ASSESSMENT OF RETINAL STRUCTURE AND VISUAL FUNCTION IN ASSOCIATION WITH DIABETIC PERIPHERAL NEUROPATHY

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Standard Automated Perimetry

Flicker sensitivity

Abstract

Diabetes is an increasingly prevalent disease worldwide. Providing early management of the complications can prevent morbidity and mortality in this population. Peripheral neuropathy, a significant complication of diabetes, is the major cause of foot ulceration and amputation in diabetes. Delay in attending to complication of the disease contributes to significant medical expenses for diabetic patients and the community.

Early structural changes to the neural components of the retina have been demonstrated to occur prior to the clinically visible retinal vasculature complication of diabetic retinopathy. Additionally visual functionloss has been shown to exist before the ophthalmoscopic manifestations of vasculature damage. The purpose of this thesis was to evaluate the relationship between diabetic peripheral neuropathy and both retinal structure and visual function. The key question was whether diabetic peripheral neuropathy is the potential underlying factor responsible for retinal anatomical change and visual functional loss in people with diabetes.

This study was conducted on a cohort with type 2 diabetes. Retinal nerve fibre layer thickness was assessed by means of Optical Coherence Tomography (OCT). Visual function was assessed using two different methods; Standard Automated Perimetry (SAP) and flicker perimetry were performed within the central 30 degrees of fixation. The level of diabetic peripheral neuropathy (DPN) was assessed using two techniques - Quantitative Sensory Testing and Neuropathy Disability Score (NDS). These techniques are known to be capable of detecting DPN at very early stages. NDS has also been shown as a gold standard for detecting 'risk of foot ulceration'.

Findings reported in this thesis showed that RNFL thickness, particularly in the inferior quadrant, has a significant association with severity of DPN when the condition has been assessed using NDS. More specifically it was observed that inferior RNFL thickness has the ability to differentiate individuals who are at higher risk of foot ulceration from those who are at lower risk, indicating that RNFL thickness can predict late-staged DPN. Investigating the association between RNFL and QST did not show any meaningful interaction, which indicates that RNFL thickness for this cohort was not as predictive of neuropathy status as NDS. In both of these studies, control participants did not have different results from the type 2 cohort who did not DPN suggesting that RNFL thickness is not a marker for diagnosing DPN at early stages. The latter finding also indicated that diabetes per se, is unlikely to affect the RNFL thickness.

Visual function as measured by SAP and flicker perimetry was found to be associated with severity of peripheral neuropathy as measured by NDS. These findings were also capable of differentiating individuals at higher risk of foot ulceration; however, visual function also proved not to be a maker for early diagnosis of DPN. It was found that neither SAP, nor flicker sensitivity have meaningful associations with DPN when neuropathy status was measured using QST.

Importantly diabetic retinopathy did not explain any of the findings in these experiments. The work described here is valuable as no other research to date has investigated the association between diabetic peripheral neuropathy and either retinal structure or visual function.

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List of Abbreviations

AR: aldosereductase
BRB: blood retinal barrier
CCM: corneal confocal microscopy
CNS: central nervous system
CRA: central retinal artery
CRV: central retinal nerve
cSLO: confocal scanning laser ophthalmoscope
DNS: diabetic neuropathy symptoms
DM: diabetes mellitus
DPN: diabetic peripheral neuropathy
DR: diabetic retinopathy
FDT: frequency doubling technology
ERG: electroretinogram
ETDRS: early treatment diabetic retinopathy study
FD: fourier domain
GCC: ganglion cell layer
GCL: ganglion cell layer
ILM: inner limiting membrane
INL: inner nuclear layer
IPL: inner plexiform layer
LADA: late auto-immune diabetes in adults
LGB: lateral geniculate body
LGN: lateral geniculate nucleus
MGC: magnocellular cells
mERG: multifocal electroretinogram
NDS: neuropathy disability score
NFL: nerve fibre layer
OCT: optical coherence tomography
OD: overall defect
OLM: outer limiting membrane
ONH: optic nerve head
ONL: outer nucleus layer
OP: oscillatory potentials
OPL: outer plexiform layer
PD: pattern defect
PERG: pattern electroretinogram
PNS: peripheral nervous system
POS: photoreceptors outer segment
PVC: parvocellular cells
QST: quantitative sensory testing
RNFL: retinal nerve fibre layer
RPE: retinal pigmented epithelium
RGC: retinal ganglion cells
SLO: scanning laser ophthalmoscope
SLP: scanning laser polarimetry
TD: time domain
QST: quantitative sensory testing
VEGF: vascular endothelium growth factor
VEP: visual evoked potential

List of Publications and Manuscripts

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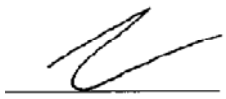
Chapter 5 has been prepared as the basis of a journal article. Dr Geoff Sampson has contributed to this and has given his permission to use the chapter, which contains some statements and language attributable to him, although the underlying work and the majority of the of the chapter text have been performed and written solely by the candidate, AydaMoaven-Shahidi" (first author of the manuscript).

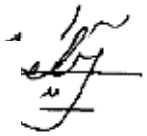
I give my permission for the candidate, Ms AydaMoaven-Shahidi, to use the content of chapter 5 as it currently stands, which contains some sections that have been co-written with, and influenced by me during manuscript preparation. I confirm that the candidate is responsible for conducting the work described in this chapter, and for the majority of the text describing this work.



Geoff P Sampson (PhD)

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: 

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1 Introduction

Diabetes is a highly prevalent chronic disease worldwide and early management of the consequent complications is the key factor to reducing morbidity and mortality in this population. Diabetic peripheral neuropathy is one of the major complications of diabetes. More than 50% of individuals with diagnosed diabetes will suffer from disturbing outcomes of peripheral neuropathy including foot ulcerations and amputations. Approximately 80% of these complications can be prevented if they are diagnosed at early stages. The majority of early and accurate diagnostic techniques are invasive. These include skin and nerve biopsy, which cannot be routinely performed in clinical examination of the patients. Therefore establishing early, less invasive and comfortable diagnostic tools for the detection and management of peripheral neuropathy should be a priority for relevant public health associations.

Recently, corneal confocal microscopy has been introduced as a novel method of assessing neuropathy *in vivo*. The technique has been shown to be capable of predicting early neuropathy by using the extent of corneal nerve damage as a marker of peripheral sensory nerve function. However, the technique still requires contact with the cornea under local anaesthesia. Hence, it can be questioned whether other ophthalmic markers, specifically retinal structure and visual function, may have similar capability in predicting neuropathy elsewhere in the body. Additionally, there is yet a need for an entirely non-invasive, non-contact and accurate method for helping to diagnose peripheral neuropathy.

Retinal vascular complications and their effect on visual function have been the main focus of most published studies on diabetes. Even though diabetes-induced complications in the peripheral nervous system are well documented, changes within the central nervous system,and particularly their relationship to retinal structure and visual function, have received much less attention. The link between diabetic retinopathy and neuropathy is not clear yet. However, evidence of neural and glial changes in the retinae of people with diabetes prior to clinically visible vascular abnormalities has introduced the possibility that retinal neuro-degeneration occurs in conjunction with, rather than a consequence of, the pathogenesis of retinopathy. It has been argued that retinopathy not only involves increased vascular permeability and blood-retinal barrier breakdown, but also is accompanied by gradual loss and apoptosis of retinal neurons. Evidence supporting this concept is outlined in detail in *Chapter 2*.

Reduced retinal nerve fibre layer thickness prior to clinically evident retinopathy has been shown in individuals with diabetes. It is has also been shown that functional changes happen in people with diabetes as a result of neuro-retinal damage. These functional abnormalities, including contrast sensitivity reduction, impaired electroretinogram (ERG) signals, prolonged latencies in visual evoked potential (VEP) and reduced flicker sensitivity, have been demonstrated to precede retinal vascular abnormalities in diabetes. The underlying mechanisms for neural abnormalities in the retina and visual system are not well understood. However, it has been shown that involvement of glial cells can lead to oxidative stress that is also a known factor in peripheral neuropathy pathogenesis. As such, the current body of evidence raises the possibility that retinal neural structure and visual function may potentially be of value in

diagnosing diabetic peripheral neuropathy. Again, the literature supporting this theory will be outlined in *Chapter 2*.

