence of neuropathy*. J Diabet Complications. In Press.

Foot bone density in diabetes may be unaffected by the presence of neuropathy

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HRV, heart rate variation; HU Hounsfield units; ICC, intra-class correlation coefficient; LFN, large fibre neuropathy; MET, metabolic equivalent minutes/week; PORH, post-occlusive reactive hyperaemia; P%BL, peak as a percentage of baseline; RMS-SD, root mean square of the R-R intervals; SDNN, standard deviation of the N-N interval: SFN, small fibre neuropathy; tPeak, time to peak

### Abstract

Neuropathies are common complications of diabetes and are proposed to influence peripheral bone principally via an altered vascular supply. This study aimed to determine the relationship between neuropathy and foot bone density in people with diabetes. The secondary aim was to investigate the effect of neuropathy subtypes and microvascular function on foot bone density. A case-control observational design was utilised with two groups: those with diabetic peripheral large fibre neuropathy (n=23) and a control group with diabetes but without neuropathy (n=23). Bone density in 12 foot bones was determined with computed tomography scanning. Neuropathy was assessed with standard clinical assessment. Additionally, post-occlusive reactive hyperaemia, presence of small fibre neuropathy and heart rate variability was determined. T-tests were used to determine differences in bone density between groups with and without neuropathy and hierarchical regression was used to examine the influence of small fibre neuropathy, heart rate variability and reactive hyperaemia on bone density. No difference in foot bone density was found between those with and those without large fibre neuropathy. Furthermore, no association between heart rate variability or reactive hyperaemia and bone density was found. Small fibre neuropathy was associated with increased cuboid trabecular bone density (p=0.006) with its presence predictive of 14% of the variance. This study found no clear association between presence of diabetic neuropathies and foot bone density. Furthermore, vascular reactivity appears to have no impact on bone density. This is despite the common assertion that diabetic neuropathy changes the blood supply to bone, reducing its density and predisposing it to fracture and Charcot foot.

#### Keywords

Bone

Diabetes

Neuropathy

Microvascular disease

#### Cardiac autonomic neuropathy

### Introduction

Neuropathy is a common complication of diabetes [1] that can affect both the somatic and autonomic nervous systems [2]. It can manifest as a loss of sensation, paraesthesia, muscle atrophy, cardiac deregulation and poor skin blood flow [2]. Such changes cause significant morbidity in the lower limb in the form of ulceration, infection and amputation [3] and are implicated in changes to bone seen in neuropathic osteoarthropathy of the foot (Charcot foot) [4].

The nervous system is involved in the maintenance of bone strength in a number of ways. Bone itself contains sensory and autonomic nerve fibres in cortical and trabecular bone including in the periosteum, bone marrow and mineralised bone [5]. Moreover, bone cells contain receptors for neuropeptides suggesting that there are direct neural influences on bone activity [5]. The autonomic nervous system plays a role in the vascular supply of bone [4] as well as regulation of metabolic pathways that impact osteoclast and osteoblast activity [6]. Despite this knowledge, the exact role of the nervous system in bone maintenance remains largely undefined and the potential impact of neuropathy on bone health is unclear.

Nevertheless, neuropathy induced bone demineralisation has long been thought to predispose to the development of Charcot foot. A longstanding theory asserts that a loss of sympathetic vascular tone occurring with neuropathy leads to increased bone blood flow that upturns osteoclast activity [7]. The resulting bone resorption predisposes the foot to neuropathic osteoarthropathy [8].

Previous research investigating the effects of neuropathy on peripheral bone density has demonstrated inconsistent results [9, 10]. Meta-analysis of available data demonstrated that, in the calcaneus of those with diabetes, there is a trend towards poorer bone health in those with neuropathy, however this failed to reach statistical significance [11]. The research to date is limited by a lack of available techniques to image foot bones and has so far mainly focused on the calcaneus. It has been suggested, however, that the other bones of the foot are more likely to be involved in Charcot foot [12] and imaging of these bones may lead to a better understanding of diabetic neuropathy.

Furthermore, the available data largely relate to the effects of generalised neuropathy on bone without examining the individual contributions of large and small fibre dysfunction and the direct effect of

changes to vascular supply on foot bone density. It has been demonstrated that there is dysregulation in blood flow responses of the lower limb in people with diabetic neuropathy [7, 13], which may in turn affect bone.

The aim of this study was to determine the relationship between subtypes of diabetic neuropathy and measures of microvascular reactivity on foot bone density in people with diabetes. The goal is to provide clarify whether neuropathy affects bone in a manner that may predispose to bone pathology such as Charcot foot. The central hypothesis is that those with diabetic neuropathy will have poorer foot bones strength than those without neuropathy. Such insight will aid in pinpointing possible clinically identifiable risk factors that may assist in the prevention, early diagnosis and treatment of the disease.

### Materials and Methods

#### Participants

A cross-sectional case-control design was utilised. A convenience volunteer sample was recruited from patients with diabetes (type 1 or 2) from podiatry clinics and newspaper advertising in the Hunter region of New South Wales, Australia, as a subset of a larger study. Recruitment took place from March 2014 to January 2015. Exclusion criteria included: pregnancy; long term use of corticosteroids, hormone replacement therapy, or bisphosphonates; osteoporosis (excluded with dual energy x-ray absorptiometry screening using the WHO criteria of a bone density more than two standard deviations below the young adult mean) [14]; chronic renal failure; current foot ulceration or neuropathic osteoarthropathy of both feet; malignancy; neuropathy not caused by diabetes; recent history of foot trauma; endocrine disorders such as thyroid disease; and participation in other research within the previous 12 months involving ionising radiation. Ethics was obtained from the University of Newcastle Human Research Ethics Committee and written informed consent was obtained from all participants prior to participation. Diagnosis of diabetes was taken from self-report and a medical history obtained from the participants' general practitioner. Most recent HbA1c was obtained from patient records. Physical activity level was measured with the International Physical Activity Questionnaire long form and is presented in metabolic equivalent (MET) minutes/week [15]. Participants were recruited concurrently and grouped into those with large fibre sensory neuropathy and those without. Groups were matched for age (within three years), body mass index (within three points), type of diabetes, gender and duration of diabetes (within five years). Equal numbers of cases and controls were recruited.

#### Equipment and Measurement

#### Computed Tomography

An Aquilion One 320 slice (Toshiba Medical Systems, Japan) computed tomography scanner was used for all examinations. One radiographer performed all aspects of the examinations, including participant positioning, scanning and acquisition of measurements. A pre-planned program was utilized for each examination. No adjustments to the pre-planned program were made for any participant. Volume acquisition was utilised with the following settings applied: CTDIvol 7.2mGy; dose-length product 115.9 (mGy x cm); 120kV; 150mA; rotation time 0.5s; range 16cm; display field of view medium or large (depending on foot size).

The right foot of all participants was scanned, except where prohibited by injury or amputation in which case the left foot was scanned. Each participant was placed in a recumbent position on the table, offset to the side contralateral to the scanned limb in order to allow a more midline position for the lower extremity to be scanned. The knee was flexed to prevent scanning of the contralateral foot. The degree of angulation of this leg was determined by the comfort of the patient to assist in maintaining the desired position throughout examination and thereby preventing any movement artefact.

The foot that was to be scanned was placed against a wooden box with the ankle in a neutral position as close to 90° to the table surface as possible. The foot was scanned using the pre-planned program and resultant images were assessed by the radiographer for any movement and to ensure that all anatomical areas were covered. Once the radiographer ratified the imaging data the participant was removed from the table.

All seven tarsals and the five meta-tarsals were assessed in the axial plane of reconstruction. Axial images were viewed using a bone algorithm so that clear differentiation was possible between

trabecular and cortical bone. All images were viewed with a window level of 350 and a window width of 2700. Images were reconstructed at 0.5mm thickness at intervals of 0.25mm.

Three random slices were obtained from the body of each of the 12 bones. The radiographer selected appropriate regions from the slices of each participant and Hounsfield units (HU) measurements were obtained. The largest region of interest possible was traced in the trabecular bone and three regions of interest were taken from the cortical bone from each slice image yielding a total of three trabecular readings and nine cortical readings for each bone. Values were averaged for the trabecular and cortical bone.

#### Neuropathy Assessment

Presence of large fibre neuropathy (LFN) was assessed using the guidelines devised by Boulton et al. [16], which recommend the 10g monofilament test and one other of five neurological exams. In this case, the second test used was vibration perception threshold as assessed with a neurothesiometer. A four site monofilament test using a Bailey Instruments (Chorlton, Manchester, UK) monofilament calibrated to buckle at 10g was performed. A score of three or less out of four is indicative of large fibre sensory loss in that foot. This test was performed three times and an average of the three was taken. Vibration perception threshold was assessed with a Horwell neurothesiometer (Scientific Laboratory Supplies, Nottingham, UK). A value of over 25V was considered abnormal [16]. The three readings collected were averaged. Abnormal readings on both tests was considered LFN as defined for this study as criteria for entry into the diabetic neuropathy group.

Presence of small fibre neuropathy (SFN) was determined in accordance with the methods used in Papanas et al. [17] which measures temperature perception with a Tiptherm device (AXON Gmbh Dusseldorf, Germany) and pain sensation with a Neurotip (Owen Mumford, Oxford, UK) installed in a calibrated Neuropen (Owen Mumford, Oxford, UK). Abnormal readings on both tests is considered SFN.

A Polar RS800cx heart monitor (Polar Electro Oy, Kempele, Finland) was utilised to assess heart rate variability (HRV) as a measure of cardiac autonomic function. Participants completed a supine five minute rest recording. The R-R interval tachogram was analysed with Kubios heart rate variability software (version 2.1, Kuopio, 2012) with ectopic beats removed using linear interpolation of

previous and subsequent beats. Both time and frequency domain parameters were assessed. Time domain measured included the standard deviation of the N-N interval (SDNN) and the root mean square of the R-R intervals (RMS-SD). Frequency domain measures were divided by spectral power analysis into high (0.15-0.40 Hz), low (0.04-0.15Hz) and very low frequency (0.00-0.04 Hz) powers with total power calculated as the sum of all powers [18].

#### Reactive Hyperaemia Assessment

Post-occlusive reactive hyperaemia (PORH) was assessed using the protocol of Barwick et al. [19]. Briefly, a MoorVMS-LDF2 Laser Doppler (Moor Instruments Ltd, Axminster, UK) was used to measure blood flux at the plantar hallux prior to, during, and post a three minute occlusion of the hallux with a pneumatic cuff. Peak flux post-occlusion expressed as a percentage of resting blood flux (P%BL) and the time to peak (tPeak) were chosen to represent the magnitude and temporal characteristics of the response.

#### Statistical analyses

A power calculation was not possible due to a lack of available data for mid foot bones examined in this study. Statistical analysis was performed in SPSS Version 22 for Windows (SPSS Inc, Chicago, USA). The reliability of outcome measurements was assessed with repeat testing on 10 participants for foot bone density, 31 for peripheral neuropathy assessments and 29 for HRV testing. Dichotomous variables (presence of SFN and LFN) were assessed with the Kappa statistic and interpreted according to Landis and Koch [20]:  $\geq 0.75 =$  excellent agreement, 0.4-0.75 = fair to good agreement and <0.40 = poor agreement. Continuous variables (foot bone density and HRV) were assessed with intra-class correlation coefficients (ICC) and interpreted according to Portney and Watkins [21]:  $\geq 0.75 =$  good, 0.50 to 0.75 = moderate, < 0.50 = poor. T-tests were run to determine significant differences between groups in age, BMI, duration of diabetes and HbA1c with significant level set at p<0.05. Activity level data were cleaned in accordance with recommendations [22] and was expressed as median and interquartile. Differences between groups was assessed with a Mann-Whitney U test.

Differences in bone density for each foot bone between groups was investigated with independent ttest with alpha level set at <0.01 for significance due to the number of tests run increasing the likelihood of error.

Hierarchical multiple regression analyses examined the extent to which other neurological factors and response to occlusion accounted for variance in observed bone density of the navicular cortical bone, navicular trabecular bone and second metatarsal trabecular bone. These bones were chosen due to their frequent involvement in Charcot foot [13]. Demographic variables (age, gender and BMI) were entered at step 1 and neurological and vascular factors at step 2. A significance value of <0.01 was chosen due to the relative small sample size. Assumptions of normality, singularity and homoscedasticity were checked prior to analysis. Non-normally distributed data were log transformed.

# Results

Forty-six participants were recruited to the study (23 cases and 23 controls). One hundred and four participants who were recruited as part of a larger study were screened for eligibility. Cases were selected first. After screening for neuropathy and exclusions in medical history (leaving 23 eligible participants), participants underwent screening for osteoporosis which did not lead to the exclusion of any participant. This resulted in a total of 23 cases. Twenty-three controls were selected from the remaining pool on the basis of absence of neuropathy and matching the case group for age, gender, BMI, diabetes type and duration. Participant characteristics are found in Table 1. All participants were Caucasian. Data cleaning of physical activity levels resulted in one participant in the non-neuropathic group being excluded from the analysis of comparison of physical activity. The left foot was used for six participants. There were no statistically differences between groups in physical activity, age, BMI, duration of diabetes or HbA1c.

Assessment of LFN (Kappa: left foot 1.00; right foot 0.93) and SFN (left foot 0.82; right foot 0.92) displayed excellent agreement. Assessment of HRV time domains were moderate (ICC: SDNN 0.70; RMS-SD 0.66) and the frequency domain (total power 0.94) were good. Assessment of bone density displayed moderate to good reliability (trabecular: talus 0.91, calcaneus 0.90, navicular 0.70, cuboid

0.68, medial cuneiform 0.83, intermediate cuneiform 0.88, lateral cuneiform 0.86, first metatarsal 0.90, second metatarsal 0.81, third metatarsal 0.82, fourth metatarsal 0.69, fifth metatarsal 0.85; cortical: talus 0.52, calcaneus 0.59, navicular 0.70, cuboid 0.69 first metatarsal 0.61).

The t-test revealed that there were no statistically significant differences in bone density between groups (Table 2). Hierarchical multiple regressions showed that neither PORH response (Table 3) nor HRV (Table 4) predicted variance in bone density after adjusting for age, gender and BMI. Small fibre neuropathy was associated with increased cuboid trabecular bone density (p=0.006) with its presence predictive 14% of the variance (Table 3).

### Discussion

This study aimed to examine differences in foot bone density in those with diabetes with and without neuropathy. No indication of the hypothesised reduced bone density in those with neuropathy was observed. Moreover, there was no clear relationship between foot bone density and clinical subtypes of diabetic neuropathy or PORH. The results of this study provide evidence that diabetic neuropathies do not alter peripheral bone density in a manner that predisposes to injury or neuropathic osteoarthropathy.

Previous studies examining the relationship between neuropathy and the development of Charcot foot are inconclusive [11]. Several studies have demonstrated a reduction in calcaneal bone density assessed by ultrasound in association with the presence of peripheral sensory neuropathy [23-25]. However, more recent studies have not reproduced this relationship [26-28] and meta-analysis of existing data does not support such an association [11].

The existing data is inconsistent in part due to the complex nature of the relationship between diabetic neuropathy and bone strength, which is complicated by a long list of potential confounds and effect modifiers including activity level, length of diabetes, control of diabetes, age and gender. The current study controlled for age, gender, BMI and duration of diabetes. Furthermore, there was no statistically significant difference in activity level between the groups. Existing data also concentrates on the calcaneus due to its accessibility with one previous study examining the cortical bone of the second metatarsal with plain radiographs and finding a reduced bone mass in those with diabetic neuropathy

[29]. Our investigation of all tarsals and metatarsals did not confirm this relationship. However, given that the participants in that study had a history of foot ulceration and likely subsequent offloading, may have contributed to the reduced BMD found in that cohort.

Previous research has mainly concentrated on large fibre sensory neuropathy. Reduced bone density is proposed to be caused more specifically by SFN induced dysregulation of blood flow to bone [30]. Even though small and large fibre neuropathy usually occur together, SFN can occur independently of LFN [1]. Therefore measuring small fibre deficits may have greater sensitivity in identifying changes to bone. To this end HRV (mediated by small autonomic fibres) representing cardiac autonomic neuropathy and small fibre sensory neuropathy were assessed in the current study. In contrast to two previous studies [23, 25], the current study did not find a relationship between measures of cardiac autonomic function and bone density. Furthermore, this study demonstrated that there was no clear link between bone density and small fibre sensory neuropathy are common in those with neuropathy [1, 31] and Charcot foot affects only a small proportion of these [12] there may be a more specific set of factors that predispose to Charcot foot involving blood flow.

There has been only limited investigation of the impact of clinical vascular measures on foot bone density. There is demonstrated increased blood flow to foot bones in the presence of diabetic neuropathy that is proposed to increased demineralisation [7] but it has not been linked to reduced bone density in vivo. In fact, the opposite has been demonstrated with a reduced blood flow to the extremities due to peripheral arterial disease being linked to low bone density in feet [32].

Microvascular flow rather than global blood flow may be a differentiating factor. Generally, the microvasculature in those with diabetic neuropathies has a reduced ability to dilate, however, this ability appears to be retained in those with Charcot foot [33-35]. Such a retention in the ability to vasodilate may cause uncontrolled blood flow to bone leading to its demineralisation. The current study examined PORH as a measure of microvascular vasodilatory capacity and did not find an association with foot bone density. However, it is possible that the increased vasodilatory response seen in Charcot foot may only be relevant in response to injury and not in a healthy state as the participants in the current study were.