The research outcomes arising from this thesis provides important insights into non-invasive assessment of retinal structure and visual function in association with diabetic peripheral neuropathy.

The thesis is structured as follows:

Chapter 2 provides a review of the literature which has examined theassociations between retinal structure and visual function in relation to diabetic peripheral neuropathy. **Chapter 3**describes the study design and the common methodology that has been used throughout the thesis. Participant recruitment procedure has also been explained in this chapter. In **Chapter 4**, a comparison of two methods for assessing diabetic neuropathy that have been used throughout the thesis has been presented. **Chapter 5** and **Chapter 6** examine the associations for retinal nerve fibre layer thickness with the neuropathy disability score and quantitative sensory testing respectively. In **Chapter 7** and **Chapter 8** the association between standard automated perimetry outcomes and these two neuropathy assessment techniques have been presented. **Chapter 9** investigates the association between flicker sensitivity and neuropathy disability score. Each of these chapters contains a brief introduction, comprehensive aims and specific hypotheses. Additionally relevant discussion has been presented at the end of individual chapters. Finally, **Chapter 10** summarises the outcomes for all chapters. The strengths and limitations of the research are presented alongwith the clinical implications of the findings.

2 Literature Review

2.1 Introduction toDiabetes

Diabetes mellitus is an increasingly prevalent chronic disease that can produce a high percentage of loss of work time, disability, morbidity and premature mortality world-wide. The condition is characterised by glycaemic level deficiencies due to certain endocrine pathologies. The prevalence of diabetes increases with age, and population growth, and is higher amongst certain racial and ethnicity minorities [1, 2].

Diabetes is one of the leading causes of blindness worldwide, generally as a result of changes to the retinal and choroidal vasculature. Peripheral neuropathy, nephropathy as well as other micro-vascular problems are among other major endpoints of diabetes with cardiovascular problems being the primary reason for mortality in this population [3]. Reducing morbidity and mortality from diabetes as well as improving quality of life in this population are amongst the major goals of public health associations and these could potentially be achieved by increasing early diagnosis of the disease and higher screening rates for diabetes complications.

2.1.1 Types of diabetes

Type 1 diabetes, formerly known as insulin-dependent, is defined by autoimmune T-cell mediated defects of pancreatic beta-cells that are responsible for controlling the level of glucose in blood. Such defects will result in shortage of insulin and requires substantial therapies to reduce the risk caused by hyperglycemia. This type of diabetes develops

mainly in children younger than 15 years of age [4] but is also possible to become evident along with or after puberty (late auto-immune diabetes in adults-LADA). Genetic factors have been implicated as initiating triggers [5]. Environmental factors such as viral infections have also been shown to be a leading cause of destroying pancreatic beta cells that contribute to occurrence of the disease [5].

Type 2 diabetes, formerly known as non-insulin-dependent, is by far the most common type of diabetes with a dramatically increasing prevalence globally [6]. The condition develops due to a combination of insulin resistance and impaired insulin secretion. In simple terms, type 2 diabetes occurs when the body is unable to respond properly to the insulin produced by the pancreas. The prevalence rate is higher in adults aged 65-75and is influenced by environmental factors as well as quality of nutrition and presence of obesity[4, 6].

Gestational diabetes resembles type 2 diabetes by having inefficient secretion of, and ineffective response to insulin. It occurs in approximately 7% of all pregnancies and women with this condition are more likely to develop type 2 diabetes after their pregnancy [7].

2.1.2 Epidemiology of diabetes

The prevalence of diabetes increases with age and with population growth[2].It has been reported that over 150 million people have diabetes worldwide with this figure expected to be doubled by year 2025 [6]. In 2005, more than 3% of the Australian population were estimated to have established diabetes, with many more cases remaining

undiagnosed[8]. Trends for international prevalence of the disease mirror this profile[2]. Ethnic background is known to have great impact on incidence of type 2 diabetes [6].

2.1.3 Complications of diabetes

Diabetes is characterised by a hyperglycemic condition, which is an underlying or contributing factor to all diabetic complications. Micro-vascular problems such as retinal haemorrhages are tightly linked to glycaemic levels both in type 1 and type 2 [9], while insulin resistance and hyperglycaemia play a strong role in creating macro-vascular complications including myocardial infarctions [10]. Three of the major complications of diabetes are retinopathy, nephropathy and peripheral neuropathy, further describedas follows:

Retinopathy –Thisis the most common micro-vascular ramification in diabetes.Retinopathy is caused by hyperglycemic-associated deficiencies in biochemical pathways[11]. The occurrence of diabetic retinopathy is highly associated with the duration of diabetes. According to a report by the American Association of Diabetes, approximately 60% of the patients with type 2 diabetes will have some scales of diabetic retinopathy after 15 years of having the disease[12].

Nephropathy - This is the most common cause of end stage renal failure and is known to be associated with various risk factors including age, ethnicity and genetic susceptibility[13]. The pathogenesis of nephropathy is better known in type 1 than in type 2 diabetes[4].

Peripheral neuropathy - Neuropathies or pathology of the peripheral nervous system are amongst the most common complications in the diabetic population worldwide[14]. Optimized glycemic control has been postulated to be the only effective potential agent in preventing the distressing consequences of peripheral neuropathies; however the condition remains to be the major cause of foot ulceration in this population. The pathogenesis of diabetic peripheral neuropathy will be described comprehensively later in this chapter.

2.2 Peripheral Nervous System

The peripheral nervous system (PNS) in humans is composed of a combination of nerve bundles that are grouped based on the type of their cell bodies to either sensory or motor fibres [15]. The cell bodies differ in shape, function and polarity condition. Sensory ganglia are oval-shaped, uni-polar and located on the root of specific cranial nerves while motor ganglia are irregular in shape, multi-polar and the majority of these ganglia are located on the sympathetic trunks[16]. Afferent (sensory) neurons originate at sensory receptors and serve to inform the central nervous system (CNS) of presence of relevant stimuli, whilst efferent (predominantly motor) neurons connect the CNS to muscles to precipitate movement. The somatic subdivision of the PNS primarily consists of efferent nerves innervating voluntary skeletal muscle but also includes afferent components from the skin, whereas the autonomic subdivision of the PNS innervates involuntary muscles as well as having visceral sensory components (Figure 1). The autonomic system has further sympathetic, parasympathetic and enteric subdivisions. The function of somatic and autonomic components is summarized inTable 1.

Table 1.Subdivisions of the peripheral nervous system and their action.

Peripheral nervous system	Action
Somatic nervous system	
Sensory	Receive sensory stimuli
Motor	Stimulates skeletal muscle contraction
Autonomic Nervous system	
Sympathetic	Fight and flight
Para-sympathetic	Vegetative state
Enteric	Digestion control

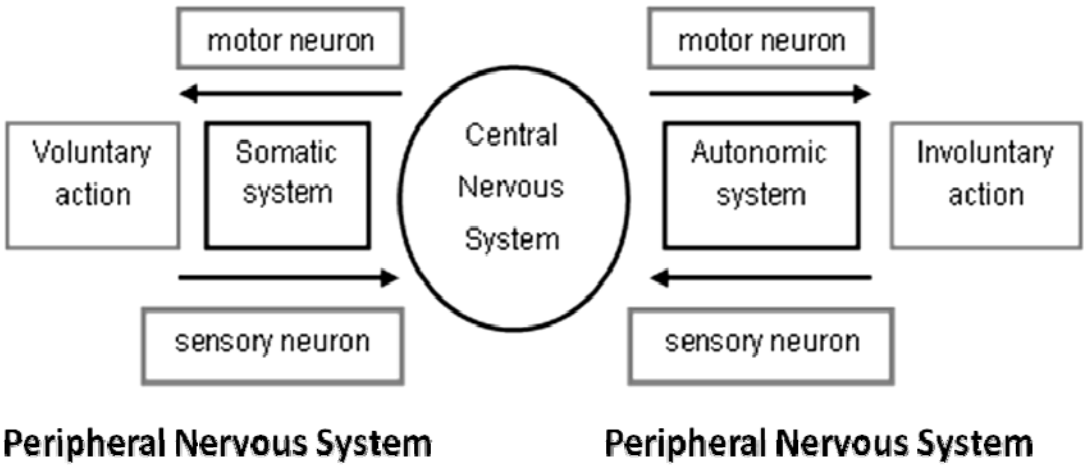


Figure 1.Illustration of interaction between the central nervous system and the peripheral nervous system.The illustration is courtesy of Kimball's Biology Pages (http://biology-pages.info), February 2010, with modification).

The axon diameter plays an important role in classification of the PNS nerve fibres with the thicker fibres having a faster conduction velocity. Based on this characteristic, fibres are categorized in three groups:

GroupA-Fibres in this group are specifically classified into α, β, δ and γ. The largest myelinated axons belong to this subdivision.

Group B - Fibres in group B are mainly the myelinated axons of autonomic preganglionic neurons but generally have not generated much interest in regards to clinical studies [17].

Group C -Group C consists of the smallest diameter unmyelinated fibres.

A second classification in this group is based on the transmission of the impulse along the nerve (conduction velocity) and contains numerical classes I – IV where I is the highest velocity and IV represents the lowest, depending on the axon diameter[17]. A comprehensive description of peripheral nerve fibres and their specific function are shown in Table 2.

Table 2.Classification of peripheral nerve fibres.

Source	Myelination	Diameter (μm)	Conduction Velocity (m/s)	Classification	
				ABC	I-IV
Efferents					
α-motoneurons to muscle	Y	8 - 13	44 - 78	Aα	NA
γ-motoneurons to muscle	Y	3 - 8	18 - 48	Aγ	NA
Afferents					
Limb position and motion	Y	12 - 20	75 - 120	Aα	I
Tactile, pressure, vibration	Y	6 - 12	30 - 75	Aβ	II
Fast pain, cold	Y	1 - 6	5 - 30	Aδ	III
Slow pain, warm	N	< 1.5	0.5 - 2	C	IV

2.3 Diabetic Peripheral Neuropathy

Diabetic peripheral neuropathy(DPN) is the major cause of pathology of lower extremities in diabetes affecting up to 50% of the population [18].The clinical features of DPN are greatly variable with negative symptoms such as loss of sensation being amongst the main characteristics of the condition. This contributes significantly to the pathogenesis of diabetic foot complications such as ulceration and amputation[19]. Another major presentation of nerve damage in diabetes is “pain”, which can result in depression, anxiety and hence reduced life quality and morbidity in affected individuals [20].

The true incidence of DPN is uncertain as the majority of epidemiological reports are for people who have sought medical care for their condition. Additionally, differing methods of assessing DPN provide yet another reason for variation in the incidence reports[21]. An intensive epidemiological report on prevalence of DPN has shown that neuropathies exist in 30% of patients who attend hospital and 20% of people in the general community [22]. In another study prevalence of DPN was shown to be approximately 54% in type 1 diabetes and 45% in type 2 diabetes [23].

2.3.1 Definition of diabetic peripheral neuropathy

According to members of an international consensusmeeting on outpatient diagnosis and management, diabetic neuropathy can be defined as "the presence of symptomsand/or signs of peripheral nerve dysfunctionin people with diabetes after exclusion of other causes" [24]. A more specific definition for diabetic neuropathy has been proposed by The San Antonio Consensus Conference described diabetic neuropathy as: "a demonstrable disorder, eitherclinically evident or subclinical, that occursin the setting of diabetes without other causes for peripheral neuropathy.The neuropathic disorder includesmanifestations in the somaticand/or autonomic parts of the peripheralnervous system"[25].

2.3.2 Pathogenesis of diabetic neuropathy

Pathogenesis of diabetic peripheral neuropathy has been a matter of controversy and a wide range of metabolic and ischemic sources has been described as possible aetiologies for DPN (Figure 2). There are several factors involved in pathophysiology of diabetic neuropathy such as:

Hyperglycemia –It has been suggested that duration and severity of exposure to hyperglycemia can influence the severity of DPN [26]. Severity of neuropathy in patients with impaired glucose tolerance has been shown to be milder than newly diagnosed patients [18].This suggests that nerve damage caused by hyperglycemia can happen at very early stages of diabetes [26, 27]. Insulin therapy and/or pancreas implant have been suggested as potential methods for improvement of impaired glycaemic control and consequently DPN [28, 29].

Polyol pathway - The aldose reductase (AR) enzyme in a polyol pathway is known to have a role in reforming glucose to sorbitol. Complications of diabetes have been hypothesized to be related with sorbitol accumulation in tissue [30]. Moreover, animal studies have shown that greatest risk of developing DPN is involved with AR over-expression [31]. Increased glucose flux through the polyol pathways can lead to peripheral nerve damage and a similar mechanism can cause changes to the crystalline lens in the eye [32].

Oxidative stress -Diabetes can cause an increase in the concentration of intracellular glucose content. Glycol-oxidation or lipoxidation compounds, as two end-points of interaction between glucose and reactive oxygen species, increase the extracellular osmotic stress [33]. This ultimately leads to aggregation of protein kinase C and a reduction in antioxidant cell defence. The increased protein kinase C will give rise to micro-vascularpermeability.

Vascular factors – Peripheral neuropathy in diabetes has been shown to be associated with micro-vascular complications and there is evidence of improvements in neuropathic condition as a consequence of improved tissue blood flow (Figure 3)

[34].The importance of vascular factors in pathogenesis of DPN has been highlighted in focal ischemic nerve lesions in association with severe blood vessels damage. This has been shown to mainly occur in diabetic focal neuropathies [35].

Other factors -There are other factors involved in pathogenesis of diabetic peripheral neuropathy including insulin-like growth factors, vascular endothelium growth factors (VEGF) and immune factors [36].

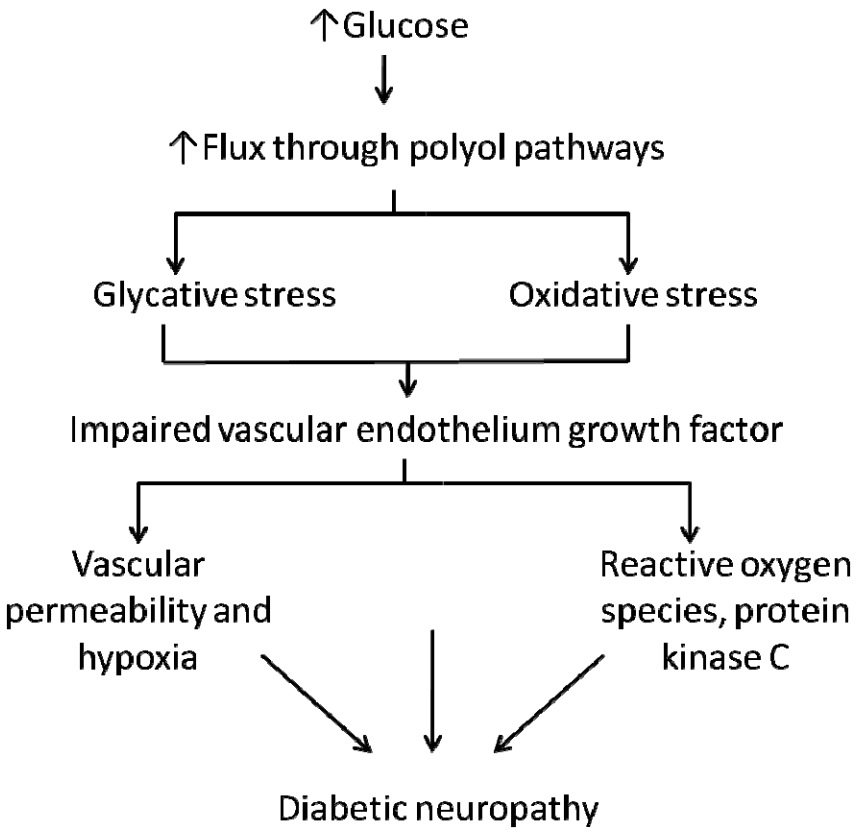


Figure 2.Pathogenesis of diabetic peripheral neuropathy. Simplified mechanism of hyperglycemic-induced nerve damage in diabetic peripheral neuropathy [37].