The results of the present study need to be considered in the context of several study limitations. The characteristics of the volunteer convenience sample used, influence the applicability of the findings to the target population. The study cohort were all overweight, mostly male (91%) with the majority having type 2 diabetes (96%). Thus, the results should be interpreted in this context. The target population are those at risk of diabetic foot complications, especially Charcot foot, which does not show preference for gender or diabetes type [12]. The study population are likely to have a higher bone density than the broader target population due to male gender, type 2 diabetes and high body mass index (although the rate of overweight is reflective of the target population). This may have masked or attenuated the effect of neuropathy on foot bones.

Furthermore, given the large number of variables that affect BMD that would have confounded our results, the exclusion criteria for this study were considered essential. Given that a large number of variables are known to affect BMD, these exclusion criteria are likely to have ensured the results of this study accurately reflected the impact of neuropathy on foot bones density, however, it is acknowledged that the tight exclusion criteria limit the generalisability to the general population with diabetes.

Another potential reason for the lack of observed relationships in this study may be the small sample size that may have been insufficient to detect an existing difference. Furthermore, the method used for BMD measurement may have lacked adequate sensitivity to detect any difference in this small sample. Bone mineral density was not converted to mg of hydroxyapatite (mg.cm<sup>3</sup>), but rather the values were expressed as HU. Hounsfield units acquired from CT are quantitative units of the radiodensity of objects [36]. The relative simplicity (conversion requires phantoms that are not readily available in the range of bone densities encountered in the foot [37] and association with bone strength and fracture risk, make it a useful measure [38], however further development of this method may increase its sensitivity, accuracy and validity.

The sample size of 46 is potentially underpowered to detect small associations between neurovascular factors and bone density especially given the range of potential influencing factors. Neuropathy in this study was measured with clinical indicators and not with nerve conduction studies which may be more sensitive to the relationship. Activity level was also assessed in this study as it will have

85

important effects on bone density that may confound the expected difference due to neuropathy, thus needed to be assessed for consistency between groups. After comparing metabolic minutes per week between groups, there was no statistically significant difference between those with neuropathy and those without. However, the difference was notable with those with without neuropathy performing almost double that of those with neuropathy. This would trend towards a lower bone density in those with neuropathy, serving to enhance the expected bone density reduction in this group, which was not observed. Additionally, HRV and small fibre sensory neuropathy were used as surrogates for peripheral autonomic neuropathy. Finally, PORH may not be a good measure of a neurovascular response as there are multiple factors responsible for the response and it may not reflect blood flow at the level of the bone.

# Conclusions

Increased fragility to bone caused by particular neural [24] and vascular factors [34] is proposed to precede and predispose to Charcot foot. This study did not find clinical neuropathy patterns or vascular reactivity to affect bone density in those with diabetes. Future prospective research of risk factors for Charcot foot is needed to establish the pathogenesis of this disease process and allow for the early diagnosis and treatment of the condition. Furthermore, development if simple clinical tests to asses these risk factors may identify those most likely to develop foot bone pathology and will therefore aid in the prevention, early detection and treatment of Charcot foot.

#### Acknowledgements

This study was supported by a Faculty of Heath Departmental Funding from the University of Newcastle (Australia). The research has not previously been published in part or in full. Conflicts of interest: none. We acknowledge the statistical support of Hunter Medical Research Institute. Guarantor: Dr Vivienne Chuter.

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Table 1: participant characteristics

Characteristic (mean, SD)	Large fibre neuropathy status	
	Absent	Present
Age	68.04 (7.95)	70.22 (8.10)
Sex	19/4	20/3
Height	169.46 (7.99)	177.98 (8.48)
Weight	95.13 (17.60)	108.65 (28.19)
BMI	33.26 (6.66)	34.22 (8.08)
Diabetes type (1/2)	1/22	1/22
Diabetes duration	15.22 (12.03)	11.54 (9.79)
HbA1c (%)	7.0 (1.49)	7.1 (1.31)
HbA1c (mmol/mol)	53 (16.20)	54 (14.22)
Met Minutes/week (median, IQ range)	3415.5 (5227.25)	1716 (2186)

IQ, interquartile; SD, standard deviation

	Ś								
come (HU)	Large fibre	e neuropatl	hy status				99% Confidence Interval		
	Absent			Present			for mean difference		
	М	SD	N	Μ	SD	Z		t	df
rtical									
alus	3009.53	233.98	23	3040.06	422.68	23	-305.23, 244.17	-0.30	34.33
alcaneus	2856.66	314.41	23	2782.63	318.69	23	-177.29, 325.35	0.79	4
Javicular	2849.88	353.43	23	2796.94	238.82	23	-188.03, 293.91	0.60	38.63
Juboid	2766.85	289.48	23	2805.36	351.48	23	-294.22, 217.21	-0.41	44
irst metatarsal	2990.35	297.83	23	2930.57	325.24	23	-187.78, 307.35	0.65	4
lbecular									
alus	443.70	65.40	23	467.36	59.14	23	-73.15, 25.84	-1.29	44
alcaneus	217.85	58.74	23	240.00	41.99	23	-62.69, 18.38	-1.47	4
lavicular	377.31	63.33	23	387.46	50.17	23	-55.50, 35.21	-0.60	44
uboid	225.71	65.36	23	249.65	84.96	23	-84.12, 36.24	-1.07	4
fedial cuneiform	374.87	75.02	23	385.69	63.31	23	-65.92, 44.29	-0.53	4
nter. Cuneiform	495.37	101.96	23	529.40	62.14	23	-101.70, 33.64	-1.37	36.36
ateral cuneiform	387.49	74.73	23	410.85	71.73	23	-81.50, 34.79	-1.08	4
irst metatarsal	261.39	84.72	23	283.67	67.60	23	-83.09, 38.53	-0.99	4
econd metatarsal	289.21	88.96	23	338.92	62.80	23	-111.15, 11.74	-2.19	39.56
hird metatarsal	273.30	72.14	23	309.93	65.52	23	-91.25, 18.17	-1.80	4
ourth metatarsal	279.23	97.39	23	305.01	64.66	23	-91.85, 40.30	-1.06	38.24
ifth metatarsal	391.01	341.52	23	608.86	484.34	23	-552.25, 116.53	-1.76	39.54

HU: Hounsfield units

Table 3: Associations between PORI	I variables, presence	e of SFN and	d bone density of the navicular ar	id cuboid cortical bor	ne and the nav	vicular, cuboid and second met	atarsal trabecula	r bone.	
tPeak (log)		c	P%BL (log)		(	SFN		(	,
	Change in R <sup>2</sup>	β	Р	Change in R <sup>2</sup>	β	b	Change in R <sup>2</sup>	β	Ρ
Navicular cortical bone									
Step 1	0.28		<0.01*	0.28		<0.01*	0.28		$<0.01^{*}$
Step 2	0.02		0.27	0.003		0.67	0.04		0.14
BMI		-0.23	0.12		-0.24	0.10	1	0.24	0.09
Gender		-0.40	<0.01*		-0.40	<0.01*	ī	0.39	<0.01*
Age		-0.24	0.14		-0.30	0.06	ī	0.29	0.05
Variable		-0.16	0.27		-0.06	0.67	1	0.20	0.14
Cuboid cortical bone									
Step 1	0.20		0.03	0.20		0.03	0.20		0.03
Step 2	0.003		0.72	0.04		0.15	0.000		0.97
BMI		0.20	0.20		0.21	0.16	0	.19	0.22
Gender		-0.21	0.14		-0.18	0.19	T	0.22	0.13
Age		-0.24	0.17		-0.19	0.23	Г	0.26	0.09
Variable		0.37	0.72		-0.21	0.15	0	.005	0.97
Navicular Trabecular bone									
Step 1	0.04			0.04		0.63	0.04		0.63
Step 2	0.03			0.001		0.85	0.007		0.58
BMI		-0.06	0.72		-0.03	0.85	Г	0.04	0.83
Gender		0.17	0.26		0.19	0.24	0	.18	0.26
Age		-0.3	0.86		0.07	0.69	0	.05	0.78
Variable		-0.16	0.23		-0.03	0.85	0	60.	0.58
Cuboid trabecular bone									
Step 1	0.19		0.03	0.19		0.03	0.19		0.03
Step 2	0.01		0.44	0.01		0.61	0.14		<0.01*
BMI		0.000	0.99		-0.02	0.88	Т	0.02	0.87
Gender		0.41	$<0.01^{*}$		0.40	<0.01*	0	.39	$<0.01^{*}$
Age		0.18	0.28		0.10	0.54	C	.07	0.62
Variable		0.12	0.44		0.08	0.61	0	.38	<0.01*
Second metatarsal trabecular									
Step 1	0.02		0.84	0.02		0.84	0.02		0.84
Step 2	0.000		0.99	0.000		0.89	0.12		0.02
BMI		0.07	0.68		0.07	0.69	0	.06	0.70
Gender		0.03	0.83		0.03	0.85	C	.01	0.94
Age		-0.09	0.62		-0.10	0.56	1	0.15	0.37
Variable		0.003	0.99		0.02	0.89	0	.35	0.02
Deals: time to need D0% D1 . Deals as	parantage of here	line CENI.	moll fibre sources thre *Cionifico	$m_{t} = m_{t} = 0.01$					

tPeak: time to peak, P%BL: Peak as a percentage of baseline, SFN: small fibre neuropathy, \*Significant at p < 0.01
SDNN			RMS-SD	· · · ·		Total P	ower			
	Change in R <sup>2</sup>	ß	D	Change in R <sup>2</sup>	ß	P	Char	nge in R <sup>2</sup>	β	Р
Navicular cortical bone	þ			þ				2		
Step 1	0.28		$<0.01^{*}$	0.28		<0.01*	0.28	2		$<0.01^{*}$
Step 2	0.01		0.50	0.01		0.52	0.00	4		0.64
BMI		-0.25	0.09		-0.254	0.08			-0.237	0.11
Gender		-0.47	<0.01*		-0 424	<0.01*			-0.392	<0.01*
		0.24	0.03		-0.336	0.03			7000-	0.05
Variahle		- 60 0	0.50		0.080 0	0.50			0.066	0.64
Cuboid cortical bone		0.0	00.0		100.0	70.0			0000	500
CUDULA CULICAL DULLE Stan 1	0 106		0.03	0.106		0.03	010	y		0.03
Step 1 Step 2	0.000		0.0 20	0.000		00.0	0.13	2 r		CU.U
DMI DMI	100.0	0100	0.0	0.000	0.10	<i>cc</i> 0	cn.n	10	0.710	0.15
Condor		0100	0.15		0.15 0.15	0.17			0.160	CT-0
Celluci		017.0-	CT.0		0.000	0.14			-01.07	0.24
Age		-0.257	0.10		-0.263	0.10			-0.206	0.19
		-0.027	0.86		0.002	0.99			0.206	0.17
Navicular Trabecular bone										
Step 1	0.040		0.63	0.04		0.63	0.04			0.63
Step 2	0.050		0.14	0.025		0.30	0.00	13		0.70
BMI		-0.045	0.78		-0.047	0.78			-0.042	0.80
Gender		0.142	0.35		0.147	0.35			-0.166	0.30
Age		0.012	0.94		0.022	0.90			0.043	0.80
Variable		0.233	0.14		0.165	0.30			-0.062	0.70
Cuboid trabecular bone										
Step 1	0.191		0.03	0.191		0.03	0.19	11		0.03
Step 2	0.007		96.0	0.004		0.66	0.00	10		0.83
BMI		-0.021	0.89		-0.011	0.94			-0.012	0.94
Gender		0.395	<0.01*		0.422	<0.01*			0.416	<0.01*
Age		0.107	0.49		0.14	0.37			0.134	0.40
Variable		0.085	0.56		-0.066	0.66			0.032	0.83
Second metatarsal trabecular										
Step 1	0.02		0.84	0.02		0.84	0.02	,		0.84
Step 2	0.003		0.71	0.000		0.99	00.0	90		0.63
BMI		0.066	0.70		0.069	0.68			0.058	0.73
Gender		0.023	0.88		0.033	0.84			0.016	0.92
Age		-0.108	0.53		-0.096	0.58			-0.117	0.50
Variable		0.06	171		0.002	66 U			-0.079	0.63

### Chapter Seven

### Conclusions

This thesis set out to investigate relationships among diabetic neuropathy, altered vascular states and poor foot bone health. Individually these pathologies are known to contribute to the common foot complications of diabetes and represent a large burden to individuals and the health care system [170]. The significance and extent of relationships between these complications are somewhat unknown. Diabetic neuropathies, primarily autonomic sympathetic deficits, are thought to alter bone blood flow in the feet causing the bone to become fragile and prone to pathology. This underlies the basis of the neurovascular theory of the development of Charcot foot [79]. The theory continues to be claimed and contested [112], but data on this topic are sparse.

The objectives of the thesis were: to review the existing literature related to differences in foot bone density in those with and without diabetic neuropathy; to develop a reliable measure of microvascular reactivity for clinical and research purposes; to develop a feasible and reliable method of foot bone density measurement; to explore relationships between clinical subtypes of diabetic neuropathy and vascular characteristics in the diabetic foot and; to explore relationships among diabetic neuropathy, microvascular reactivity and foot bone density in those with diabetes. The central hypotheses of the thesis were that those with diabetic neuropathy have altered vascular reactivity in the feet and that neuropathy induced vascular changes in those with diabetes, contribute to a reduction in bone mineral density in the feet. With respect to the aims and objectives of this thesis, this chapter discusses the strength and limitations of the research, implications of the findings and directions for future research.

The first objective of the thesis was to review the existing literature related to differences in foot bone density in those with and without diabetic neuropathy. To achieve this, a systematic review and meta-

analysis was undertaken (see Chapter Three). Databases were searched for studies that investigated foot bone health and measures of neuropathy in diabetic populations. Assessments of study quality and publication bias were undertaken as well as a meta-analysis of the included studies.

Of the 10 studies that met the inclusion criteria of the review, four found a statistically significant relationship between the presence of neuropathy and poorer bone health. Meta-analysis was performed on seven studies representing a total of 364 participants. A non-significant trend towards poorer bone health in those with diabetic neuropathy was found. The included studies were limited methodologically with incomplete reporting of methods and failure to account for potential confounds the primary concerns. The review also highlighted a lack of research into foot bones other than the calcaneus due to methodological restrictions in measuring BMD in foot bones.

The strengths of this review include the use of an extensive search of multiple databases without language restrictions using broad search terms along with a hand search of reference lists, thus representing all current research at the time of searching. Authors were contacted in cases where there was missing information. Standardised statistical methods including a random effects model were used in pooling data to account for differences among the studies in statistical and methodological approach. Furthermore, publication bias and heterogeneity were statistically assessed. Independent authors assessed articles for inclusion into the study and the quality assessment of included studies was undertaken with a standardised tool. The review is presented as per PRISMA guidelines (preferred reporting items for systematic reviews and meta-analyses) and published in a peer reviewed journal.

Limitations of this review are that a minimum quality standard for studies to be included in the review was not used. Unfortunately, data from three of the10 studies could not be pooled in the meta-analysis due to inadequate reporting. There was insufficient data to pool into diabetes types to assess the potential for differences between type 1 and 2 diabetes, as is indicated in the literature. Furthermore, there was insufficient data to assess different types of neuropathy, such as autonomic neuropathy, which may be more likely to affect bone density. The meta-analysis instead is a reflection of large fibre neuropathy. Additionally, study methodologies were varied resulting to a large amount of heterogeneity (71%) in the meta-analysis and results of publication bias assessment were inconsistent showing potential for this bias to be present.

Overall, the review and meta-analysis highlighted the complex nature of the relationship between diabetes and bone. It exposed the need for a focused investigation of this relationship that carefully accounts for confounding factors, assesses different subtypes of neuropathy that are most likely to affect bone based on current theoretical assertions and to investigate foot bones other than the calcaneus.

The second objective of this thesis was to develop a reliable measure of microvascular reactivity for clinical and research purposes. In order to achieve this, intra-tester and inter-tester reliability of four methods of assessing PORH were assessed. This is a non-invasive measure of the cutaneous blood flow reaction to a period of ischaemia that is indicative of microvascular dysfunction [171]. The main findings of this study were that the measurement of PORH using laser Doppler is most reliable when using automated settings for the protocol and automatically calculated parameters opposed to manual methods. The reliability of measurement of PORH using laser Doppler improved with the use of local heating to a thermoneutral temperature. The parameters with the best intra- and inter-tester reliability are peak expressed as a percentage of baseline and the index of the area under the curve post-occlusion to pre-occlusion. Finally, measuring PORH as the index of pre-occlusive blood pressure to post-occlusive blood pressure at the hallux has poor reliability and should not be used clinically using this method.

The strengths of this study were that the measures were taken in a large sample from a pathological population, which has high clinical relevance. A variety of methods were examined allowing the assessment of the most reliable method and parameters and both intra and inter-tester reliability were assessed. Assessors were blinded to each others results and their own previous results as much as possible. Random error was minimised by using a standardised time of day for each participant, using a standardised protocol across testers and testing sessions, calibrating equipment prior to each session and allowing warm up of the equipment as per the manufacturer's instructions. Finally and limits of agreement (LOA) were utilised. Limits of agreement represent the test-retest differences for 95% of the population, therefore this is a better statistical approach for a clinically applied test such as this involving blood pressures [172]. The study was published in a peer reviewed journal.

Limitations of the study include that the physiological nature of the measures used in this study is limited by the fact that laser Doppler measures arbitrary perfusion units and not flow in ml/mm<sup>2</sup> relative to volume of tissue, that skin was heated to improve reliability. Furthermore, the cutaneous vascular conductance which takes into account variations in existing blood pressure was not used and time to resting flux which has been identified as a potentially useful measure was not used. The study was performed in a participant group at risk of peripheral arterial disease and such reliability may be different amongst other populations. Furthermore, because the PORH response is dependent on the location and length of occlusion, the results cannot be extrapolated to other techniques. Another consideration is the large proportion of participants with diabetes, meaning that medial wall calcinosis may be present, influencing the occlusion of arteries and potentially the PORH response. Finally, although several measures were identified as having acceptable ICC values, the LOA were wide indicating that 95% of cases will fall within a large range. This means that any change or difference in the parameter found in any investigation will need to be large to ensure that a true change has occurred.

This study establishes the reliability of PORH measurement at the hallux. It has provided methodology and parameters that can be used in research using PORH as an outcome. It assessed the reliability of a blood pressure index, a method that was clinically simple to perform and requires low cost equipment. Although this method was unfortunately not reliable. Further research should investigate whether altering the methodological procedure such as the timing of the second blood pressure measurement improves the reliability of this method. Furthermore, research into the predictive value of PORH as a measure of risk of ulceration and other diabetic foot complications such as Charcot foot and its usefulness as a tool to assess wound healing capacity is recommended.

The third objective of this thesis was to develop a feasible and reliable method of foot bone density measurement. In order to do this, the reliability of averaging regions of interest across slices of obtained three-dimensional computed tomography images yielding a measure of density in Hounsfield units (HU) of cortical and trabecular bone of each foot bone was assessed. The study found that the method could be used to reliably assess the trabecular component of all tarsals and metatarsals and the cortical component of select foot bones in a cost and time efficient manner.

One of the strengths of the study is that the method was assessed in living participants from a pathological population, which increases the generalisability of the findings. Random error was minimised with calibration of the equipment, standardising the procedure for all participants and by using a trained radiographer. Furthermore, the repeat scans were performed on the same day ensuring that there is no chance that the clinical measure actually changed. Statistical analysis was performed by a different researcher and included ICC and standard error of measurement (SEM). SEM estimates how repeated measures are distributed around the 'true' score [173]. The advantage of calculating the SEM allows for easier interpretation of the magnitude of the error as the estimate is in the same units as the original measurement, in this case, HU [173].

Foot bone densitometry is plagued by difficulties. This hampers research into pathology of foot bones that takes place in primarily peripherally dominant disease processes such as diabetic neuropathy. This study presents a novel, simple, cost-efficient, time-efficient and reliable method of assessing bone density of feet. The study presents enough detail for the method to be reproduced and as the method is novel, it represents a valuable contribution to the study of foot bone pathology.

The limitations to this study were that the whole bone could not be assessed, instead regions of interest from several slices were averaged together. Additionally, the regions of interest taken from the cortical bone were small. However, this was essential for the simplicity of the method. Similarly, the study obtained HU and did not convert this to density in mg.cm<sup>3</sup> with the use of a phantom, again to simplify the method. Assessment of the validity of the method was limited to comparison to a previous study, though the values we obtained were comparable to those in that study [174]. Another limitation is that a modest sample size of 10 was used due to ethical considerations. This may have been one reason why some obtained ICC values were negative. This indicates that the variability within the sample was greater than between testing sessions, which means there may not have been enough variability within the outcome measure to accurately assess reliability in this small sample. The radiographer assessing the scans was blinded only to a limited extent. The scans were taken on the same day after repositioning. They were assessed by a single radiographer at least a week apart with the assessor unable to review previous results but without de-identifying the scans.

The generalisability of the findings is limited by the fact that only intra-tester reliability was assessed in one context using one piece of equipment. Furthermore, the participant group were mostly male

(80%), older (mean age 73) and overweight (mean BMI 31) and all had diabetes, thus results reflect only this very specific population. Nevertheless, the study shows the potential for the method to be used in other settings once reliability has been assessed. Further research is needed to establish its reliability in other settings and between assessors and to establish the validity of the method with respect to three-dimensional analysis.

Together, the above research along with methodological reliability results provided in Appendix E, provide the rationale and reliability for the independent and dependent variable measurement used in the subsequent studies.

The fourth objective of the thesis was to explore relationships between clinical subtypes of diabetic neuropathy and vascular characteristics in the diabetic foot to test the hypothesis that those with diabetic neuropathy have altered vascular reactivity in the feet. To achieve this, a sample of 99 participants were recruited. Peripheral sensory neuropathy, including that of small and large fibres, was diagnosed and HRV was measured to indicate cardiac autonomic function. Microvascular function was assessed with PORH. Relationships between diabetic neuropathy variables and PORH were examined with hierarchical regression analyses. The main findings of this study were that the presence of peripheral sensory neuropathy was indicative of a slower time to peak perfusion following occlusion but did not predict the magnitude of the peak and HRV parameters were not predictive of the variance in the PORH response.

The strengths of the study include that clinically relevant, validated and reliable tests of neuropathy were used. Investigating clinical subtypes of neuropathy in this manner allowed for the investigation of different subtypes (sensory and autonomic) of neuropathy on blood flow. PORH was measured in the periphery, which is more likely to be affected by neuropathy and more relevant for diabetic foot disease than previous studies that measured PORH in the upper limb. Some confounds were accounted for by excluding conditions and medications know to affect BMD, whilst appropriate statistical analyses dealt with other potential confounders including age, gender and duration of diabetes.

Prior to conducting the hierarchical multiple regression models, the relevant assumptions of this analysis including adequate sample size considering the five variables in the analysis [175],

singularity of the independent variables contained with each model (see correlations table of independent variables in Appendix F), normality, linearity and homoscedasticity through examination of scatter plots were tested with non-normally distributed data being log transformed [176]. By using a hierarchical multiple regression, an investigation of relationships between post-occlusive reactive hyperaemia and multiple neuropathy variables was possible also estimating the relative contribution of neuropathies to the PORH response. This robust analysis and interpretation of the data whilst factoring in potential confounds was necessary in what is likely a complex interrelationship.

The limitations to the study were that a convenience sample was used, no nerve conduction studies were conducted and the assessor of PORH was not blinded to the neuropathy status of the participant (although the automatic nature of the measurement means potential for bias is limited). In terms of the parameters used, the area under the curve (index) identified as a reliable measure in Chapter Three was not utilized in the study. This was due to the need to capture both temporality (with time to peak) and magnitude (peak as a percentage of baseline) of the response whilst reducing the amount of variables in the analysis. Peak as a percentage of baseline and the index had comparable reliability in the study, however the peak was chosen as a measure of the magnitude of the response due it being used more widely in the literature allowing for greater interpretation. Finally, the cross-sectional study cannot determine the cause and effect of the relationship between neuropathy and PORH. Because the regression analysis demonstrates only an association between the variables it is not possible to determine whether the two were causally related or which direction this causality might function especially given the potentially bidirectionality of the relationship between neuropathy and microvascular dysfunction [62].

The implications of the study are that there is a potential for neuropathy assessment to identify a vascular reactivity deficiency. The research provides an initial indication that peripheral sensory neuropathy may have implications for microvascular reactivity that may be relevant in the response to injury. Further research should look further into this relationship and the clinical relevance of a delayed onset of PORH in terms of implications for ulceration, wound healing and Charcot foot.

The final objective of the thesis was to explore relationships among diabetic neuropathy, microvascular reactivity and foot bone density in those with diabetes in order to test the hypothesis that neuropathy induced vascular changes in those with diabetes, contribute to a reduction in bone mineral density in the feet. In order to do this a case control study was conducted.

Two groups of diabetic participants, one with LFN (n=23) and one without (n=23) underwent foot computed tomography scanning using the methodology in Chapter Four. The trabecular bone of all tarsals and metatarsals and the cortical bone of the talus, calcaneus, navicular, cuboid and first metatarsal were assessed for density (HU). Bone density for individual bones between the groups were compared using t-tests with no significant differences found. Secondly, in this group of 46 participants, SFN, HRV and PORH were measured. Hierarchical regression was performed to assess whether these factors predicted bone density of the on the navicular cortical and trabecular bone, cuboid cortical and trabecular bone and the trabecular bone of the second metatarsal with no consistent relationships being found.

Strengths of the study were that clinically relevant, valid and reliable methods of testing of neuropathy and bone density were utilised and a protocol strictly followed, participant groups were able to be matched for gender, age, type and BMI and cases and controls were recruited concurrently. Other potential confounds were dealt with by excluding those comorbidities and medications that could affect both neuropathy status and bone density. Bias was reduced by blinding the assessor of bone to the neuropathy and vascular status of the participant. Activity level was also assessed in this study as it will have important effects on bone density that may confound the expected difference due to neuropathy, thus needed to be assessed for consistency between groups. After comparing metabolic minutes per week between groups, there was no statistically significant difference between those with neuropathy and those without. However, the difference was notable with those with without neuropathy performing almost double that of those with neuropathy. This would trend towards a lower bone density in those with neuropathy, serving to enhance the expected bone density reduction in this group, which was not observed. In the hierarchical regression analysis, age, gender and BMI were accounted for statistically. Assumptions of normality, singularity and homoscedasticity were checked prior to analysis. The study was the first to assess relationship of neuropathies to the density of midfoot bones.

Limitations included use of a convenience sample with no power calculation due to a lack of available data for mid foot bones examined in this study and funding restraints due to costs associated with foot

scans. In addition, nerve conduction studies were not used which would have been a more specific method of neuropathy diagnosis. Statistically, the large number of t-tests performed increases the chance of error, increasing the chance of finding a significant result that is due to chance alone and not due to a real relationship between neuropathy and bone density. However, this risk was adjusted for by using a significance level at <0.01. The hierarchical regression performed in this study also has its limitations in the small sample size, with a large number of regressions performed, potentially, being underpowered to detect these relationships. Furthermore, with a large number of regressions performed, this singular relationship observed between navicular cortical bone and small fibre neuropathy, may be due to chance.

Another limitations is that HRV and SFN were used as proxy for peripheral autonomic function which is more directly linked to bone health though these may not always co-exist [62]. This study assessed the density of bone as a measure of propensity for pathology. Whilst density is indicative of such risk, further investigation into the geometric strength properties of bone may provide additional information [177]. Additionally, this study also aimed to assess microvascular reactivity and how it may affect bone. Microvascular reactivity was measured in the skin, however, this may not be indicative of such dysfunction in the bone.

The characteristics of the volunteer convenience sample used in this study influence the applicability of the findings to the target population. The study cohort were all overweight, mostly male (91%) with the majority having type 2 diabetes (96%). Thus, the results should be interpreted in this context. The target population are those at risk of diabetic foot complications, especially Charcot foot, which does not show preference for gender or diabetes type [67]. The study population are likely to have a higher bone density than the broader target population due to male gender, type 2 diabetes and high BMI (although the rate of overweight is reflective of the target population). This may have masked or attenuated the effect of neuropathy on foot bones.

Furthermore, given the large number of variables that affect BMD that would have confounded our results, the exclusion criteria for this study were considered essential. Given that a large number of variables are known to affect BMD, these exclusion criteria are likely to have ensured the results of this study accurately reflected the impact of neuropathy on foot bones density, however, it is

acknowledged that the tight exclusion criteria limit the generalisability to the general population with diabetes.

Another potential reason for the lack of observed relationships in this study may be the small sample size that may have been insufficient to detect an existing difference. Furthermore, the method used for BMD measurement may have lacked adequate sensitivity to detect any difference in this small sample. Bone mineral density was not converted to mg of hydroxyapatite (mg.cm<sup>3</sup>), but rather the values were expressed as HU. Hounsfield units acquired from CT are quantitative units of the radiodensity of objects [178]. The relative simplicity (conversion requires phantoms that are not readily available in the range of bone densities encountered in the foot [174] and association with bone strength and fracture risk, make it a useful measure [179], however further development of this method may increase its sensitivity, accuracy and validity.

This study adds to evidence that the long hypothesised reduction in bone density that predisposes to Charcot foot is not present in general neuropathic populations. Although limited by the cross-sectional design of the study, the implication is that diabetic neuropathies do not influence bone density that predisposes to injury in this population. If the relationship between neuropathy and bone exists it is not likely to be detectable with clinically diagnosed neuropathy and may be overshadowed by the influence of other factors such as activity level and weight. Microvascular reactivity was also not related to bone density. Further research should assess potential risk factors such as osteopenia and altered microvascular flow in Charcot foot populations and in at risk populations in large scale prospective cohort trials to establish whether there is a temporal relationship between these proposed predisposing factors and the occurrence of Charcot foot.

#### **Concluding remarks**

In spite of the well cited theory that neuropathy induces blood flow changes to bone that cause osteopaenia, this research failed to show differences in foot bone density in those with neuropathy or any interaction with types of neuropathy or microvascular reactivity parameters. The neurovascular theory states that autonomic dysfunction leads to an increase in blood flow in bone which results in reduced bone density predisposing to pathology such as fractures and Charcot foot. This research disputes such a proposition by showing that in general neuropathic populations neuropathy does not appear to affect bone density. This research found peripheral sensory neuropathy to be associated with microvascular reactivity. This may have implications for the healing of injury, however, was not associated with foot bone density that may predispose to Charcot foot. This is an important and underresearched area that has increasing relevance in the context of increasing diabetes prevalence. These relationships are relevant to the prevention, early diagnosis and treatment of disease states in the diabetic foot. Statements of Authorship

I hereby certify the following contribution to the publication entitled '*The effect of diabetic neuropathy on foot bones: a systematic review and meta-analysis*' submitted by Alex Barwick in partial fulfilment of the requirements for the degree Doctor of Philosophy.

 Alex Barwick designed and performed the search strategy, assessed studies for inclusion, assessed studies for quality, extracted and entered data for statistical analysis, interpreted results, prepared the manuscript, managed submission, and responded to peer review.

Supervisor

Candidate

Vivienne Chuter

I hereby certify the following contribution to the publication entitled '*Intra-tester and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux.*' submitted by Alex Barwick in partial fulfilment of the requirements for the degree Doctor of Philosophy.

 Alex Barwick designed the protocol, recruited and screened participants, collected data as one of two testers, entered and analysed data, interpreted results, prepared the manuscript, managed submission, and responded to peer review.

Supervisor

Candidate

Vivienne Chuter

I hereby certify the following contribution to the manuscript entitled 'Reliability of computed tomography derived foot bone density measurements in people with diabetes.' submitted by Alex Barwick in partial fulfilment of the requirements for the degree Doctor of Philosophy.

- Alex Barwick designed the protocol, recruited and screened participants, oversaw data collection, analysed data, interpreted results, prepared the manuscript, and managed submission.

Supervisor

Candidate

Vivienne Chuter

I hereby certify the following contribution to the manuscript entitled 'Peripheral sensory neuropathy is associated with altered post-occlusive reactive hyperemia in the diabetic foot' submitted by Alex Barwick in partial fulfilment of the requirements for the degree Doctor of Philosophy.

- Alex Barwick designed the protocol, recruited and screened participants, collected all data, analysed data, interpreted results, prepared the manuscript, and managed submission.

Supervisor

Candidate

Vivienne Chuter

I hereby certify the following contribution to the manuscript entitled 'Foot bone density in diabetes may be unaffected by the presence of neuropathy.' submitted by Alex Barwick in partial fulfilment of the requirements for the degree Doctor of Philosophy.

- Alex Barwick designed the protocol, recruited and screened participants, collected data relating to neuropathy, demographic characteristics and vascular assessments, analysed data, interpreted results, prepared the manuscript, and managed submission.

Supervisor

Candidate

Vivienne Chuter

## Materials for the Study in Chapter Four

#### **Notification of Expedited Approval**

To Chief Investigator or Project Supervisor: Cc Co-investigators / Research Students:	Doctor Viv Chuter Miss Alex Barwick Miss Jennifer Sonter Ms Peta Craike Mrs Sarah Casey Mr Priten Solanki Doctor Fiona Hawke
Re Protocol:	The validity, reliability and predictive value of the Toe-Brachial Index as a measure of peripheral blood flow in people with diabetes mellitus
Date:	19-Mar-2013
Reference No:	H-2010-1230

Thank you for your **Response to Conditional Approval (minor amendments)** submission to the Human Research Ethics Committee (HREC) seeking approval in relation to a variation to the above protocol.

Variation to:

1. Add Sean Lanting and Alex Barwick to the research team.

2. Recruit a new participant group made up of an additional 30 participants from the same population as the existing protocol

3. Invite the new participant group to undertake measurements of resting toe pressure and reactive hyperaemia. Reactive hyperaemia will be measured first using a photoplethysmographic probe and then a laser Doppler.

4. Conduct a second measurement of toe pressure following a period of occlusion. (blocking of the blood vessels)

This process will occur twice with two different clinicians in session one and will then repeated at session two, 7 -10 days later

- Participant Information Statement, version 2 dated 27.2.2013

Your submission was considered under Expedited review by the Ethics Administrator.