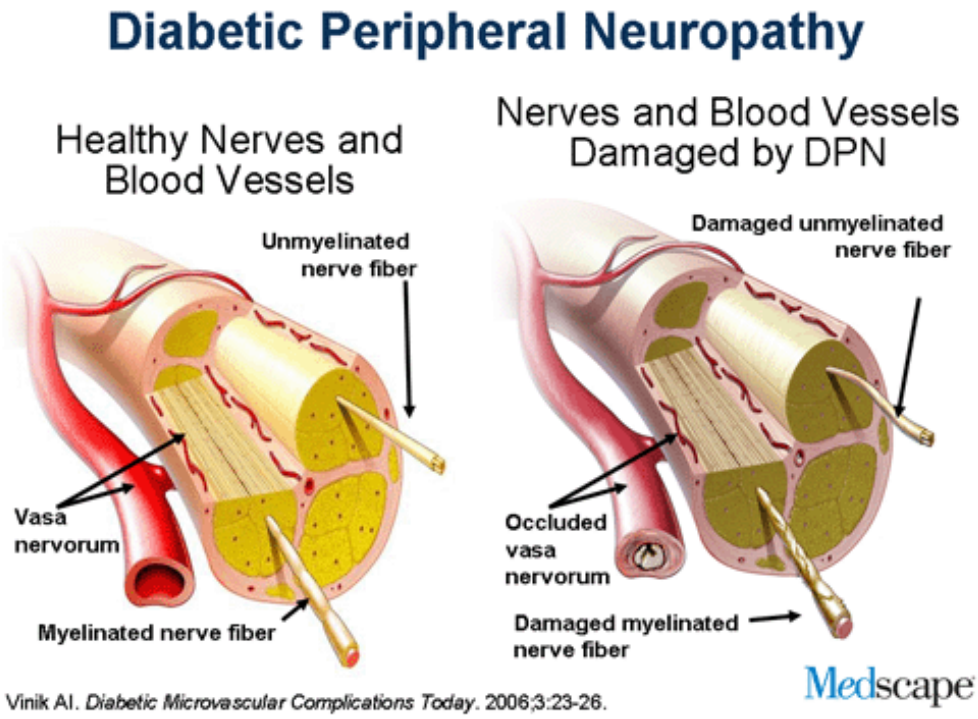


Figure 3.Vascular-related nerve damage in diabetic peripheral neuropathy.

2.3.3 Classification of diabetic peripheral neuropathy

There are a number of ways of classifying diabetic neuropathy based on anatomical, pathological and pathogenic features of neuropathy, but those based on clinical manifestations are the most widely used [38]. Thomas proposed a classification based on the anatomical site of the body and clinical findings (Table 3). According to this classification diabetic neuropathy is not a sole condition but is the outcome of several disorders in the peripheral nerves [39]. Chronic sensori-motor neuropathies are the most common type encountered [36].

Table 3.Classification of diabetic peripheral neuropathy. (from Thomas [39])

Type of diabetic neuropathy	Sub-types
Rapidly reversible	Hyperglycemic neuropathy
Generalized symmetrical polyneuropathies	Sensori-motor (chronic) neuropathy Acute sensory neuropathy Autonomic neuropathy
Focal and multifocal neuropathies	Cranial neuropathy Thoracolumbar radiculoneuropathy Focal limb neuropathy
Superimposed chronic inflammatory demyelinating neuropathy	N/A

2.3.4 Methods of assessing diabetic peripheral neuropathy

Clinical assessment of peripheral neuropathy begins with evaluation of the symptoms associated with the condition. These symptoms usually involve distal extremities and are often described as burning, tingling, stabbing and glove-stocking sensations. Some patients also experience pain and/or paraesthesia. These should be handled carefully by the clinicians as individuals have different perceptions of their symptoms [36]. A number of pain questionnaires have been developed to facilitate the interpretation of patients' symptoms. However a thorough assessment of neuropathy would require more sophisticated methods. A brief description of commonly used assessment techniques is as follows:

Screening procedure– This procedure involves clinical assessment of skin temperature and colour, foot pulses (for assessment of vascular flow and peripheral vascular disease) as well as any kind of foot deformity including Charcot and muscle wasting.

Semmes-Weinstein monofilament – The monofilaments are among the most widely used tools in rapid screening of diabetic patients for peripheral neuropathy; when applied to the skin surface the presence of absence of sensation is noted. These are available in various diameters, however, the most widely used is the 10-gram monofilament, which has been shown to be a good predictor for diabetic foot ulcers [40].

Diabetic neuropathy symptom (DNS) score – This questionnaire contains four general questions including patients' gait condition (steady or unsteady), experience of pain or burning sensation in legs or feet, prickling sensation in legs or feet, and numbness in legs or feet and is known to be a valid and sensitive way of assessing symptoms [41]. There are a few other symptom questionaries available for neuropathy clinical screening such asMcGill Pain Questionnaire [42], Michigan Neuropathy Screening [43]., Neuropathy Symptom Profile and the University of Texas subjective verbal questionnaire [44].

Neuropathy disability score (NDS) -The test involves neurological examination of three sensory modalities:vibration perception with a 128-Hz tuning fork, sharp and blunt sensation using a Neurotip device, and temperature sensation using hot and cold rods. A score of 0 isnormal and 1 is abnormal for each individual test component. Additionally, the ankle reflex is assessed using a reflex hammer with the scores being 0 for normal, 1 for reinforcementand 2 for absent. A reinforcement of the reflex is when fingers of each hand are hooked together so each arm can forcefully pull against the other. This helps distracting the individual and hence obtaining the reflex if presents. NDS score of more

than 5/10 predicts foot ulceration [45]. Each foot can have maximum score of 5 resulting in a total score of 10 in both feet (lower scores mean the foot is less severely affected).The North West Diabetes Foot Care Study investigated the incidence of, and clinically relevant risk factors for, new foot ulcerationin 9,710 diabetic patients using this technique and recommended the method as a useful screening tool in clinical practice [45].

Quantitative sensory testing (QST) - Quantitative sensory testing is used to identify the sensorymodalities affected by damage to the peripheral nerves in diabetes and to estimatethe magnitude of the deficit. It is known to be a valuable method for diagnosing subclinical neuropathy in the diabeticpopulation using vibration, thermal, andpain thresholds [46]. The intensity of stimuli is well-controlled and the individuals test results can be compared with normative databases. The technique in non-invasive [36]; however the subjective nature of the test can influence the test outcomes. Additionally the technique cannot be used in evaluation of all types of neuropathy in diabetes as it is more capable of predicting small fibre neuropathy [47].

Electrophysiology - Electrophysiological examinations include several different procedures such as nerve conduction studies, F-waves testing, and sensory, and/or motor amplitudes assessments. It is an objective, non-invasive, reliable and reproducible method of assessing peripheral neuropathy; however, the results reflect neural activity in a small subset of large-diameterand heavily myelinated axons [36].

Nerve and skin biopsy - Sural nerve biopsy has been used for many years for diagnosing diabetic neuropathy. However, the technique is invasive and may result in postoperative pain and sensory loss in diabetic patients [48]. Skin biopsy is an

alternative less invasive method for assessing small fibre neuropathy. This technique, although relatively quick to heal, still requires resection of a3-mm piece of skin tissue [36].

2.3.5 A new ophthalmic method for assessing diabetic neuropathy

The cornea is the most densely innervated part of the human body, housing C and Aδ fibres. This tissue has recently gained considerable attention in neuropathy studies as a potentially useful ophthalmic marker of DPN. Corneal confocal microscopy (CCM) is a highly sophisticated method of observing corneal sub-basal nerve plexus [49] and is capable of detecting changes in nerve fibre density and branching at early stages of diabetic neuropathy in a non-invasive manner [48, 50].Corneal nerve fibre density is reduced in people with diabetes when compared with healthy non-diabetic individuals, and greater density reductionhappens along with increased severity of neuropathy [50].It is noteworthy to mention that CCM, despite being non-invasive and reiterative, yet requires contact with the cornea under local anaesthesia. Corneal sensitivity has also been explored as another tool in assessing diabetic neuropathy and reduced sensitivity seems to be related to the severity of diabetic neuropathy [51].Examples of CCM images of individuals with and without neuropathy areshown inFigure 4.

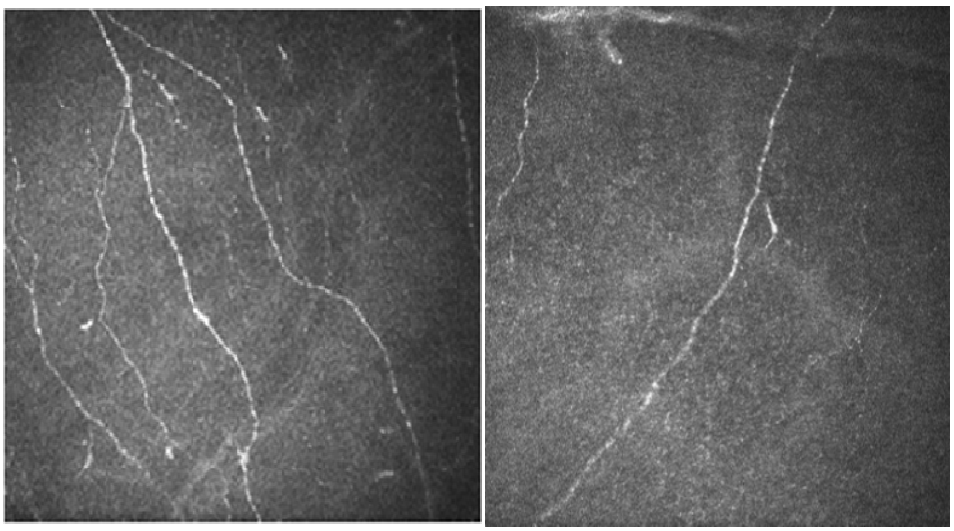


Figure 4.Corneal confocal microscopy (CCM) images of the sub-basal nerve plexus of a healthy individual without neuropathy (left) and a diabetic individual with severe neuropathy (right)

2.3.6 Treatment of diabetic peripheral neuropathy

Treatment of diabetic neuropathy has been the major focus of many research studies; however no treatment to-date has been suggested to successfully prevent or reverse the progression of diabetic peripheral neuropathy. In general, good control of the blood glucose levels, as the underlying cause of diabetes complication, is considered as the most important factor in management of peripheral neuropathy. Additionally, pharmacological treatments can be useful in managing neuropathic pain syndromes in affected patients. Some of the medication types that are prescribed for this purpose are listed below:

Opioid analgesics – A group of strong pain killers that act at the peripheral and spinal levels. The use of this treatment is mainly for controlling more severe pain in older patients; however its general use is not widely accepted [52].

Antidepressants - Tricyclic anti-depressants are thought to affect pain transmission in the spinal cord. These have been the most common treatment for pain in neuropathy as they provide treatment for depression as well controlling the pain [53].

Topical agents – Capsaicin cream has been shown to be a useful method of reducing pain symptoms when applied topically to the painful area of the limb. The cream has been extracted from the alkaloid in the pepper and it alters pain neuro-transmision[53].

The information provided above has summarised important factors that are involved in pathogenesis of diabetic peripheral neuropathy, various available methods of assessing the condition as well as few of the potential treatments of neuropathy major endpoints. Early diagnosis of DPN is the most important factor in reducing the risk of foot ulceration and amputation in affected population. It has been discussed that the most accurate techniques of diagnosing DPN at early stages are invasive. Therefore there is a need for techniques that are less-painful and non-invasive which can also be used in routine clinical examination. Corneal nerve morphology in association with peripheral neuropathy is a promising marker of this condition, occurring elsewhere in the body. However the technique of corneal confocal microscopy requires contact with the cornea. The current thesis aims to investigate the anatomy of retina and visual function, as other promising ophthalmic indicators of diabetic peripheral neuropathy by means of non-invase, non-contact techniques. The following sections will discuss retinal structure and

visual function as well as their availablemeasuring techniques. Growing body of evidence supporting potential roles for retinal nerve fibre layer and visual function in assessment and monitoring diabetic peripheral neuropathy will also be discussed in the subsequent sections.

2.4 The Retina

The retina is a ten-layered sensory tissue forming the internal surface of the posterior eye [54](Figure 5). The thickness of the retina varies between 0.56 mm near the optic disc to 0.1 mm at the oraserrata with the thinnest part at the centre of the fovea (252 μm). The retina is firmly attached at the margins of the optic disc and at its termination at the oraserrata. The outer surface of the retina is in contact with Bruch's membrane (the innermost layer of the choroid) while the inner surface is in contact with vitreous body [54].

2.4.1 Retinal Vasculature

The retina is an extremely metabolically active tissue which consumes the highest percentage of oxygen per weight of any human tissue [55]. Blood supply and drainage to the retina is provided by two main sources as described below:

Central retinal artery and vein

The central retinal artery (CRA) is the first branch of the main ophthalmic artery that nourishes about two-third of the entire retina. The CRA originates as a separate stem from the first part of the ophthalmic artery. It measures about 0.3 mm in diameter and

runs adherent to the dural sheath of the optic nerve [54]. The arterial branches run in the retinal nerve fibre layer closer to the inner limiting membrane. Transparency of this layer helps the visibility of retinal vessels when examined using ophthalmoscopy. These arteries are mainly innervated by sympathetic fibres of the PNS. The central retinal vein (CRV) is prominently responsible for conducting blood from the retina.

Choriocapillaris

The choroid has high blood flow and low oxygen exchange with the outer layers of the retina (retinal pigmented epithelium and outer nuclear layer). The vessels do not enter the retinal layers; however, fluidfrom the choriocapillarisexudes between the cells [54].

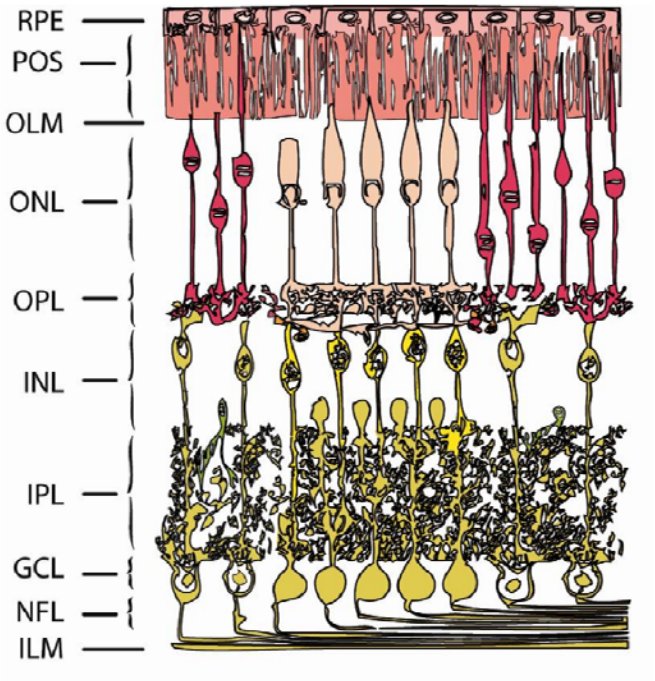


Figure 5.Individual layers of the retina.RPE: retinal pigmented epithelium, POS: photoreceptors outer segment, OLM: outer limiting membrane, ONL: outer nucleus layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer, NFL: nerve fibre layer, ILM: inner limiting membrane [56].

2.4.2 Retinal neural components

The retina is a complex structure, with a large number of neural components. The light energy is processed in a spatio-temporal pattern and converted into a visual response through the neural elements. Figure6 illustrates various neural components of the retina. These are summarized as three broad layers as follows:

Outer neural layer – This layer contains photoreceptive cells that are specialized for converting light energy to nerve impulses and the two main photoreceptors are rods and cones with the approximate ratio of 20:1 [57].