I am pleased to advise that the decision on your submission is **Approved** effective **19-Mar-2013**. The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal *Certificate of Approval* will be available upon request.

Professor Allyson Holbrook Chair, Human Research Ethics Committee

For communications and enquiries: Human Research Ethics Administration

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RIMS website - https://RIMS.newcastle.edu.au/login.asp

#### Linked University of Newcastle administered funding:

Funding body	Funding project title	First named investigator	Grant Ref
University of Newcastle/New Staff Grant(**)	The sensitivity of the Toe-Brachial Index as a measure of blood flow and predictor of peripheral arterial disease-related morbidity in diabetes mellitus	Casey Sarah,	G1100060
Ramaciotti Foundations/Establishment Grant(**)	The reliability of the toe-brachial index as a measure of blood flow and predictor of peripheral arterial disease-related morbity and mortality in diabetes mellitus	Chuter Viv,	G0190501
University of Newcastle/New Staff Grant(**)	The sensitivity and specificity of the Toe-Brachial Index (TBI) as a measure of blood flow in the presence of peripheral arterial disease, and development of a more comprehensive TBI value classification system.	Craike Peta,	G1100272

Itact Alex Barwick: ph: 0432

Chief investigator: Dr Vivienne Chuter- Contact details: 02 43494424 or Vivienne. Chuter@newcastle.edu.au



#### PODIATRY

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#### Information Statement for the Research Project:

## The reliability of measurement of reactive hyperaemia in the toe using photoplethesmography and laser Doppler measurement

#### HREC Approval number: H-2010-1230

Document Version 2: dated 27/02/13

You are invited to participate in the research project identified above which is being conducted by Dr Vivienne Chuter (Senior Lecturer), Ms Alex Barwick (PhD candidate) and Sean Lanting (PhD candidate) from the School of Health Sciences at the University of Newcastle. The research is part of Ms Alex Barwick's and Mr Sean Lanting's postgraduate studies at the University of Newcastle, supervised by Dr Vivienne Chuter.

#### Why is the research being done?

The purpose of this research is to determine reliability of measurements of toe pressure at rest and in response to a period of occlusion (blocking) using two different devices. This research may improve the evidence base for practice and clinical efficiency.

#### Who can participate in the research?

We are seeking men and women over the age of 50. However, if you are a non-smoker and not a person with diabetes you have to be 65 years and older to participate in this study. Unfortunately, if you have any of the following conditions you cannot participate in the study:

- Ulceration, wound, infection or amputation of both big toes
- Severe lymphoedema (swelling)
- Connective tissue diseases such as Scleroderma
- Vasospastic conditions (e.g. skin pigments and thickening) such as Raynaud's disease

- Any recent injury, to the hallux (big toe) or foot, that may be exacerbated or result in pain due to inflation of the pressure cuff
- Any problems that prevent you from lying on your back for approximately 60 minutes

#### What choice do you have?

Participation in this research is entirely your choice. Only those who give their informed consent will be included in the project. Whether or not you decide to participate, your decision will not disadvantage you.

If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data which identifies you.

#### What would you be asked to do?

The following tests will be performed by either Alex Barwick or Sean Lanting who are registered podiatrists

If you agree to participate, you will be asked to:

- Abstain from smoking, caffeine and exercise for at least eight hours before testing.
- Have your toe pressure measured after 10 minutes lying flat which involves a probe being attached to your big toe and a cuff around the base of the toe being inflated until the blood flow stops and then gradually deflated.
- This process will occur again one minute after the cuff is inflated for 1 minute, occluding the blood vessels and released.
- After 10 minutes rest a different probe will then be attached to your toe and the cuff will be inflated again for a further minute and released.
- This process will occur again with the other examiner
- A second testing session involving the same tests with the same examiners 7 -10 days later.

#### How much time will it take?

Both testing sessions are anticipated to take approximately 1 hour each. The two testing sessions will be scheduled on a day and time convenient for the participant. Testing sessions need to be performed within 7-10 days of each other.

#### What are the risks and benefits of participating?

As with any research of this nature there are some potential risks and discomforts of which you must be aware. The researchers will attempt to minimise these through careful, consistent monitoring of your condition during the testing procedures. Every effort will be made by the researchers to ensure your safety, comfort and familiarity with all requirements and testing procedures.

#### How will your privacy be protected?

Any information collected by the researchers, which might identify you will be stored securely and only accessed by the researchers. Your confidentiality will be ensured by replacing your name with a numerical code. Data will be retained for at least 5 years in the Health Precinct at the University of Newcastle. Whilst the study is being conducted there is a need to transport data which will be done on a secure password protected laptop.

#### How will the information collected be used?

The results of this project will be used in a thesis to be submitted Ms Barwick's PhD (Podiatry) and Mr Sean lanting's PhD (Podiatry) degree. The results of this project may also be reported in papers in scientific journals and may be presented at conferences. Individual participants will not be identified in any reports or presentations arising from this research. You will be provided with a summary of your own results and the findings of the research. Researchers will also be available to further explain you individual results to you.

#### What do you need to do to participate?

Please carefully read this information statement and be sure you understand its contents before you participate. If there is anything you do not understand, or you have questions, contact the researcher.

If you would like to participate, please inform your health care provider and we will arrange a time convenient to you to conduct the testing sessions.

#### Further information

Thank you for considering this invitation.

If you would like further information please contact the University of Newcastle Podiatry clinic at Wyong Hospital on 02 4394 7280 to leave your contact details and a member from the research team will then contact you.

#### Thank you for considering this invitation

Alex Barwick	Sean Lanting
School of Health Sciences	School of Health Sciences
Health Precinct, Ourimbah Campus	Health Precinct, Ourimbah
Email: <u>Alex.Barwick@uon.edu.au</u>	Email: Sean.Lanting@uon.edu.au
u.au	
	Alex Barwick School of Health Sciences Health Precinct, Ourimbah Campus Email: <u>Alex.Barwick@uon.edu.au</u> u.au

#### Complaints about this research

This project has been approved by the University's Human Research Ethics Committee, Approval No. H-2010-1230

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email <u>Human-Ethics@newcastle.edu.au</u>.

Dr. Vivienne Chuter School of Health Sciences] Health Precinct, Ourimbah Campus Ph: 02 43494424 Fax: 02 43494538 Email: Vivienne.Chuter@newcastle.edu.au

#### **Consent Form for the Research Project:**

## The reliability of measurement of reactive hyperaemia in the toe using photoplethesmography and laser Doppler measurement

#### HREC Approval number: H-2010-1230

#### Dr. Vivienne Chuter, Ms. Alex Barwick, Mr Sean Lanting

Document Version 1 dated: 25/01/13

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to (please circle):

- Have my toe pressure taken before and after a period of occlusion twice by two clinicians on one occasion: yes / no
- Have my toe pressure taken before and after a period of occlusion twice by two clinicians on a second occasion approximately one week later: yes / no

I have had the opportunity to have questions answered to my satisfaction.

Print		
Name:	 	 

#### Signature:

Date:

If you would like to receive a plain language summary of the study results, please supply your email or postal address below.

Participant #	
Name	Phone
DOB	Age
Male/female	
Medical history	Medications

Smoker	Diabetes
Order	
Temperature	
Session 1	Tester 1
	Tester 2
Session 2	
	Tester 1
	Tester 2

# Materials for the Studies in Chapters Four, Five and Six

#### HUMAN RESEARCH ETHICS COMMITTEE



#### **Notification of Expedited Approval**

To Chief Investigator or Project Supervisor:	Doctor Viv Chuter
Cc Co-investigators / Research Students:	Mr John Tessier Dr Nathan Johnson Miss Alex Barwick Doctor Xanne Janse De Jonge
Re Protocol:	The effect of diabetic neuropathy on foot bones
Date:	13-Jan-2014
Reference No:	H-2013-0404
Date of Initial Approval:	13-Jan-2014

Thank you for your **Response to Conditional Approval (minor amendments)** submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under **Expedited** review by the Ethics Administrator.

I am pleased to advise that the decision on your submission is **Approved** effective **13-Jan-2014**.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research, 2007, and the requirements within this University relating to human research.

Approval will remain valid subject to the submission, and satisfactory assessment, of annual progress reports. *If the approval of an External HREC has been "noted" the approval period is as determined by that HREC.* 

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal *Certificate of Approval* will be available

upon request. Your approval number is **H-2013-0404**.

If the research requires the use of an Information Statement, ensure this number is inserted at the relevant point in the Complaints paragraph prior to distribution to potential participants You may then proceed with the research.

#### **Conditions of Approval**

This approval has been granted subject to you complying with the requirements for *Monitoring of Progress, Reporting of Adverse Events*, and *Variations to the Approved Protocol* as <u>detailed below</u>.

#### PLEASE NOTE:

In the case where the HREC has "noted" the approval of an External HREC, progress reports and reports of adverse events are to be submitted to the External HREC only. In the case of Variations to the approved protocol, or a Renewal of approval, you will apply to the External HREC for approval in the first instance and then Register that approval with the University's HREC.

#### • Monitoring of Progress

Other than above, the University is obliged to monitor the progress of research projects involving human participants to ensure that they are conducted according to the protocol as approved by the HREC. A progress report is required on an annual basis. Continuation of your HREC approval for this project is conditional upon receipt, and satisfactory assessment, of annual progress reports. You will be advised when a report is due.

#### Reporting of Adverse Events

- 1. It is the responsibility of the person **first named on this Approval Advice** to report adverse events.
- 2. Adverse events, however minor, must be recorded by the investigator as observed by the investigator or as volunteered by a participant in the research. Full details are to be documented, whether or not the investigator, or his/her deputies, consider the event to be related to the research substance or procedure.
- 3. Serious or unforeseen adverse events that occur during the research or within six (6) months of completion of the research, must be reported by the person first named on the Approval Advice to the (HREC) by way of the Adverse Event Report form (via RIMS at <a href="https://rims.newcastle.edu.au/login.asp">https://rims.newcastle.edu.au/login.asp</a>) within 72 hours of the occurrence of the event or the investigator receiving advice of the event.
- 4. Serious adverse events are defined as:
  - o Causing death, life threatening or serious disability.

- Causing or prolonging hospitalisation.
- Overdoses, cancers, congenital abnormalities, tissue damage, whether or not they are judged to be caused by the investigational agent or procedure.
- Causing psycho-social and/or financial harm. This covers everything from perceived invasion of privacy, breach of confidentiality, or the diminution of social reputation, to the creation of psychological fears and trauma.
- Any other event which might affect the continued ethical acceptability of the project.
- 5. Reports of adverse events must include:
  - Participant's study identification number;
  - o date of birth;
  - o date of entry into the study;
  - o treatment arm (if applicable);
  - o date of event;
  - o details of event;
  - the investigator's opinion as to whether the event is related to the research procedures; and
  - o action taken in response to the event.
- 6. Adverse events which do not fall within the definition of serious or unexpected, including those reported from other sites involved in the research, are to be reported in detail at the time of the annual progress report to the HREC.

#### • Variations to approved protocol

If you wish to change, or deviate from, the approved protocol, you will need to submit an *Application for Variation to Approved Human Research* (via RIMS at <u>https://rims.newcastle.edu.au/login.asp</u>). Variations may include, but are not limited to, changes or additions to investigators, study design, study population, number of participants, methods of recruitment, or participant information/consent documentation. **Variations must be approved by the (HREC) before they are implemented** except when Registering an approval of a variation from an external HREC which has been designated the lead HREC, in which case you may proceed as soon as you receive an acknowledgement of your Registration.

#### Linkage of ethics approval to a new Grant

HREC approvals cannot be assigned to a new grant or award (ie those that were not identified on the application for ethics approval)

without confirmation of the approval from the Human Research Ethics Officer on behalf of the HREC.

Best wishes for a successful project.

Professor Allyson Holbrook

#### **Chair, Human Research Ethics Committee**

For communications and enquiries:

#### Human Research Ethics Administration

Research Services Research Integrity Unit The Chancellery The University of Newcastle Callaghan NSW 2308 T +61 2 492 17894 F +61 2 492 17164 <u>Human-Ethics@newcastle.edu.au</u>

RIMS website - https://RIMS.newcastle.edu.au/login.asp

#### Linked University of Newcastle administered funding:

Funding body	Funding project title	First named	Grant Ref
r analig body		investigator	

Ð.	Foot Bones	earch?		members to participate in ovascular function.	at reception or contact	THE UNIVERSITY OF NEWCASTLE	AUSTRALIA
r to Participat	ropathy and F	pating in clinical res	betes	rtment are seeking community uropathy on foot bones and micr	e ask for an information sheet a	Thank you	one. Human Ethics Protocol Number: 2013-
Opportunity	Research on Neu	Are you interested in particil	We need people with dia	University of Newcastle Podiatry Depairs research on the effect of diabetes and nei	If you are interested in participating please Alex Barwick: ph: 0432 994 84 em	Alex.Barwick@newcastle.edu.au	Research project: The effect of diabetic neuropathy on peripheral b

Chief investigator: Dr Vivienne Chuter- Contact details: 02 43494424 or Vivienne.Chuter@newcastle.edu.au



Newspaper story published in the Newcastle Herald on Thursday November 19th 2014.

# Diabetes researcher recruits for study

#### By MARK CONNORS

 AMPUTATIONS and foot wounds can be a major setback for anyone with diabetes.

With this in mind, University of Newcastle PhD student Alex Barwick has started a research project focused on diabetesrelated foot problems and looking at the best way to treat them. Specifically, she is looking at damaged nerve and blood vessels in feet after exposure to high levels of sugar.

"The contribution of diabetic nerve damage to other foot problems is not well understood," she said.

"This research compares characteristics of people with diabetic nerve damage in the feet to those who don't have nerve damage."

She said this research would allow the medical community to better understand how nerve damage is related to these characteristics and whether it can be used to prevent, identify or treat foot problems.

The research would also improve understanding of



FEET FIRST: Alex Barwick at the Newcastle Community Health Centre on Hunter Street, Newcastle.

the rare disorder Charcot foot, she said.

Charcot foot is a condition that causes a weakening of the bones, which often occurs in people with significant nerve damage.

Ms Barwick is in the middle of a recruitment drive based at the Newcastle Community Health Centre in Hunter Street.

She is on the hunt for about 60 more people with diabetes, especially those with neuropathy (numb feet) and Charcot foot. Ms Barwick said

participation in the study was a great opportunity fo locals to have an in-depth assessment of their feet, free of charge.

Participation involves having a bone density scan a variety of clinical feet tes and maybe a foot scan. To assess your eligibility to be part of the study email Alex Barwick on Alex.Barwick@newcastle. edu.au or phone her on 0432 994 849.

Newspaper story published in the Newcastle Star on Wednesday 26th November.



#### PODIATRY

Dr. Vivienne Chuter Senior Lecturer Podiatry Program Convener School of Health Sciences University of Newcastle Brush Rd, Ourimbah NSW 2258 Tel: + 61 2 43494424 Fax:+ 61 2 43494538 Vivienne.Chuter@newcastle.edu.au

#### Information Statement for the Research Project:

#### The effect of diabetic neuropathy on foot bones

Document Version 2: dated 18/12/13

You are invited to participate in the research project identified above which is being conducted by Dr Vivienne Chuter (Senior Lecturer), Ms Alex Barwick (PhD candidate) from the School of Health Sciences and Dr Xanne Janse de Jonge (Lecturer) from the School of Environmental and Life Sciences at the University of Newcastle. The research is part of Ms Alex Barwick's postgraduate studies at the University of Newcastle, supervised by Dr Vivienne Chuter and Dr Xanne Janse de Jonge.

#### Why is the research being done?

The purpose of the research is to determine whether neuropathy (damage to nerves) occurring in diabetes affects the blood flow and bones in the feet. Previous research has shown that people with diabetes have an increased risk of injury to their foot bones. This study will investigate one of the possible reasons for this – neuropathy and how this may interact with blood flow. We will compare the foot bones of people with and without diabetic neuropathy as well as in those with a joint condition called Charcot foot. The results of this research will shed light on causes of foot bone injury and disease in people with diabetes.

#### Who can participate in the research?

We are seeking people aged over 18 to participate in this research. We are recruiting these people from podiatry clinics. We require a group of people with diabetes, a group of people without diabetes mellitus and a group of people with Charcot foot (in only one foot) to participate in this research. Unfortunately you cannot participate if any of the following apply to you:
- You are currently pregnant
- You take any of the following medications
  - Long term use of corticosteroids (such as prednisone for rheumatoid arthritis)
  - Hormone replacement therapy (medication for menopause)
- You have any of the following conditions
  - o Osteoporosis (bone loss)
  - o Insulin resistance/pre-diabetes (high blood sugar levels)
  - o Chronic renal failure (kidney damage)
  - Current foot ulceration of both feet
  - Both feet are affected by Charcot neuroarthropathy (joint condition involving collapse of the arches of the feet). Those with Charcot neuroarthopathy in one foot are able to participate.
  - Malignancy (cancer)
  - Neuropathy (nerve damage) not caused by diabetes
  - o Recent history of foot trauma
  - Endocrine disorders such as thyroid disease
- You have participated in other research within the last 12 months involving radiation such as x-rays

#### What choice do you have?

Participation in this research is entirely your choice. Only those who give their informed consent will be included in the project. Whether or not you decide to participate, your decision will not disadvantage you or have any impact on your current treatment or relationship with the clinic.

If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data which identifies you.

#### What would you be asked to do?

If you agree to participate, you will undergo the following testing procedures:

*DEXA scan:* This is a routine painless scan for assessing bone density and identifying osteoporosis. You will be required to lie still on a table for approximately ten minutes, a low dose x-ray will pass underneath the table to a scanning arm above you. If you are female and of reproductive age you will be required to undertake a pregnancy test beforehand. If this test identifies that you have osteoporosis, then you will be excluded from participation in the study. This will take place at either the Gateshead (6-8 Sydney Street, Gateshead NSW) or Maitland (24 Elgin Street, Maitland NSW) branch of Hunter Imaging Group and will be at no cost you.

*Neuropathy testing:* This will take place at Newcastle Community Health in the podiatry clinic. You will be required to refrain from caffeine, alcohol and nicotine consumption as well as exercise for eight hours prior to these tests. If you have had a hypoglycaemic event in the previous 12 hours your test will be postponed.

 Peripheral neuropathy: this will involve a series of non-painful tests of the sensations in your feet including monofilament test where a fishing wire-like tool is placed on your foot and you are asked to detect its presence or absence, identifying sharp versus blunt objects, temperature sensation and vibration perception. This will be performed by Alex Barwick who is a registered podiatrist.

- Testing for autonomic neuropathy: this will involve being attached to an ECG machine and having your heart monitored during a period of deep breathing, standing and blowing into a small device. This will be performed by Dr Nathan Johnson who is an accredited exercise physiologist. This will require electrodes to be placed on your chest.
- Testing for peripheral autonomic neuropathy: this will involve a probe being attached to your big toe. You will be asked to perform two simple tasks. Holding your breath for 10 seconds and placing an ice pack on your upper arm, close to the trunk for 60 seconds. This will be performed by Alex Barwick.
- Blood flow (microvascular) testing: the blood pressure will be taken in your toe in a similar manner to how blood pressure is taken in your arm at the doctors. A cuff will be placed around your big toe as well as a probe that measures blood flow. The cuff is inflated and then slowly released. Following this the cuff will be inflated for three to five minutes until the blood flow stops. It will then be deflated and we will measure how long it takes to return. The toe pressure will then be performed again. These measures will be taken by Alex Barwick.
- Other: Your height will be measured using a tape measure and weight by standing on a scale.
- You will also be asked to fill out a survey about your exercise level and we will ask you
  questions regarding your smoking and drinking habits and history of foot problems
  including ulceration and muscle cramps.

We require a subset of participants to undergo the neuropathy testing a second time at a further testing session in order to establish how reproducible our measurements are. Participation in this testing session is entirely voluntary, if you choose to participate in the other testing sessions you are under no obligation to participate in this one.

*Foot bone density testing:* One computed tomography (CT) scan of your feet: CT is a routine painless test for assessing bone and soft tissue integrity. This requires you to keep your foot still in a relaxed position for approximately 10 minutes. This will take place at the Cardiff branch of Hunter Imaging Group at 48 Thomas Street, Cardiff NSW and will be at no cost to you.

We require a subset of participants to undergo this CT scan a second time at a further testing session in order to establish how reproducible our measurements are. Participation in this testing session is entirely voluntary, if you choose to participate in the other testing sessions you are under no obligation to participate in this one.

With your consent we will obtain a health summary from your general practitioner including information on health conditions, medications and diabetes history (if applicable). In the event that any of the procedures results in identifying an anomalous results both you and your general practitioner will be advised.

#### How much time will it take?

The DEXA scan will take approximately 30 minutes. The neuropathy testing will take around one hour. The CT scan will take around 20 minutes.

#### What are the risks and benefits of participating?

As with any research of this nature there are some potential risks and discomforts of which you must be aware. The researchers will attempt to minimise these through careful, consistent monitoring of your condition during the testing procedures. Every effort will be

made by the researchers to ensure your safety, comfort and familiarity with all requirements and testing procedures. Should you choose to participate in this research you are advised not to participate in any research requiring further imaging for the next five years. If you need to undergo further imaging for clinical purposes within this period please advise the radiographer that you have been involved in the research.

The benefits to participating in this study include free of charge testing of bone density, autonomic and peripheral neuropathy testing and foot bone quality. These results will be reported both to you and to your GP in the form of a letter if requested.

This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 to 3 millisieverts (mSv) each year. The effective dose from this study is no more than 0.15 mSv. At this dose level, no harmful effects of radiation have been demonstrated and the risk is minimal.

Females with any chance of being pregnant should not undergo DEXA or CT scanning. If you become pregnant during the course of the study you must inform us immediately and withdraw from the study.

#### How will your privacy be protected?

Any information collected by the researchers, which might identify you will be stored securely and only accessed by the researchers. Your confidentiality will be ensured by replacing your name with a numerical code. Data will be retained for at least 5 years in the Health Precinct at the University of Newcastle on a password protected computer or in a locked filing cabinet. Whilst the study is being conducted there is a need to transport data which will be done on a secure password protected laptop. Should you agree, your de-identified data may be used in future studies with ethical approval conducted by the researchers of this project or conducted by researchers supervised by the researchers of this project.

#### How will the information collected be used?

The results of this project will be used in a thesis to be submitted Ms Barwick's PhD (Podiatry) degree. The results of this project may also be reported in papers in scientific journals and may be presented at conferences. Individual participants will not be identified in any reports or presentations arising from this research. You will be provided with a summary of your own results and the findings of the research. Researchers will also be available to further explain your individual results to you. You may also choose to allow for the data collected in this study, once de-identified (your name and other personal information is removed from the results), to be used in further studies.

#### What do you need to do to participate?

Please carefully read this information statement and be sure you understand its contents before you participate. If there is anything you do not understand, or you have questions, contact the researcher.

On the day of neuropathy testing you will be required to refrain from caffeine, alcohol and nicotine consumption as well as exercise for eight hours prior. You will also be required to wear loose fitting clothing to allow for the placement of equipment including electrocardiogram of the heart.

If you would like to participate, please inform your health care provider and we will arrange a time convenient to you to conduct the testing sessions.

#### Further information

If you would like further information or wish to participate please contact:

Ms Alex Barwick 0432 994 849

Thank you for considering this invitation.

Dr Vivienne Chuter

Senior Lecturer in Podiatry

Alex Barwick

PhD Candidate

#### Complaints about this research

This project has been approved by the University's Human Research Ethics Committee, Approval No. 2013-0404

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email <u>Human-Ethics@newcastle.edu.au</u>.

# **Consent Form**



#### PODIATRY

Dr. Vivienne Chuter Senior Lecturer Podiatry Program Convener School of Health Sciences University of Newcastle Brush Rd, Ourimbah NSW 2258 Tel: + 61 2 43494424 Fax:+ 61 2 43494538 Vivienne.Chuter@newcastle.edu.au

#### **Consent Form for the Research Project:**

#### Effect of diabetic neuropathy on foot bones

#### Dr Vivienne Chuter, Ms Alex Barwick, Dr Xanne Janse de Jonge, Mr John Tessier

#### Document Version 2; dated 18/12/2013

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to

- The researchers accessing my medical records via my general practitioner to extract information on medical conditions, diabetes history and medications
- Have my height and weight measured
- Answer questions relating to nicotine, alcohol consumption and foot health history
- Complete a questionnaire detailing my activity level
- Refrain from alcohol, caffeine and nicotine consumption as well as exercise for eight hours prior to neuropathy testing

- Undergo testing for sensory neuropathy: sharp/blunt, temperature, monofilament and vibration perception
- Undergo testing for peripheral autonomic neuropathy involving placing an ice pack against your arm 60 seconds and holding a breath for 10 seconds
- Undergo testing for cardiac autonomic neuropathy involving an electrocardiogram during deep breathing, standing and forceful outward breath
- Undergo a computed tomography scan of one foot and one DEXA scan
- Undergo blood flow measurement testing during occlusion of the big toe

I understand that my personal information will remain confidential to the researchers

I have had the opportunity to have questions answered to my satisfaction.

\* I consent to have my de-identified data to be used in future studies with ethical approval

\* I consent to undergo a second set of testing of sensory, peripheral autonomic and cardiac autonomic neuropathy and blood flow measurement at a further testing session

\* I consent to undergo a second computed tomography scan of my foot at a further testing session

Print Name:\_\_\_\_\_

Signature:

-

Date:

Please provide the name and contact details of your general practitioner below.

This project has been approved by the University's Human Research Ethics Committee, Approval No. 2013-0404



#### PODIATRY

Dr. Vivienne Chuter Senior Lecturer Podiatry Program Convener School of Health Sciences University of Newcastle Brush Rd, Ourimbah NSW 2258 Tel: + 61 2 43494424 Fax:+ 61 2 43494538 Vivienne.Chuter@newcastle.edu.au

#### Information on release of medical information in the research project:

#### The effect of diabetic neuropathy on foot bones

As part of this project we require information about your current and previous medical history which we will request, with your permission (Medical Information Release form attached), from your current general practitioner. Should you agree we will request the following information:

- Details of current and previous medical conditions such as diabetes, kidney disease, heart disease, thyroid disease, heart attacks, strokes etc.
- Details of current and previous medications.
- Details of diabetes history (if applicable) including date of diagnosis, medication history and the latest obtained HbA1c value.

Should you have any questions or concerns, please don't hesitate to speak to the researchers.

Thank you,

Dr Vivienne Chuter

Senior Lecturer in Podiatry

Alex Barwick

PhD Candidate

This project has been approved by the University's Human Research Ethics Committee, Approval No. 2013-0404



Dr Vivienne Chuter

School of Health Sciences

PO Box 127 Ourimbah, 2258

Ph: 02 4349 4424

#### AUTHORITY TO RELEASE HEALTHCARE INFORMATION Fax: 02 43494538

Project Title: The effect of diabetic neuropathy on foot bones.

Dr Vivienne Chuter, Ms Alex Barwick, Dr Xanne Janse de Jonge, Mr John Tessier

Document Version 1 dated: 07/01/14

Patient's Name:	Date of Birth:
I request	to
release healthcare inf	ormation of the patient named above to:
Name:	Ms. Alex Barwick
Address:	Podiatry Discipline, University of Newcastle, P.O Box 127, Ourimbah
	Postcode: 2258
<ul> <li>This request and auth</li> <li>Details of cudisease, heat</li> <li>Details of cudisease, heat</li> <li>Details of cudisease, heat</li> <li>Details of diagenergy and the story as a story</li></ul>	iorisation applies to: rrent and previous medical conditions such as diabetes, kidney rt disease, thyroid disease, heart attacks, strokes etc. rrent and previous medications. abetes history (if applicable) including date of diagnosis, medication the latest obtained HbA1c value.
This information will I the feet. Any informa procedures regarding I authorise the releas project	be used to help us determine if there a link between health conditions and bone strength in tion provided will be held in accordance with the University of Newcastle's policies and the storage and protection of confidential data. e of release of information detailed above, which is relevant to this research
Patient Signature:	Date Signed:

Print Name:\_

This project has been approved by the University's Human Research Ethics Committee, Approval No. 2013-0404



#### PODIATRY

Dr. Vivienne Chuter Senior Lecturer Podiatry Program Convener School of Health Sciences University of Newcastle Brush Rd, Ourimbah NSW 2258 Tel: + 61 2 43494424 Fax:+ 61 2 43494538 Vivienne.Chuter@newcastle.edu.au

#### Information on the Research Project:

#### The effect of diabetic neuropathy on foot bones

**RE: Participant NAME** 

Address:

DOB:

Dear Dr General Practitioner,

Mr/s Participant has volunteered to participate in the above mentioned research project. This research is being conducted by Dr Vivienne Chuter (Senior Lecturer), Ms Alex Barwick (PhD candidate) from the School of Health Sciences and Dr Xanne Janse de Jonge (Lecturer) from the School of Environmental and Life Sciences at the University of Newcastle.

The purpose of the research is to determine whether neuropathy occurring in diabetes affects blood flow and bone parameters in the feet. We will compare the foot bones of people with and without diabetic neuropathy as well as in those with Charcot neuroarthropathy. The results of this research will shed light on causes of foot bone injury and disease in people with diabetes.

As part of this research we require details of the participants' medical history from their general practitioner. This information will be used to determine eligibility for the study and determine whether any conditions, medications or diabetes duration and severity (latest HbA1c) may account for our results. Attached is the Medical Information Release form signed by Mr Participant. The information required includes the following:

- A list of current and previous medical conditions.
- A list of current and previous medications.
- If the patient has diabetes: the type and date of diagnosis and the medication (if applicable) as well as the latest obtained HbA1c value .

If you could provide as much information from the above as possible would be highly appreciated.

The information should be forwarded to using the envelope provided:

Alex Barwick Health Precinct University of Newcastle 10 Chittaway Rd, Ourimbah NSW 2258

If you require any further information on the study, please don't hesitate to contact us.

Thank you for your co-operation,

Dr Vivienne Chuter Senior Lecturer in Podiatry

Alex Barwick PhD Candidate Contact: 0432 994

849

# Demographic Information

Demographics		
Sex		
DOB		
Height		
Weight		
Ethnicity		
Smoking		
Do you currently smoke?		
Have you previously	Years since	
smoked?	quitting	
Alcohol consumption		
How many standard		
drinks do you consume on		
average per week? – see		
guide for standard drinks		
History of ulceration		
Have you ever had an		
ulceration on your lower		
leg or foot?		
If yes, where?		
Time since healed?		
Neuropathy symptoms		
Do you ever experience in		
your feet?	Burning / tingling / numbro	ess / Shooting pains

# **Clinical Testing**

Have you had any caffeine (coffee, tea, coke) today?	
Have you had any cigarettes today?	
Have you had any alcohol today?	
Have you had a hypoglycaemic attack in the last 12 hours?	
Have you done any vigorous exercise today?	

Test	Right				
Monofilament					
VPT					
Pain perception					
Temperature					
perception					
	Right only	·			
Heart rate lying	Yes				
PORH	Yes	Dorsum Temp			
Toe Pressure	Yes	Dorsum Temp			
Heart rate Standing	Yes				

#### FACULTY OF HEALTH

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#### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

#### Research Project: The effect of diabetic neuropathy on foot bones

Investigators: Dr Vivienne Chuter, Ms Alex Barwick , Mr John Tessier, Dr Xanne Janse de Jonge and Dr Nathan Johnson



### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

## (October 2002)

### LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

#### FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

#### Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

#### Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

#### Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at <u>www.ipaq.ki.se</u>. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

#### Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

#### More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at <u>www.ipaq.ki.se</u> and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

#### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the <u>last 7 days</u>. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

#### PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?



The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

No vigorous job-related physical activity - Skip to question 4

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

\_\_\_\_\_ hours per day \_\_\_\_\_ minutes per day

days per week

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

\_\_\_\_\_ days per week

No moderate job-related physical activity **No Skip to question 6** 

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

\_\_\_\_\_ hours per day \_\_\_\_\_ minutes per day

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

\_\_\_\_ days per week

No job-related walking - Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days **walking** as part of your work?

\_\_\_\_ hours per day

\_\_\_\_\_ minutes per day

#### PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

\_\_\_\_ days per week

No traveling in a motor vehicle 🔶	Skip to question 10
-----------------------------------	---------------------

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

 hours per day
 minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

	days per week
	No bicycling from place to place
11.	How much time did you usually spend on one of those days to ${f I}$

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

\_\_\_\_\_ hours per day

\_\_\_\_\_ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

\_\_\_\_ days per week

		No walking from place to place	$\rightarrow$	Skip to PART 3:
HOUSE	EWC	DRK,		

HOUSE MAINTENANCE, AND CARING FOR FAMILY

13. How much time did you usually spend on one of those days **walking** from place to place?

\_\_\_\_ hours per day

\_\_\_\_\_ minutes per day

#### PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

\_\_\_\_ days per week

No vigorous activity in garden or yard **Skip to question 16** 

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

	hours per day
	minutes per day
16.	Again, think about only those physical activities that you did for at least 10 minutes at a time. During the <b>last 7 days</b> , on how many days did you do <b>moderate</b> activities like carrying light loads, sweeping, washing windows, and raking <b>in the</b> <b>garden or yard</b> ?
	days per week
	Io moderate activity in garden or yard - Skip to question 18
17.	How much time did you usually spend on one of those days doing <b>moderate</b> physical activities in the garden or yard?
	bours por day
	nours per day
	minutes per day
18.	Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the <b>last 7 days</b> , on how many days did you do <b>moderate</b> activities like carrying light loads, washing windows, scrubbing floors and sweeping <b>inside your home</b> ?
	days per week
RECF	No moderate activity inside home Skip to PART 4:
SPOF	RT AND LEISURE-TIME PHYSICAL ACTIVITY
19	How much time did you usually spend on one of those days doing <b>moderate</b>

19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

\_\_\_\_\_ hours per day \_\_\_\_\_ minutes per day

#### PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

\_\_\_\_ days per week

21. How much time did you usually spend on one of those days **walking** in your leisure time?

\_\_\_\_ hours per day

\_\_\_\_\_ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

\_\_\_\_ days per week

	Io vigorous activity in leisure time - Skip to question 24
23.	How much time did you usually spend on one of those days doing <b>vigorous</b> physical activities in your leisure time?
	hours per day
	minutes per day
24.	Again, think about only those physical activities that you did for at least 10 minutes at a time. During the <b>last 7 days</b> , on how many days did you do <b>moderate</b> physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis <b>in your leisure time</b> ?
	days per week
SPEN	No moderate activity in leisure time Skip to PART 5: TIME
	SITTING
25.	How much time did you usually spend on one of those days doing <b>moderate</b> physical activities in your leisure time?
	hours per day
	minutes per day

#### PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

\_\_\_\_\_ hours per day

\_\_\_\_\_ minutes per day

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

\_\_\_\_\_ hours per day

\_\_\_\_\_ minutes per day

# This is the end of the questionnaire, thank you for participating.

Computed Tomography Foot Scan Method for the Studies in Chapters Four and Six



Figure 1: participant positioning and set up for foot computed tomography scanning.