Middle neural layer- This layer forms the middle order neurons and is composed of the following cells:

Bipolar cells in this layer act as connectors between the photoreceptors and ganglion cells. The axons of these cells are referred to as *afferents* (this term, in the peripheral nervous system is used for the axons that relay sensory information to the central nervous system). These axons often act through a *distal process* where they receive the signal in the periphery via their bare nerve endings, pass the nerve body and propagate along the *proximal process* until they reach the synapse in the posterior horn of the spinal cord [58].

Horizontal cells run parallel with the retinal surface. They are associated with rods and cones via their long and short processes; respectively. These cells respond to hyperpolarisation of photoreceptors following light-stimulation.

Amacrine cells integrate the retinal circuits [57]. They can be recognized by their large cell bodies and are known to have no axons. These cells are located close to ganglion cells and hence excite them via the stimulation received from bipolar cells [54].

Müller cells serve as one of the major glial cells of the retina (a similar role as the Schwann cells in the peripheral nervous system). Their cell bodies are long and narrow and their processes extend all throughout the neural retina [54]. The function of Müller cells is to structurally support the retina [59]. Retinal neurons are nourished by Müller cells as they are comprehensively connected to the walls of capillaries and the amount of glycogen stored in the retina is limited by these cells [60]. The cells are known to regulate the retinal blood flow and they can influence the retinal-blood barrier characteristics in endothelial cells [59].

Inner neural layer- This layer mainly contains the retinal ganglion cells (RGC) and their long axons. The input to a single RGC originates from photoreceptor cells servicing a spatial area known as the "receptive field" of the cell [61]. Most retinal ganglion cells in a primate retina are classified into two functional categories:

1) Magnocellular cells: These are known to have a large receptive field. They respond to larger objects and are able to track down rapid changes in the stimulus.

2) Parvocellular cells: These cells arise mainly from the foveal area. They have a smaller receptive field and respond to selective wavelengths only and are responsible for mediating visual acuity and colour perception.

Retinal ganglion cells relay processed visual information from the retina to the lateral geniculate nucleus (LGN). RGCs have different diameters from 10-30 μm and most of them are small in size (midget ganglion cells). They form one single layer in most parts of the retina however the number of the layers increases from the periphery to the macula. They decrease towards the fovea, where they are absent. The ganglion cell axons form the nerve fibre layer[54].

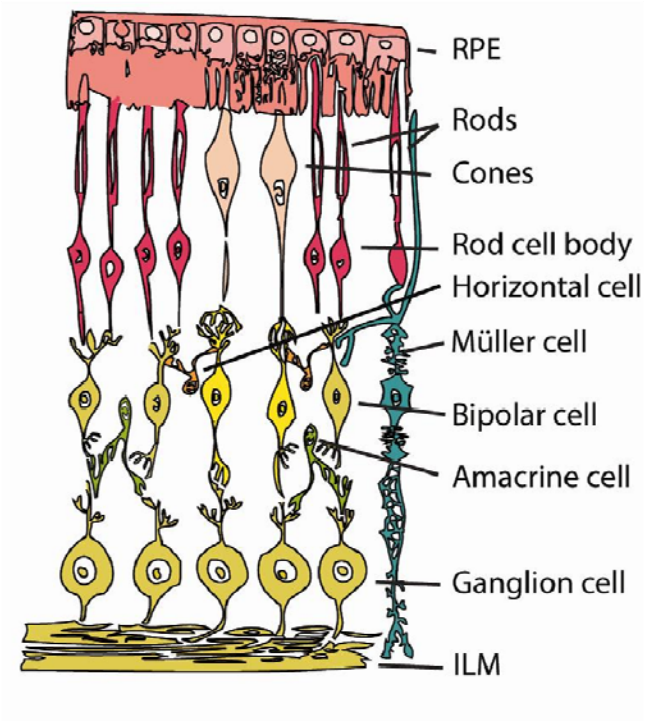


Figure6.Neural components of the retina.

2.4.3 Blood-retinal barrier

The neural component of the retina needs a barrier to protect it from the large molecules and toxic substances entering via the choriocapillaris where most fluid exchange happens. The movement of fluid and ions to/from the retina is restrictedly

controlledbecause of the tight junctions between the endothelial cells of blood vessels. Blood retinal barrier (BRB) is known to be made of two components: the retinal vascular endothelium and the RPE. These two components provide a barrier for outer and inner retina, respectively. Reciprocal function of both barriers helps maintaining homeostasis of the retina and normal nerve metabolism and conduction [62]. BRB break-down has been shown to be one of the early signs of diabetic retinopathy which exposes the retina to excessive amount of fluid. Such BRB impairment can be a result of glial cell death [63].

2.5 Retinal Nerve Fibre Layer

Retinal nerve fibre layer (RNFL) mainly consists of the unmyelinated axons of ganglion cells as well as astrocytes and glial cells [64]. The fibres converge together in a unique pattern to build the optic disc and leave the eye at this level, however they remain a part of the optic nerve [57].The RNFL is responsible for carrying information to the lateral geniculate body (LGB) where they synapse with the brain. These axons lose their myelination once they enter the eye at the lamina cribrosa.

The spread-pattern of retinal nerve fibres is specifically dependant on their origin in the retina [65], such that fibres that originate from the macula region find a relatively direct path to the optic nerve head, while those appearing in the temporal region of the macula find an arched path around the earlier-developing ones(Figure7). Axon size is closely related to eccentricity of the RGCs which indicates that longer fibres originate from areas closer to the fovea [66]. Retinal nerve fibre layer is the main focus of this thesis; hence determinants of the layer and methods of assessing the layer and quantifying the thickness will be explained in more details.

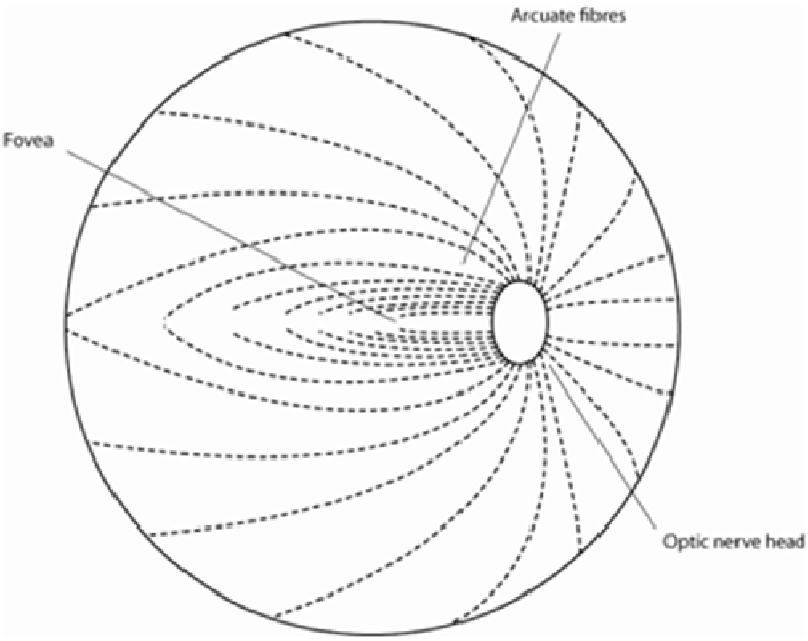


Figure7.Spread-pattern of the retinal nerve fibres [67].

2.6 Determinants of Retinal Nerve Fibre Layer Thickness

Quantification of retinal nerve fibre layer (RNFL) is of great value in assessing retinal pathologies including glaucoma. The thickness of the nerve fibre layer can be influenced by multiple factors. Such factors may include RNFL anatomical variations or maybe related to individual ophthalmic and systemic conditions. Understanding these factors as well as pathological changes to the RNFL can decrease random variability in findings and improve diagnostic value of RNFL measurements [68]. A number of these factors are discussed below:

2.6.1 Retinal nerve fibre layer thickness and age

Retinal nerve fibres, like many other parts of the body, change with increasing age. A few studies have investigated the relationship between the thicknesses of RNFL and age [69-73]. The average peripapillary RNFL thickness has been shown to vary between 80 - 110 micrometers (μm). Table 4outlines a summary of several studies on RNFL thickness using various measurement techniques. Age of the cohort in these studies vary between 5 – 90 years old and the disparity observed among these studies can be attributed to the ethnicity of the study population, sample size, different age range and different instrumentation.