Figure 2: example of cortical bone region of interest selection of the cuboid.



Figure 3: example of trabecular bone region of interest selection of the cuboid.

# Intra-tester Reliability of Outcome Measures used in Chapters Five and Six

# Objective

To ascertain intra-tester reliability for measurements used in Chapters Five and Six: diagnosis of large fibre neuropathy, diagnosis of small fibre neuropathy, and heart rate variation testing.

# Methodology

#### Participants

Participants were recruited from a subset of the participants in Chapter Five who agreed to a second round of testing. They were recruited from local newspaper articles and posters in podiatry clinics in NSW, Australia (see Appendix C). All participants were adults with type 1 or 2 diabetes mellitus and were excluded if they were pregnant, took corticosteroids, or hormone replacement therapy, had osteoporosis, chronic renal failure, current bilateral foot ulceration, neuropathic osteoarthropathy, malignancy, endocrine disorders (other than diabetes), or a recent history of foot trauma.

#### Equipment and measurement

#### Large fibre neuropathy

Presence of large fibre neuropathy was performed using the guidelines devised by Boulton et al. [36] which recommends the 10g monofilament test and one other of five neurological exams for the diagnosis of large fibre neuropathy. A combination of two tests gives an 87% sensitivity for peripheral neuropathy, being able to predict ulceration [10]. In this case, the second test used was vibration perception threshold (VPT) as assessed with a neurothesiometer. The sensitivity and specificity of both monofilament testing and VPT has been established [180]. Abnormal readings on both tests is considered large fibre neuropathy as defined for this study.

Monofilament was performed in accordance with Boulton et al. [36]. A Bailey Instruments (Chorlton, Manchester, U.K) monofilament calibrated to buckle at 10g was utilised (deemed to be accurate by Booth and Young [181]). Participants were shown the sensation that was to be detected on their hand. They were then asked to close their eyes and the monofilament was placed perpendicular to the skin until it buckled then held for one second on each site. The participant was first asked whether they felt the touch and then its location on their foot. A 'yes' response followed by correct identification of the site was considered 'detected'. The four sites include the plantar surface of the metatarsal heads one, three and five as well as the plantar surface of the hallux [36]. A score out of four was given. A score of three or less out of four is indicative of large fibre sensory loss. This test was performed three times and an average of the three was taken. Reliability may be affected by the testing procedure (number and location of sites), patient factors, factors intrinsic to the device (newness) and humidity. Recommendations for maintenance of calibration was followed [181].

Vibration perception threshold was assessed with a Horwell neurothesiometer (Scientific Laboratory Supplies ltd., Nottingham, UK) .Vibration perception threshold is sensitive and specific [182]. The procedure was explained to the participant and demonstrated first on the hand. The participant lay supine and the instrument was placed on the dorsal hallux proximal to the nail fold. The amplitude of the instrument was gradually increased until the participant indicated they could feel vibration. This voltage was recorded as the VPT. The mean of three readings was taken. A value of over 25V was considered abnormal [36].

#### Small fibre neuropathy

Presence or absence of small fibre neuropathy was determined in accordance with Papanas et al. [44] which measures temperature perception and pain sensation with a neurotip. Abnormal readings on both tests is considered small fibre neuropathy as defined for this study.

Temperature perception was assessed using a TipTherm device (AXON Gmbh Dusseldorf, Germany). This is a cylindrical device with a 14mm diameter circular surface at both ends. One end is metallic and the other plastic. The action of ambient temperature with the materials on either end serve to provide the temperature difference. This technique is validated in temperatures of below 24 degrees. The sensations to be detected were demonstrated first on the participant's hand. Random ends of the device were placed on the dorsum of each foot for 5 seconds, three times. Participants were asked to identify which end of the device was in contact with their skin. Where the participant gave two or

more incorrect responses, the test was seen as abnormal. The test was performed a total of three times and an average of the three was taken. The sensitivity and specificity of this test has been established [183].

Pain sensation was assessed with a Neuropen that applies consistent force of 40g and attached Neurotip (Owen Mumford, Oxford, UK) which consists of a plastic blunt probe and a metallic sharp end. The sensation to be detected was first demonstrated on the participant's hand. Random ends of the device were placed on the plantar surface of the hallux three times. Where the participant gave two or more incorrect responses, the test was seen as abnormal. The test was performed a total of three times and an average of the three was taken.

#### Heart rate variation

A Polar RS800cx heart monitor (Polar Electro Oy, Kempele, Finland) was utilised to assess heart rate variability (HRV) as a measure of cardiac autonomic function. Participants completed a supine five minute rest recording. The R-R interval tachogram was analysed with Kubios heart rate variability software (2.1, Kuopio, 2012) with ectopic beats removed using linear interpolation of previous and subsequent beats. Both time and frequency domain parameters were assessed. Time domain measured included the standard deviation of the N-N interval (SDNN) and the root mean square of the R-R intervals (RMS-SD). Frequency domain measures were divided by spectral power analysis into high (0.15-0.40 Hz), low (0.04-0.15Hz) and very low frequency (0.00-0.04 Hz) powers with total power calculated as the sum of all powers.

#### Procedure

Participants refrained from nicotine, alcohol, vigorous exercise and caffeine for eight hours prior to testing. All measurements were taken in a room with a temperature of 23°C. All measurements were made by a podiatrist (AB).

Participants lay supine whilst neuropathy measures were assessed in the following order – monofilament, VPT, pain and temperature detection. Finally, lying then standing heart rate testing was performed. Tests were repeated in an identical fashion three to 14 days later at the same time of day. The assessor was blinded to previous results as best as possible.

#### **Statistics**

Statistical analysis was performed in SPSS Version 22 for Windows (SPSS Inc, Chicago, USA). For tests yielding dichotomous data (presence/absence of large fibre neuropathy, and small fibre neuropathy) Cohen's Kappa statistics with standard error of measurement (SEM) were calculated. These were interpreted as per Landis and Koch (1977):  $\geq 0.75 =$  excellent agreement, 0.4-0.75 = fair to good agreement and <0.40 = poor agreement [184]. Intra-tester reliability for tests yielding continuous data (heart rate variation) between session 1 and 2 was determined with intra-class correlation coefficients (ICC) and 95% confidence intervals. Interpretation of ICC was in accordance with Portney and Watkins: > 0.75 = good, 0.50 to 0.75 = moderate, < 0.50 = poor [185].

# Results

Thirty-one participants were included in the analysis of neurological measures. The average age of the participant group was 67.29 (SD 9.64) with a range of 53 to 86 and a male to female ratio of 21 to 10. All dichotomous measures demonstrated excellent agreement (Table 1). Time domains of heart rate variation demonstrated moderate reliability and the frequency domain demonstrated good reliability (see Table 2).

	Kappa	р	
Large fibre neuropathy			
Right	0.93**	< 0.01	
Left	1.00**	< 0.01	
Small fibre neuropathy			
Right	0.92**	< 0.01	
Left	0.82**	< 0.01	

 Table 1: Reliability statistics for dichotomous variables

\*\*measurement demonstrates excellent agreement

Variable	Mean (SD)		ICC (95%CI)	Р
	Session 1	Session 2		
SDNN	23.34 (9.73)	23.09 (9.53)	0.70 (0.45; 0.85)*	< 0.01
RMS-SD	16.37 (8.04)	17.10 (10.23)	0.66 (0.39; 0.83)*	< 0.01
Total	380.72 (151.49)	387.30 (156.02)	0.94 (0.87; 0.97)**	< 0.01

Table 2: Reliability statistics for heart rate variation (n=29)

\*Measurement demonstrates moderate reliability

\*\*Measurement demonstrates good reliability

# Conclusions

Diagnosis of large fibre neuropathy displayed excellent reliability. Vibration perception threshold with neurothesiometer or biothesiometer has previously demonstrated good reliability [186-189] with the monofilament showing moderate reliability testing [182, 186]. A combination of tests is recommended by the American Diabetes Association [36]. The combination of these two tests demonstrated excellent reliability in the diagnosis of large fibre neuropathy (Kappa = 0.92 -right foot, 1.0 -left foot).

There is less previous research into the reliability of pain perception and temperature perception with specialised instruments as measures of small fibre neuropathy. Previous reliability on the neuropen, has shown good reliability especially when used in combination with other tests [189]. Previous reliability studies on the TipThem show it to have adequate reliability when used in temperatures up to  $23^{\circ}$ C [183]. We found the combination of these tests to have excellent intra-tester reliability in diagnosing small fibre neuropathy (Kappa = 0.92 -right, 0.82 -left).

Strengths of the study are that we had 30 participants, within the population we want to extrapolate to. Random error was reduced by using equipment as per guidelines and manufacturer's instructions, standardising the procedure and using a trained assessor. The time of day was standardised across testing sessions for each participant and the duration between testing sessions was short enough to not have affected the true value but allow for reasonable blinding of the assessor to previous results.

In conclusion, diagnosis of large fibre neuropathy, small fibre neuropathy and cardiac autonomic neuropathy displayed adequate reliability to be used in the studies in Chapters Five and Six.

# Table of Correlations for the Study in Chapter Five

					Sensory			Total
		Duration	Gender	Age	neuropathy	SDNN	RMSSD	power
Duration	Pearson Correlation	1	.045	$.230^{*}$	.047	087	053	.054
	Sig. (2-tailed)		.656	.022	.643	399.	.605	.601
	Z	66	66	66	66	96	96	96
1 M 2 F	Pearson Correlation	.045	1	028	193	007	078	.026
	Sig. (2-tailed)	.656		.786	.056	.950	.448	<i>799</i>
	Z	66	66	66	66	96	96	96
Age	Pearson Correlation	$.230^{*}$	028	1	.145	.008	.012	065
	Sig. (2-tailed)	.022	.786		.152	.939	907.	.528
	Z	66	66	66	66	96	96	96
Sensory	Pearson Correlation	.047	193	.145	1	.042	.059	521**
neuropathy	Sig. (2-tailed)	.643	.056	.152		.682	.569	000.
	Z	66	66	66	66	96	96	96
SDNN	Pearson Correlation	087	007	.008	.042	1	.864**	.012
	Sig. (2-tailed)	.399	.950	.939	.682		000.	907.
	Z	96	96	96	96	96	96	96
RMSSD	Pearson Correlation	053	078	.012	.059	$.864^{**}$	1	.064
	Sig. (2-tailed)	.605	.448	907	.569	000.		.533
	Z	96	96	96	96	96	96	96
Total	Pearson Correlation	.054	.026	065	521**	.012	.064	1
power	Sig. (2-tailed)	.601	799.	.528	000.	907.	.533	
	Ν	96	96	96	96	96	96	96
F, female; M,	male; RMSSD, root mean s	quare of the R	-R interval;	SDNN, st	andard deviatio	n of the N-N	V interval	

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\* significant at p<0.05; \*\*significant at <0.01

# STROBE Checklist for Case-control Study in Chapter Six

#### STROBE Statement for the case-control study in Chapter Six Checklist of items that should be included in reports of *case-control studies* [190]

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	( <i>a</i> ) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and	2
		balanced summary of what was done and what	
Introduction		was found	
Introduction	T		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods	•		
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates,	4-7
		including periods of recruitment, exposure,	
		follow-up, and data collection	
Participants	6	(a) Give the eligibility criteria, and the sources	4
		and methods of case ascertainment and control	
		cases and controls	
		(b) For matched studies, give matching criteria	4
		and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures,	5-7
		predictors, potential confounders, and effect	
		modifiers. Give diagnostic criteria, if applicable	
Data sources/	8*	For each variable of interest, give sources of data	5-7
measurement		and details of methods of assessment	
		(measurement). Describe comparability of	
		group	
Bias	9	Describe any efforts to address potential sources	4-7
		of bias	
Study size	10	Explain how the study size was arrived at	Not provided
Quantitative variables	11	Explain how quantitative variables were handled	6-7
		in the analyses. If applicable, describe which	
Statistical matheda	12	groupings were chosen and why	67
Statistical methods	12	(a) Describe all statistical methods, including	0-7
		(b) Describe any methods used to examine	7
		subgroups and interactions	,
		(c) Explain how missing data were addressed	7
		( <i>d</i> ) If applicable, explain how matching of cases	7
		and controls was addressed	
		(e) Describe any sensitivity analyses	n/a
Results	10*		-
Participants	13*	(a) Report numbers of individuals at each stage of	/
		for eligibility confirmed eligible included in the	
		study, completing follow-up, and analysed	
	İ	(b) Give reasons for non-participation at each	7
		stage	
		(c) Consider use of a flow diagram	Not provided
Descriptive data	14*	(a) Give characteristics of study participants (eg	Table 1
		demographic, clinical, social) and information on	
		exposures and potential confounders	
		(b) indicate number of participants with missing	n/a
	I	uata foi cacii variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	7
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Main results	16	( <i>a</i> ) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 7, 8 Tables 2- 4
		( <i>b</i> ) Report category boundaries when continuous variables were categorized	Tables 2-4
		( <i>c</i> ) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 8 Tables 3 and 4
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9, 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Publication of the Study in Chapter Six

Journal of Diabetes and Its Complications xxx (2016) xxx-xxx



Contents lists available at ScienceDirect

# Journal of Diabetes and Its Complications



journal homepage: WWW.JDCJOURNAL.COM

# Foot bone density in diabetes may be unaffected by the presence of neuropathy

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#### ARTICLE INFO

Article history: Received 19 January 2016 Received in revised form 15 March 2016 Accepted 18 April 2016 Available online xxxx

Keywords: Bone Diabetes Foot Neuropathy Microvascular disease

#### ABSTRACT

*Aims:* Neuropathies are common complications of diabetes and are proposed to influence peripheral bone, principally via an altered vascular supply. This study aimed to determine the relationship between subtypes of neuropathy and vascular reactivity on foot bone density in people with diabetes.

*Methods:* A case–control observational design was utilised with two groups: those with diabetic peripheral large fibre neuropathy (n = 23) and a control group with diabetes but without neuropathy (n = 23). Bone density in 12 foot bones was determined with computed tomography scanning. Additionally, post-occlusive reactive hyperemia, presence of small fibre neuropathy and heart rate variability were determined. T-tests and hierarchical regression were used to examine the relationships among the variables.

*Results:* No difference in foot bone density was found between those with and those without large fibre neuropathy. Furthermore, no association between heart rate variability or reactive hyperemia and bone density was found. Small fibre neuropathy was associated with increased cuboid trabecular bone density (p = 0.006) with its presence predictive of 14% of the variance.

*Conclusions:* This study found no clear association between presence of diabetic neuropathies and foot bone density. Furthermore, vascular reactivity appears to have no impact on bone density.

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### 1. Introduction

Neuropathy is a common complication of diabetes (Karvestedt et al., 2011) that can affect both the somatic and autonomic nervous systems (Boulton et al., 2005). It can manifest as a loss of sensation, paraesthesia, muscle atrophy, cardiac deregulation and poor skin blood flow (Boulton et al., 2005). Such changes cause significant morbidity in the lower limb in the form of ulceration, infection and amputation (Boulton, 2005) and are implicated in changes to bone seen in neuropathic osteoarthropathy of the foot (Charcot foot) (La Fontaine, Shibuya, Sampson, & Valderrama, 2011).

The nervous system is involved in the maintenance of bone strength in a number of ways. Bone itself contains sensory and autonomic nerve fibres in cortical and trabecular bone including in the periosteum, bone marrow and mineralised bone (Chenu, 2004). Moreover, bone cells contain receptors for neuropeptides suggesting that there are direct neural influences on bone activity (Chenu, 2004). The autonomic nervous system plays a role in the vascular supply of bone (La Fontaine et al., 2011) as well as regulation of metabolic

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pathways that impact osteoclast and osteoblast activity (Elefteriou et al., 2005). Despite this knowledge, the exact role of the nervous system in bone maintenance remains largely undefined and the potential impact of neuropathy on bone health is unclear.

Nevertheless, neuropathy induced bone demineralisation has long been thought to predispose to the development of Charcot foot. A longstanding theory asserts that a loss of sympathetic vascular tone occurring with neuropathy leads to increased bone blood flow that upturns osteoclast activity (Edmonds, Clarke, Newton, Barrett, & Watkins, 1985). The resulting bone resorption predisposes the foot to neuropathic osteoarthropathy (Jeffcoate, 2005).