Histological measurements of optic nerve fibre count in post-mortem eyes have shown a loss of 4000-5000 fibres per year (age 3.5 – 88 years) [74, 75].Others have reported an age-related thinning of RNFL [76] with one study reporting a loss of approximately 8% per decade [73] and another reporting 0.39 μm per year (age ≤10 – ≥77 years) [69]. A few recent studies have also shown thinning of the RNFL layer (using optical coherence tomography) with an increase in age, particularly in the temporal sector [77, 78]. Bundez and colleagues [79]reported a 2% loss of overall RNFL thickness for every decade of age. Hougard and associates [68] also reported a thinning of approximately 2.6 – 2.9 μm per increasing decade of age. Another recent study has shown that thickness of the superior quadrant can be reduced due to advanced aging[80].

Table 4.Summary of key studies on retinal nerve fibre layer thickness.

				Retinal nerve fibre layer thickness (μm)				
Author	**N**	**Age (yr)**	**Method**	**Global**	**Temporal**	**Superior**	**Nasal**	**Inferior**
Schuman et al.[81]	21	28 ± 5	OCT	153 ± 13	126 ± 11	179 ± 16	131 ± 26	175 ± 14
Poinoosawmyet al.[69]	150	5 - 90	SLP	78 ± 10	-	-	-	-
Mistlbergeret al.[82]	17	53 ± 13	OCT	99 ± 14	-	-	-	-
Bowdet al.[83]	30	63 ± 10	OCT	86 ± 6	66 ± 5	106 ± 8	62 ± 9	107 ± 8
Jones et al.[84]	15	25 - 30	OCT	129 ± 10	-	-	-	-
Alamoutiet al.[77]	100	6 -79	OCT	109 ± 22	148 ± 18.4	-	-	-
Kanamoriet al.[78]	144	16 - 44	OCT	123 ± 11	101 ± 18	148 ± 18	96 ± 19	146 ± 19
Ramakrishnanet al.[85]	118	21 - 76	OCT	105 ± 38	66 ± 17	138 ± 22	85 ± 21	129 ± 25
Bundezet al.[79]	328	51± 33	OCT	101 ± 11	69 ± 13	124 ± 18	81 ± 18	126 ± 18

OCT: optical coherence tomography, SLP: scanning laser polarimetry

2.6.2 Retinal nerve fibre layer and optic nerve head parameters

Retinal nerve fibre layer measurements using OCT (a very common and accurate technique of quantifying RNFL) are captured at 3.4 mm diameter around the optic disc by default, regardless of the size of the optic nerve head (ONH) since it captures the majority of the fibres that spread out of the optic disc [81]. However variability of ONH parameters such as disc area, vertical diameter and rim area are good indicators that the thickness of RNFL thickness may be expected to vary among healthy, non-diseased individuals[75]. Therefore, using the same fixed diameter scan can result in obtaining measurements from dissimilar areas around the optic nerve head. Savini and associates [86]investigated the relationship between RNFL thickness using OCT and optic disc size on 54 healthy individuals and found a significant thicker RNFL in those with large discs. They argued that a fixed diameter scan can result in an over-estimation of RNFL thickness in cases of larger ONH diameter. Another OCT study on 328 healthy individuals by Bundez and colleagues also confirmed the association between RNFL thickness and ONH size [79]. Histological studies have also shown that for every millimetre increase in diameter of ONH (base-line diameter of 1.89 millimeter), there is an increase in nerve fibre counts by up to 780 fibres [87] and that RNFL thickness reduces at greater distances from the margin of the ONH [88]. In contrast, Mardin and associates [89]showed a negative correlation between RNFL thickness measurements and disc area using Heidelberg Retina Tomographmethod. These findings suggest that characteristics of the ONH should be considered in assessment of retinal nerve fibre layer.

2.6.3 Retinal nerve fibre layer thickness and axial length

It has been shown that longer axial lengths (greater myopia) can affect RNFL thickness measurements using OCT, resulting in an underestimation of the thickness [79]. This is due to enlargement of the scan circle size that is used in the OCT protocol for assessment of RNFL thickness. Budenz and associates [79] have shown that that for every one millimetre increase in axial length, RNFL thickness will be approximately 2.2 μmthinner. Hougard and colleagues [68]also found a significant inverse correlation for axial length and refractive error as two main factors which affect the RNFL thickness measurements.

2.6.4 Retinal nerve fibre layer and ethnicity

Individuals from different ethnic backgrounds have been shown to have different ONH anatomical features which result in different RNFL thicknesses [90]. Pooinosowmy and colleagues found lower values of RNFL thickness in Afro-Caribbeans compared with Caucasians [69]. Budenz*et al.*[79] also found thicker RNFL measurements for Caucasians compared with other ethnic groups. They suggested that OCT instruments' normative databases perhaps do not include a large number of Afro-Americans, Asians and Indians.

2.6.5 Retinal nerve fibre layer and gender

Three research groups did not find any between gender differences for RNFL thickness measurements [79, 81, 83]. Varma*et al.* [91], however, found gender-related differences but only in the inferior RNFL quadrant with male participants having thicker readings.

2.7 Methods of assessing retinal nerve fibre layer thickness

Clinical evaluation of retinal nerve fibre layer thickness is of great importance in early detection of retinal pathologies. Several techniques have been used for this purpose as follows:

2.7.1 Ophthalmoscopy

Ophthalmoscopy is amongst the early methods developed for assessment of the RNFL changes. The method is used to observe fundus features such as blood vessels *in vivo*. However the method does not provide quantitative information regarding RNFL pathologies.

2.7.2 Photography

Photography of the peripapillary RNFL is one of the earliest methods of diagnosing glaucoma; it gained prominence in the early 1970s. Numerous cameras have been invented with a variety of optical properties. Hoyt and co-workers [92] used red-free photography to evaluate diffuse and local loss in peripapillary retina and described the fundoscopic signs of early RNFL loss in glaucoma. The method of photography was then improved using black and white negatives [93]. Other groups enhanced the RNFL red-free photographs by computer programmes [94], or used an image analyser to measure grey levels in red-free photographs in normal and glaucomatous eyes [95].Yamazaki and colleagues [96] also developed an analysis programme to detect changes to RNFL thickness at early stages of glaucoma. The aforementioned techniques are limited by factors including pupil size and media opacities. Additionally,

photography contrast levels have a considerable impact on accurate assessment of RNFL.

2.7.3 Scanning laser ophthalmoscopy

Application of laser for imaging retinal tissue started with the invention of the Scanning Laser Ophthalmoscope (SLO) in the 1980s. The use of laser in a joint ophthalmoscope - fundus camera brought major advantages to retinal imaging technology including the possibility of non-mydriatic imaging without the use of bright intensity flash. Additionally the new technique had better resolution and could be used in the presence of media opacities [97]. SLO was introduced by Webb and colleagues [98] and was designed based on the principles of traditional ophthalmoscopes. The technique was very similar to scanning laser microscopes with the imaging sample always being the retina. Colour SLO images, which were introduced by Manivannan and co-workers [99], were a novel approach in differentiating pathological conditions; for instance, cotton wool spots and drusen which appear with the same colour can be easily differentiated from one another by features such as shape, size and texture.

Confocal scanning laser ophthalmoscopy (cSLO) provides an indirect measure of the RNFL by creating topographic images of the optic nerve head meaning that detection of back-reflecting light happens at a point that is conjugate to the focus of the illumination spot on the retina[100]. cSLO is commercially available as the Heidelberg retina tomograph (HRT; Heidelberg Engineering GmbH, Dossenheim, Germany). Tomographic information about the retina is then obtained from analysis of the amount of reflectedlight. The technique is capable of measuring elevation and volume as well as

estimating the retinal thickness. Previous research have also shown good reproducibility of topographicanalysis of optic disc by this method [101, 102].