Previous research investigating the effects of neuropathy on peripheral bone density has demonstrated inconsistent results (Christensen & Svendsen, 1999; Wang, Xie, & Yu, 2010). Metaanalysis of available data demonstrated that, in the calcaneus of those with diabetes, there is a trend towards poorer bone health in those with neuropathy; however this failed to reach statistical significance (Barwick, Janse de Jonge, Tessier, Ho, & Chuter, 2014). The research to date is limited by a lack of available techniques to image foot bones and has so far mainly focused on the calcaneus. It has been suggested, however, that the other bones of the foot are more likely to be involved in Charcot foot (Frykberg & Belczyk, 2008) and imaging of these bones may lead to a better understanding of diabetic neuropathy.

http://dx.doi.org/10.1016/j.jdiacomp.2016.04.013

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Conflict of interest: The authors declare no conflicts of interest.

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Furthermore, the available data largely relate to the effects of generalised neuropathy on bone without examining the individual contributions of large and small fibre dysfunction and the direct effect of changes to vascular supply on foot bone density. It has been demonstrated that there is dysregulation in blood flow responses of the lower limb in people with diabetic neuropathy (Edmonds et al., 1985; Hile & Veves, 2003), which may in turn affect bone.

The aim of this study was to determine the relationship between subtypes of diabetic neuropathy and measures of microvascular reactivity on foot bone density in people with diabetes. The goal is to clarify whether neuropathy affects bone in a manner that may predispose to bone pathology such as Charcot foot.

#### 2. Materials and methods

#### 2.1. Subjects

A convenience volunteer sample was recruited from patients with diabetes (type 1 or 2) from podiatry clinics in the Hunter region of New South Wales, Australia. Exclusion criteria included: pregnancy; long term use of corticosteroids, hormone replacement therapy, or bisphosphonates; osteoporosis (excluded with dual energy x-ray absorptiometry screening); chronic renal failure; current foot ulceration or neuropathic osteoarthropathy of both feet; malignancy; neuropathy not caused by diabetes; recent history of foot trauma; endocrine disorders such as thyroid disease; and participation in other research within the previous 12 months involving ionising radiation. Ethics was obtained from the University of Newcastle Human Research Ethics Committee and written informed consent was obtained from all participants prior to participation. Diagnosis of diabetes was taken from self-report and a medical history obtained from the participants' general practitioner. Most recent HbA1c was obtained from patient records. Physical activity level was measured with the International Physical Activity Questionnaire long form and is presented in metabolic equivalent (MET) minutes/week (IPAQ Group, 2002). Participants were recruited concurrently and grouped into those with large fibre sensory neuropathy and those without. Groups were matched for age (within three years), BMI (within three points), type of diabetes, gender and duration of diabetes (within five years).

#### 2.2. Computed tomography

An Aquilion One 320 slice (Toshiba Medical Systems, Japan) computed tomography scanner was used for all examinations. One radiographer performed all aspects of the examinations, including participant positioning, scanning and acquisition of measurements. A pre-planned program was utilised for each examination. No adjustments to the pre-planned program were made for any participant. Volume acquisition was utilised with the following settings applied: CTDIvol 7.2 mGy; dose–length product 115.9 (mGy × cm); 120 kV; 150 mA; rotation time 0.5 s; range 16 cm; display field of view medium or large (depending on foot size).

The right foot of all participants was scanned, except where prohibited by injury or amputation in which case the left foot was scanned. Each participant was placed in a recumbent position on the table, offset to the side contralateral to the scanned limb in order to allow a more midline position for the lower extremity to be scanned. The knee was flexed to prevent scanning of the contralateral foot. The degree of angulation of this leg was determined by the comfort of the patient to assist in maintaining the desired position throughout examination and thereby preventing any movement artefact.

The foot that was to be scanned was placed against a wooden box with the ankle in a neutral position as close to 90° to the table surface as possible. The foot was scanned using the pre-planned program and resultant images were assessed by the radiographer for any movement and to ensure that all anatomical areas were covered. Once the radiographer ratified the imaging data the participant was removed from the table.

All seven tarsals and the five meta-tarsals were assessed in the axial plane of reconstruction. Axial images were viewed using a bone algorithm so that clear differentiation was possible between trabecular and cortical bone. All images were viewed with a window level of 350 and a window width of 2700. Images were reconstructed at 0.5 mm thickness at intervals of 0.25 mm.

Three random slices were obtained from the body of each of the 12 bones. The radiographer selected appropriate regions from the slices of each participant and Hounsfield units (HU) measurements were obtained. The largest region of interest possible was traced in the trabecular bone and three regions of interest were taken from the cortical bone from each slice image yielding a total of three trabecular readings and nine cortical readings for each bone. Values were averaged for the trabecular and cortical bone.

#### 2.3. Neuropathy assessment

Presence of large fibre neuropathy (LFN) was assessed using the guidelines devised by Boulton et al. (2008) which recommend the 10 g monofilament test and one other of five neurological exams. In this case, the second test used was vibration perception threshold as assessed with a neurothesiometer. A four site monofilament test using a Bailey Instruments (Chorlton, Manchester, UK) monofilament calibrated to buckle at 10 g was performed. A score of three or less out of four is indicative of large fibre sensory loss in that foot. This test was performed three times and an average of the three was taken. Vibration perception threshold was assessed with a Horwell neurothesiometer (Scientific Laboratory Supplies, Nottingham, UK) on the dorsal hallux. A value of over 25 V was considered abnormal (Boulton et al., 2008). The three readings collected were averaged. Abnormal readings on both tests were considered LFN as defined for this study as criteria for entry into the diabetic neuropathy group.

Presence of small fibre neuropathy (SFN) was determined in accordance with the methods used in Papanas et al. (2007) which measures temperature perception with a Tiptherm device (AXON Gmbh Dusseldorf, Germany) on the dorsum of the foot and pain sensation with a Neurotip (Owen Mumford, Oxford, UK) installed in a calibrated Neuropen (Owen Mumford, Oxford, UK) on the plantar surface of the hallux. Abnormal readings on both tests are considered SFN.

A Polar RS800cx heart monitor (Polar Electro Oy, Kempele, Finland) was utilised to assess heart rate variability (HRV) as a measure of cardiac autonomic function. Participants completed a supine five minute rest recording. The R-R interval tachogram was analysed with Kubios heart rate variability software (Version 2.1, Kuopio, Finland) with ectopic beats removed using linear interpolation of previous and subsequent beats. Both time and frequency domain parameters were assessed. Time domain measured included the standard deviation of the N-N interval (SDNN) and the root mean square of the R-R intervals (RMS-SD). Frequency domain measures were divided by spectral power analysis into high (0.15–0.40 Hz), low (0.04–0.15 Hz) and very low frequency (0.00–0.04 Hz) powers with total power calculated as the sum of all powers (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014).

#### 2.4. Reactive hyperemia assessment

Post-occlusive reactive hyperemia (PORH) was assessed using the protocol of Barwick, Lanting, and Chuter (2015). Briefly, a MoorVMS-LDF2 Laser Doppler (Moor Instruments Ltd, Axminster, UK) was used to measure blood flux at the plantar hallux prior to, during, and post a three minute occlusion of the hallux with a pneumatic cuff. Peak flux post-occlusion expressed as a percentage of

A.L. Barwick et al. / Journal of Diabetes and Its Complications xxx (2016) xxx-xxx

Table 1Participant characteristics.

Characteristic (mean, SD)	Large fibre neu		
	Absent	Present	p-value
Age	68 (8)	70 (8)	p = 0.37
Sex	19/4	20/3	
Height	169 (8)	178 (8)	
Weight	95 (18)	109 (28)	
BMI	33 (7)	34 (8)	p = 0.67
Diabetes type (1/2)	1/22	1/22	
Diabetes duration	15 (12)	12 (10)	p = 0.19
HbA1c (%)	7(1)	7(1)	p = 0.22
HbA1c (mmol/mol)	53 (16)	54 (14)	
Met Minutes/week (median, interquartile range)	3416 (5227)	1716 (2186)	p = 0.09

BMI: body mass index.

resting blood flux (P%BL) and the time to peak (tPeak) were chosen to represent the magnitude and temporal characteristics of the response.

#### 2.5. Statistical analyses

Statistical analysis was performed in SPSS Version 22 for Windows (SPSS Inc, Chicago, USA). The reliability of outcome measurements was assessed with repeat testing on 10 participants for foot bone density, 31 for peripheral neuropathy assessments and 29 for HRV testing. Dichotomous variables (presence of SFN and LFN) were assessed with the Kappa statistic and interpreted according to Landis and Koch (Landis & Koch, 1977):  $\geq 0.75 =$  excellent agreement, 0.4– 0.75 = fair to good agreement and <0.40 = poor agreement. Continuous variables (foot bone density and HRV) were assessed with intra-class correlation coefficients (ICC) and interpreted according to Portney and Watkins (2000):  $\geq 0.75 = \text{good}$ , 0.50 to 0.75 = moderate, <0.50 = poor. T-tests were run to determine significant differences between groups in age, BMI, duration of diabetes and HbA1c with significant level set at p < 0.05. Activity level data were cleaned in accordance with recommendations (IPAQ Group, 2005) and were expressed as median and interquartile ranges. Differences between groups were assessed with a Mann-Whitney U test.

#### Table 2

Comparison of bone density (HU) in those with and without large fibre neuropathy.

Differences in bone density for each foot bone between groups
were investigated with independent t-test with alpha level set at
< 0.01 for significance due to the number of tests run increasing the
likelihood of error.

Hierarchical multiple regression analyses examined the extent to which other neurological factors and response to occlusion accounted for variance in observed bone density of the navicular cortical bone, navicular trabecular bone and second metatarsal trabecular bone. These bones were chosen due to their frequent involvement in Charcot foot (Frykberg & Belczyk, 2008). Demographic variables (age, gender and BMI) were entered at step 1 and neurological and vascular factors at step 2. A significance value of <0.01 was chosen due to the relative small sample size. Non-normally distributed data were log transformed.

### 3. Results

Forty-six participants were recruited (23 in each group). Participant characteristics are found in Table 1. All participants were Caucasian. The left foot was used for six participants. There were no statistically significant differences between groups in physical activity, age, BMI, duration of diabetes or HbA1c.

Assessment of LFN (Kappa: left foot 1.00; right foot 0.93) and SFN (left foot 0.82; right foot 0.92) displayed excellent agreement. Assessment of HRV time domains was moderate (ICC: SDNN 0.70; RMS-SD 0.66) and the frequency domain (total power 0.94) was good. Assessment of bone density displayed moderate to good reliability (trabecular: talus 0.91, calcaneus 0.90, navicular 0.70, cuboid 0.68, medial cuneiform 0.83, intermediate cuneiform 0.88, lateral cuneiform 0.86, first metatarsal 0.90, second metatarsal 0.81, third metatarsal 0.82, fourth metatarsal 0.69, fifth metatarsal 0.85; cortical: talus 0.52, calcaneus 0.59, navicular 0.70, cuboid 0.69 first metatarsal 0.61).

T-tests revealed no statistically significant differences in bone density between groups (Table 2). Hierarchical multiple regressions showed that neither PORH response (Table 3) nor HRV (Table 4) predicted variance in bone density after adjusting for age, gender and BMI. Small fibre neuropathy was associated with increased cuboid trabecular bone density (p = 0.006) with its presence predictive 14% of the variance (Table 3).

Outcome (HU)	Large fibre n	europathy status			99% Confidence				
	Absent			Present			Interval for mean difference		
	М	SD	Ν	М	SD N			t	df
Cortical									
Talus	3009.53	233.98	23	3040.06	422.68	23	- 305.23, 244.17	-0.30	34.33
Calcaneus	2856.66	314.41	23	2782.63	318.69	23	- 177.29, 325.35	0.79	44
Navicular	2849.88	353.43	23	2796.94	238.82	23	- 188.03, 293.91	0.60	38.63
Cuboid	2766.85	289.48	23	2805.36	351.48	23	-294.22, 217.21	-0.41	44
First metatarsal	2990.35	297.83	23	2930.57	325.24	23	- 187.78, 307.35	0.65	44
Trabecular									
Talus	443.70	65.40	23	467.36	59.14	23	-73.15, 25.84	-1.29	44
Calcaneus	217.85	58.74	23	240.00	41.99	23	-62.69, 18.38	-1.47	44
Navicular	377.31	63.33	23	387.46	50.17	23	- 55.50, 35.21	-0.60	44
Cuboid	225.71	65.36	23	249.65	84.96	23	-84.12, 36.24	-1.07	44
Medial cuneiform	374.87	75.02	23	385.69	63.31	23	-65.92, 44.29	-0.53	44
Inter. cuneiform	495.37	101.96	23	529.40	62.14	23	-101.70, 33.64	-1.37	36.36
Lateral cuneiform	387.49	74.73	23	410.85	71.73	23	-81.50, 34.79	-1.08	44
First metatarsal	261.39	84.72	23	283.67	67.60	23	-83.09, 38.53	-0.99	44
Second metatarsal	289.21	88.96	23	338.92	62.80	23	- 111.15, 11.74	-2.19	39.56
Third metatarsal	273.30	72.14	23	309.93	65.52	23	-91.25, 18.17	-1.80	44
Fourth metatarsal	279.23	97.39	23	305.01	64.66	23	-91.85, 40.30	-1.06	38.24
Fifth metatarsal	391.01	341.52	23	608.86	484.34	23	- 552.25, 116.53	- 1.76	39.54

HU: Hounsfield units.

#### A.L. Barwick et al. / Journal of Diabetes and Its Complications xxx (2016) xxx-xxx

#### 4

### Table 3

Associations between PORH variables, presence of SFN and bone density of the navicular and cuboid cortical bone and the navicular, cuboid and second metatarsal trabecular bone.

tPeak (log)	Change in $\mathbb{R}^2$	β	р	P%BL (log)	Change in R <sup>2</sup>	β	р	SFN	Change in R <sup>2</sup>	β	р
Navicular cortical bone											
Step 1	0.28		< 0.01*		0.28		< 0.01*		0.28		< 0.01*
Step 2	0.02		0.27		0.003		0.67		0.04		0.14
BMI		-0.23	0.12			-0.24	0.10			-0.24	0.09
Gender		-0.40	< 0.01*			-0.40	< 0.01*			-0.39	< 0.01*
Age		-0.24	0.14			-0.30	0.06			-0.29	0.05
PORH variable		-0.16	0.27			-0.06	0.67			-0.20	0.14
Cuboid cortical bone											
Step 1	0.20		0.03		0.20		0.03		0.20		0.03
Step 2	0.003		0.72		0.04		0.15		0.000		0.97
BMI		0.20	0.20			0.21	0.16			0.19	0.22
Gender		-0.21	0.14			-0.18	0.19			-0.22	0.13
Age		-0.24	0.17			-0.19	0.23			-0.26	0.09
PORH variable		0.37	0.72			-0.21	0.15			0.005	0.97
Navicular Trabecular bone											
Step 1	0.04				0.04		0.63		0.04		0.63
Step 2	0.03				0.001		0.85		0.007		0.58
BMI		-0.06	0.72			-0.03	0.85			-0.04	0.83
Gender		0.17	0.26			0.19	0.24			0.18	0.26
Age		-0.3	0.86			0.07	0.69			0.05	0.78
PORH variable		-0.16	0.23			-0.03	0.85			0.09	0.58
Cuboid trabecular bone											
Step 1	0.19		0.03		0.19		0.03		0.19		0.03
Step 2	0.01		0.44		0.01		0.61		0.14		< 0.01*
BMI		0.000	0.99			-0.02	0.88			-0.02	0.87
Gender		0.41	< 0.01*			0.40	< 0.01*			0.39	< 0.01*
Age		0.18	0.28			0.10	0.54			0.07	0.62
PORH variable		0.12	0.44			0.08	0.61			0.38	< 0.01*
Second metatarsal trabecular											
Step 1	0.02		0.84		0.02		0.84		0.02		0.84
Step 2	0.000		0.99		0.000		0.89		0.12		0.02
BMI		0.07	0.68			0.07	0.69			0.06	0.70
Gender		0.03	0.83			0.03	0.85			0.01	0.94
Age		-0.09	0.62			-0.10	0.56			-0.15	0.37
PORH variable		0.003	0.99			0.02	0.89			0.35	0.02

BMI: body mass index, Peak: time to peak, PORH: post-occlusive reactive hyperemia, P%BL: peak as a percentage of baseline, SFN: small fibre neuropathy. \* Significant at p < 0.01.

#### 4. Discussion

This study aimed to examine differences in foot bone density in those with diabetes with and without neuropathy. No indication of the hypothesised reduced bone density in those with neuropathy was observed. Moreover, there was no clear relationship between foot bone density and clinical subtypes of diabetic neuropathy or PORH. The results of this study provide evidence that diabetic neuropathies do not alter peripheral bone density in a manner that predisposes to injury or neuropathic osteoarthropathy.

Previous studies examining the relationship between neuropathy and the development of Charcot foot are inconclusive (Barwick et al., 2014). Several studies have demonstrated a reduction in calcaneal bone density assessed by ultrasound in association with the presence of peripheral sensory neuropathy (Conti et al., 2010; Rix, Andreassen, & Eskildsen, 1999; Sieradzki, Trznadel-Morawska, & Olszanecki, 1995). However, more recent studies have not reproduced this relationship (Barbaro, Orsini, Lapi, Turco, & Pasquini, 2008; Chakrabarty et al., 2004; Christensen, Bulow, Simonsen, Holstein, & Svendsen, 2010), and meta-analysis of existing data does not support such an association (Barwick et al., 2014).

The existing data are inconsistent in part due to the complex nature of the relationship between diabetic neuropathy and bone strength, which is complicated by a long list of potential confounds and effect modifiers including activity level, length of diabetes, control of diabetes, age and gender. The current study controlled for age, gender, BMI and duration of diabetes. Furthermore, there was no statistically significant difference in activity level between the groups. Existing data also concentrate on the calcaneus due to its accessibility with one previous study examining the cortical bone of the second metatarsal with plain radiographs and finding a reduced bone mass in those with diabetic neuropathy (Cundy, Edmonds, & Watkins, 1985). Our investigation of all tarsals and metatarsals did not confirm this relationship.

Previous research has mainly concentrated on large fibre sensory neuropathy. Reduced bone density is proposed to be caused more specifically by SFN induced dysregulation of blood flow to bone (Edmonds, 1986). Even though small and large fibre neuropathies usually occur together, SFN can occur independently of LFN (Karvestedt et al., 2011). Therefore, measuring small fibre deficits may have greater sensitivity in identifying changes to bone. To this end HRV (mediated by small autonomic fibres) representing cardiac autonomic neuropathy and small fibre sensory neuropathy was assessed in the current study. In contrast to two previous studies (Conti et al., 2010; Sieradzki et al., 1995), the current study did not find a relationship between measures of cardiac autonomic function and bone density. Furthermore, this study demonstrated that there was no clear link between bone density and small fibre sensory neuropathy. As all neuropathy types including LFN, SFN and cardiac autonomic neuropathy are common in those with diabetes (Karvestedt et al., 2011; Vinik, Mitchell, Maser, & Freeman, 2003) and Charcot foot affects only a small proportion of these (Frykberg & Belczyk, 2008) there may be a more specific set of factors that predispose to Charcot foot involving blood flow.

There has been only limited investigation of the impact of clinical vascular measures on foot bone density. There is demonstrated

#### A.L. Barwick et al. / Journal of Diabetes and Its Complications xxx (2016) xxx-xxx

#### Table 4

Associations between heart rate variability time (SDNN and RMS-SD) and frequency (total power) domains and bone density.

SDNN	Change in R <sup>2</sup>	β	р	RMS-SD	Change in $\mathbb{R}^2$	β	р	Total Power	Change in $\mathbb{R}^2$	β	р
Navicular cortical bone											
Step 1	0.28		< 0.01*		0.28		< 0.01*		0.282		< 0.01*
Step 2	0.01		0.50		0.01		0.52		0.004		0.64
BMI		-0.25	0.09			-0.254	0.08			-0.237	0.11
Gender		-0.42	< 0.01*			-0.424	< 0.01*			-0.392	< 0.01*
Age		-0.34	0.03			-0.336	0.03			-0.297	0.05
Neuropathy variable		0.09	0.50			0.089	0.52			0.066	0.64
Cuboid cortical bone											
Step 1	0.196		0.03		0.196		0.03		0.196		0.03
Step 2	0.001		0.86		0.000		0.99		0.037		0.17
BMI		0.192	0.21			0.19	0.22			0.219	0.15
Gender		-0.210	0.15			-0.215	0.14			-0.169	0.24
Age		-0.257	0.10			-0.263	0.10			-0.206	0.19
Neuropathy variable		-0.027	0.86			0.002	0.99			0.206	0.17
Navicular Trabecular bone											
Step 1	0.040		0.63		0.04		0.63		0.04		0.63
Step 2	0.050		0.14		0.025		0.30		0.003		0.70
BMI		-0.045	0.78			-0.047	0.78			-0.042	0.80
Gender		0.142	0.35			0.147	0.35			-0.166	0.30
Age		0.012	0.94			0.022	0.90			0.043	0.80
Neuropathy variable		0.233	0.14			0.165	0.30			-0.062	0.70
Cuboid trabecular bone											
Step 1	0.191		0.03		0.191		0.03		0.191		0.03
Step 2	0.007		0.56		0.004		0.66		0.001		0.83
BMI		-0.021	0.89			-0.011	0.94			-0.012	0.94
Gender		0.395	<0.01*			0.422	< 0.01*			0.416	<0.01*
Age		0.107	0.49			0.14	0.37			0.134	0.40
Neuropathy variable		0.085	0.56			-0.066	0.66			0.032	0.83
Second metatarsal trabecular											
Step 1	0.02		0.84		0.02		0.84		0.02		0.84
Step 2	0.003		0.71		0.000		0.99		0.006		0.63
BMI		0.066	0.70			0.069	0.68			0.058	0.73
Gender		0.023	0.88			0.033	0.84			0.016	0.92
Age		-0.108	0.53			-0.096	0.58			-0.117	0.50
Neuropathy variable		0.06	0.71			0.002	0.99			-0.079	0.63

BMI: body mass index, SDNN: standard deviation of the N-N interval, RMS-SD: root mean square of the R-R intervals.

\* Significant at p < 0.01.

increased blood flow to foot bones in the presence of diabetic neuropathy that is proposed to increased demineralisation (Edmonds et al., 1985) but it has not been linked to reduced bone density in vivo. In fact, the opposite has been demonstrated with a reduced blood flow to the extremities due to peripheral arterial disease being linked to low bone density in feet (Vogt, Cauley, Kuller, & Nevitt, 1997).

Microvascular flow rather than global blood flow may be a differentiating factor. Generally, the microvasculature in those with diabetic neuropathies has a reduced ability to dilate; however, this ability appears to be retained in those with Charcot foot (Baker, Green, Krishnan, & Rayman, 2007; Shapiro et al., 1998; Veves, Akbari, Primavera, Donaghue, et al., 1998). Such a retention in the ability to vasodilate may cause uncontrolled blood flow to bone leading to its demineralisation. The current study examined PORH as a measure of microvascular vasodilatory capacity and did not find an association with foot bone density. However, it is possible that the increased vasodilatory response seen in Charcot foot may only be relevant in response to injury and not in a healthy state as the participants in the current study were.

The primary limitation of the current study is the method of BMD measurement. Bone mineral density was not converted to mg of hydroxyapatite (mg.cm<sup>3</sup>), but rather the values were expressed as HU. Hounsfield units acquired from CT are quantitative units of the radiodensity of objects (Engelke et al., 2008). The relative simplicity (conversion requires phantoms that are not readily available in the range of bone densities encountered in the foot Commean et al., 2009) and association with bone strength and fracture risk, make it a useful measure (Schreiber, Anderson, Rosas, Buchholz, & Au, 2011); however further development of this method may increase its accuracy and validity.

The results of the present study need to be considered in the context of several other limitations. The sample size of 46 is potentially underpowered to detect small associations between neurovascular factors and bone density especially given the range of potential influencing factors. Neuropathy in this study was measured with clinical indicators and not with nerve conduction studies which may be more sensitive to the relationship. Additionally, HRV and small fibre sensory neuropathy were used as surrogates for peripheral autonomic neuropathy. Finally, PORH may not be a good measure of a neurovascular response as there are multiple factors responsible for the response and it may not reflect blood flow at the level of the bone.

Increased fragility to bone caused by particular neural (Rix et al., 1999) and vascular factors (Baker et al., 2007) is proposed to precede and predispose to Charcot foot. This study did not find clinical neuropathy patterns or vascular reactivity to affect bone density in those with diabetes. Future prospective research of risk factors for Charcot foot is needed to establish the pathogenesis of this disease process and allow for the early diagnosis and treatment of the condition. Furthermore, development of simple clinical tests to asses these risk factors may identify those most likely to develop foot bone pathology and will therefore aid in the prevention, early detection and treatment of Charcot foot.

#### Acknowledgements

This study was supported by a Faculty of Heath Departmental Funding from the University of Newcastle (Australia). The research has not previously been published in part or in full. We acknowledge the statistical support of Hunter Medical Research Institute. Guarantor: Dr Vivienne Chuter.

#### A.L. Barwick et al. / Journal of Diabetes and Its Complications xxx (2016) xxx-xxx

#### 6

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Appendix I

**Conference** Abstracts

Sydney Diabetic Foot Conference - May 2013

# The effect of diabetic neuropathy on peripheral bone: a systematic review and meta-analysis

# A. L. Barwick<sup>1</sup>, X. Janne de Jonge<sup>2</sup>, A. Ho<sup>1</sup>, V. H. Chuter<sup>1</sup>

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University of Newcastle , Australia	University of Newcastle, Australia			

*Aim/Hypothesis:* Diabetic peripheral and autonomic neuropathies are proposed to reduce bone strength, especially in the periphery, contributing to the occurrence foot fractures and Charcot neuroarthropathy. This systematic review aims to examine the literature related to the effect diabetic neuropathy on foot bones.

*Methods:* Studies examining relationships between neuropathy and peripheral bone quality in diabetic populations were sought. Relevant publications were obtained from searches in Medline, Embase and CINAHL in the period up to December 2012. Meta-analysis was performed using a random effects model in the statistical package STRATA v12.1.

*Results:* Nine studies met the inclusion criteria and were included in the qualitative review. Eight of the publications provided sufficient data to be included in meta-analysis. Studies predominantly examined the calcaneus. The mean effect was moderate and statistically significant at -0.52 (95% CI: -0.98, -0.06; p < 0.05) indicating reduced bone quality in those with neuropathy. Subgroup analysis by neuropathy type demonstrated a greater mean effect in participants diagnosed with both peripheral and autonomic neuropathy (-0.97; 95% CI: -1.98, 0.04: p = 0.06) compared to those with peripheral neuropathy where autonomic neurological function was unknown (-0.3; 95% CI: -0.98-0.06; p = 0.095), however neither reached statistical significance.

*Conclusion/interpretation:* We found a significant association of diabetic neuropathy with peripheral bone quality. However, methodological limitations require results to be interpreted with caution. Based on current data it is unknown if a combination of peripheral and autonomic neuropathy contributes to greater bone loss. Further studies of high methodological quality are required to make firm conclusions including the possibility of a causal relationship.

NSW Australian Podiatry Association Conference - Sydney - April 2014

## The effect of diabetic neuropathy on peripheral bone: a systematic review and meta-analysis

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# Background

Diabetic peripheral and autonomic neuropathies are proposed to reduce bone strength, especially in the periphery, contributing to the occurrence foot fractures and Charcot neuroarthropathy. This systematic review aims to examine the literature related to the effect diabetic neuropathy on foot bones.

## Methods

Studies examining relationships between diabetic neuropathy and peripheral bone quality were sought. Relevant publications were obtained from searches in Medline, Embase and CINAHL in the period up to December 2012. Meta-analysis was performed using a random effects model in the statistical package STRATA v12.1.

# Results

Nine studies met the inclusion criteria and were included in the qualitative review. Eight of the publications provided sufficient data to be included in meta-analysis. Meta-analysis demonstrated a moderate significant mean effect of -0.52 (95% CI: -0.98, -0.06; p < 0.05) indicating reduced bone quality in those with diabetic neuropathy. Subgroup analysis by neuropathy type demonstrated a greater mean effect in participants diagnosed with both peripheral and autonomic neuropathy compared to those with peripheral neuropathy where autonomic neurological function was unknown, however neither reached statistical significance.

### **Conclusion and clinical relevance**

We found a significant association between diabetic neuropathy and reduced peripheral bone quality indicating that diabetic neuropathy may be a risk factor for foot bone pathology. Based on current data it is unknown if a combination of peripheral and autonomic neuropathy contributes to greater bone loss. Further studies of high methodological quality are required to make firm conclusions including the possibility of a causal relationship.

# Keywords

Diabetic neuropathy

Autonomic neuropathy

Bone

Australasian Podiatry Council Conference - Dunedin, New Zealand - November 2014

Author(s)

Alex Barwick	University of Newcastle
Xanne Janse de Jonge	University of Newcastle
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Title

The effect of diabetic neuropathy on foot bones: systematic review and meta-analysis Abstract

Diabetic neuropathies are proposed to affect peripheral bone due to changes to its direct innervation and neural control over bone blood flow. Associated changes to bone may contribute to the occurrence of foot bone pathology in this population. If diabetic neuropathies cause changes to bone health as theorised, then this may aid in the clinical identification of those at risk of foot bone pathology. This will allow for targeted prevention and provide potential treatment targets. A systematic review was performed to examine the existing literature into this area. Studies examining relationships between diabetic neuropathy and indicators of bone health (e.g. bone mineral density) were sought in Medline, CINAHL and Embase in the period up to March 2013. Ten studies met the inclusion criteria and were included in the narrative synthesis. Four of the 10 included studies found results indicating poorer bone health in those with neuropathy compared to those with without neuropathy in diabetic populations. Seven of the 10 studies were able to be included in a meta-analysis. A non-significant trend towards poorer bone health in those with diabetic neuropathy was found with a pooled effect of -0.36 (95% CI: -0.76, 0.04; p = 0.08). Few studies have examined the relationship between diabetic neuropathy and peripheral bone health. Although four of 10 studies found significantly worse peripheral bone health in the presence of neuropathy in those with diabetes, meta-analysis of seven studies failed to show a significant association. However, methodological limitations of these studies mean further research is required.

Australasian Podiatry Conference - Gold Coast - May 2015

Barwick, A., Lanting, S., & Chuter, V. Intra- and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux. Australasian Podiatry Conference. JFAR, 2015. 8(2): p. O1

Intra- and inter-tester reliability of post-occlusive reactive hyperaemia measurement at the hallux

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## Background

Post-occlusive reactive hyperaemia (PORH) is a measurement of the vasodilatory capacity of the microvasculature that is associated with cardiovascular disease, peripheral arterial disease and foot ulceration. Reliability of its measurement in the toe for clinical and research purposes has not been adequately assessed. This study assesses both the intra and inter-tester reliability of four methods of assessing PORH in the toe.

# Methods

A within-subject repeated measures design was used. Forty-two participants underwent PORH testing using four methods: pressure measurement with photoplethysmography; an automated laser Doppler technique with local heating; an automated laser Doppler technique without local heating; and a manual laser Doppler technique. Participants underwent testing on two occasions with a three to 14 day interval. Intra-class correlation coefficients and limits of agreement were used to assess reliability.

### Results

Laser Doppler measurement with a heating probe was found to be the most reliable method of PORH measurement. Index of the area under the curve of pre- and post-occlusion and peak perfusion as a percentage of baseline were the most reliable variables.

# Conclusion and clinical relevance

PORH can be reliably measured using laser Doppler when combined with a heating probe. Further research is required to determine clinical utility of photoplethysmography in the measurement of PORH as a measure of microvascular dysfunction in the periphery.

Keywords: Microvascular dysfunction; Peripheral arterial disease; Reliability; Post-occlusive reactive hyperemia

European Association for the Study of Diabetes Conference – Stockholm, Sweden – September – 2015

Barwick, A., Tessier, J., Janse de Jonge, X., & Chuter, V. Initial findings in the relationship between

diabetic peripheral neuropathy and microvascular reactivity in the foot. European Association for the

Study of Diabetes Annual Meeting. Diabetologia, 2015. 58(S1): p. S502

# 1039

Initial findings in the relationship between diabetic peripheral neuropathy and microvascular reactivity in the foot

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Background and aims:

Microvascular dysfunction is common in people with diabetes resulting in diabetic nephropathy, retinopathy and neuropathy of both the large and small fibre nerves. In the periphery it contributes to diabetic ulceration via both peripheral neuropathy and changes to microvascular function. Post-occlusive reactive hyperaemia is a measure of microvascular reactivity (vasodilation capacity) that has been implicated in the diabetic foot complications. This study aimed to investigate the relationship between large and small fibre neuropathy and the post-occlusive reactive hyperaemia response in the diabetic foot.

Materials and methods:

Diabetic participants were recruited from podiatry clinics for this cross-sectional study. They underwent testing for large fibre neuropathy (vibration perception threshold and monofilament detection), small fibre neuropathy (temperature and pain perception) as well as post occlusive reactive hyperaemia at the hallux (using laser Doppler).

Correlations between presence of large and small fibre neuropathy, post-occlusive reactive hyperaemia parameters (time to peak and peak as a % of baseline) and demographics characteristics including sex, HbA1c, age, height, weight and duration of diabetes were performed. Binary logistic regressions were performed on factors associated with the presence of large fibre neuropathy and small fibre neuropathy.

Results:

Eighty-eight participants were included in the analysis. Significant but weak correlations were observed between presence of large fibre neuropathy and age (r=0.20; p<0.05), height (r=-0.35.; p<0.05), and time to peak (r=-0.21; p<0.05). Correlations were observed between small fibre neuropathy and height (r=0.34; p<0.05), and time to peak (r=0.24; p<0.05). Binary logistic regression analyses demonstrated presence of large fibre neuropathy was associated with several demographic factors including increasing age (OR of 1.08 (95%CI: 1.02-1.15, p<0.05) and greater height (OR of 1.1 (95%CI: 1.04-1.15, p<0.05) and time to peak perfusion after occlusion (OR of 1.02 (95%CI: 1-1.04, p<0.05). Presence of small fibre neuropathy in taller people (OR of 1.09 (95%CI of 1.03-1.15, p<0.05) and those with an increased time to peak perfusion after occlusion (OR1.02, 95%CI 1-1.03). Conclusion:

Greater height is associated with increased likelihood of the presence of both large and small fibre peripheral neuropathy. An increased time to peak following occlusion was also associated with increased likelihood of both types of neuropathy suggesting microvascular dysfunction measured by post occlusive reactive hyperaemia (specifically increased time to peak) is associated with higher likelihood of the presence of peripheral neuropathy.

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