2.7.4 Scanning laser polarimetry

Scanning Laser Polarimetry (SLP) is a modified form of SLOand was the next approach used for quantitative measurement of RNFL thickness. The method was developed by Weinreb and associates [76] and it was based on the assumption that the birefringent properties of RNFL change the polarisation of the laser beam that is reflected on the retina. Clinical application of the early SLO instruments was limited due to birefringence properties and artefacts caused by the cornea [103].

The first prototype of SLP was commercialised as the GDx Nerve fibre analyser (Laser Diagnostic Technologies Inc). Measurement is obtained at 1.75 disc diameters concentric to the disc and the method is developed in a way that works well even with dense cataracts. The field of view is 15 degrees and scanning is preferably performed through an undilated pupil. The polarized light is projected into the pupil and passes though the NFL. The problem with the birefringence effect of the cornea was overcome in the next generation, GDx VCC (Laser Diagnostic Technologies Inc) by application of a variable corneal compensator (VCC). Scanning a bigger field of view (20 × 20 degrees) of the parapapillary RNFL, the GDx VCC showed a better discrimination between the healthy and glaucomatous eyes [104, 105].

2.7.5 Optical coherence tomography

For over two decades, optical coherence tomography (OCT) has been used as a powerful diagnostic technique for chorio-retinal pathology along with ophthalmoscopy and fluorescein angiography. Retinal nerve fibre layer thicknesses in the current thesis have been measured by means of OCT. As such, principals of this technique and different types of OCT will be discussed comprehensively.

Optical coherence tomography is a reliable non-invasive method of optical imaging, capable of producing quantitative analysis of retinal morphology [106].It is currently the gold standard for assessment of posterior segment morphology [107]. OCT is comparable to ultra-sonic echo imaging technique except that light is being used instead of sound. OCT was first commercialized by Humphrey Instruments, Inc (now purchased by Carl Zeiss Meditec, Inc) in the mid-1990s and three different generations of OCT have been released since then. The first generation became commercialised in 1996 (OCT1, Carl Zeiss Meditec, Dublin, CA, USA) followed by OCT2 with axial resolution of 12-15 μm [108]. Stratus OCT (Humphrey Instruments, Dublin, CA, USA) is the third generation and was commercialised in 2002. Taking an image with a resolution of almost 10 μmin less acquisition time and therefore better recognition of the retinal layers, the technique has shown great diagnostic power in monitoring retinal pathologies [109]. Recent generations of OCT are based on a different optical system that acts quicker and provides higher resolution. Further information regarding these types of OCT has been provided later in this chapter (*Time domain vs Fourier domain*).

2.7.6 Optical principals of optical coherence tomography

Optical coherence tomography is based on the principle of low-coherence interferometry where distance to samples is measured by coherence property of light [110]. This occurs via a Michelson interferometer, which splits the light waves into two light beams (Figure 8).

An easy way of describing OCT is to compare it with ultrasound technique with light being used instead of sound. It is known that the velocity of light is almost 3 × 108 per meter, which is approximately a million times faster than sound waves. This allows imaging at a higher resolution [111]. Basic optical principle of OCT is that a low coherent beam of light is split into a reference beam and a sample beam by a mirror (beam-splitter). In order to create an optical echo (A-scan), the sample beam is reflected from the tissue at a particular distance while the back-reflection from the mirror occurs at variable distances. These two lights are recombined at the beam-splitter; however interference happens only when the path length travelled by the reference light matches the echo delay of the sample light [112]. Because of the natural differences in optical properties of tissues, various amounts of backscattering will happen. The echo time delay of back-reflected light can measure such differences in distance and dimension of tissue structures [111].

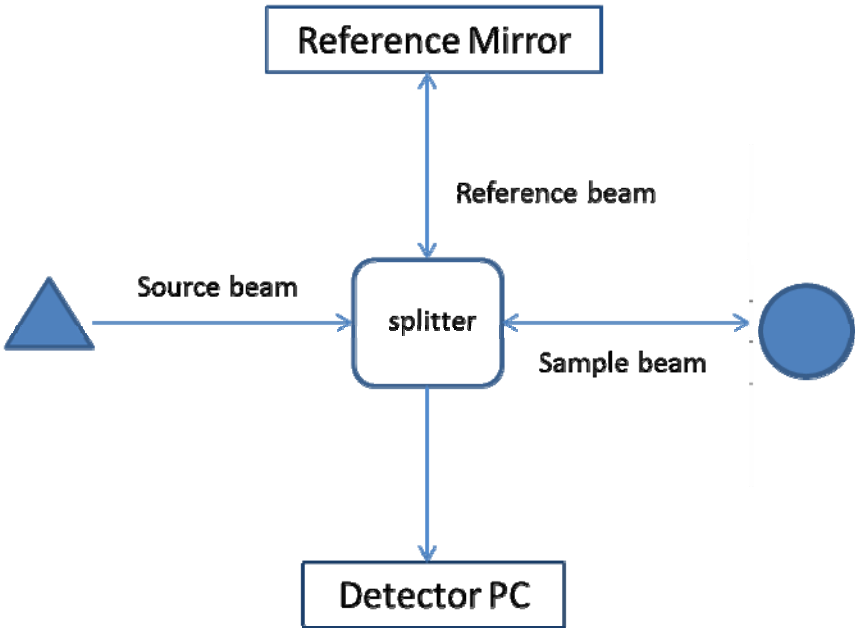


Figure 8.Optical basics of an OCT. Cross sectional images are captured by axially scanning the position of the reference arm and calculating the relative position of the retina. A two-dimensional cross sectional image (B-scan) is created by performing A-scans at various lateral positions.

Time domain vs Fourier domain optical coherence tomography

In a Time domain (TD) OCT the delay time of the beam reflected from the reference mirror is compared with the same beam back-reflected from retinal tissue at a known distance. The distance travelled by A-scans is determined by changing the distance to the reference mirror [113]. The light source then moves across the retina to create two-dimensional images of retinal tissue and the images are processed digitally for quality improvement [113]. Earlier models of OCT had a resolution of about 12-15 µm with the improvement of the technology in the latest commercial TD OCT. The instrument acquires 512 A-scans in 1.3 msec with an improved resolution of 10 µm compared with

previous instruments. Subtle eye movements seem to limit image acquisition in this system [113].

Fourier domain (FD) OCT was first introduced and used in vivo by Fercher and colleagues [114]. The method comes with a fixed reference arm and the OCT signal is acquired by changing the wave length of the light source or using an spectrometer as a detector [115]. FD OCT eliminates the artefacts caused by involuntary eye movements to a great degree [113]. Three-dimensional maps of the retina, increased scan acquisition speed to 18000 – 40000 A-scans per second and high resolution of the scans are among the many advantages of FD OCT. These factors improve visualization of macular pathologies, better definition for optic disc and RNFL thickness [116]as well as other layers of the retina [117].

2.7.7 Assessment of retinal nerve fibre layer thickness using Time domain and Fourier domain optical coherence tomography

Given that the RNFL measurements in the current thesis have been taken using FD OCT (RTVue instrument), it is important to understand the differences and the agreement between TD and FD OCT in quantification of RNFL.

Optical coherence tomography is compatible with other technologies in quantitative assessment of RNFL thickness and the method has been used widely in identifying retinal pathologies including glaucoma [110, 118]. The algorithm in the OCT system calculates the thickness of RNFL based on the reflectivity of the layer [119, 120]. A standard circum-papillary OCT image is acquired in a cylindrical scan pattern around

the optic disc at 3.4 mm diameter and the analysis is displayed in12clock position sectors around the disc. The graphical figure of RNFL thickness is often referred to as temporal-superior-nasal-inferior-temporal (TSNIT) or a "double-hump pattern" [121] with inferior quadrant in normal eyes having the greatest thickness followed by superior, nasal and temporal (Figure9).

There are only a few studies that have evaluated the agreement between TD and FD measurements. Vizzeri and associates [122]assessed reproducibility of FD and TD OCT outcomes for healthy and glaucoma subjects and reported agreement between the two methods; however, the thickness measured by TD was slightly higher than FD. They argued that the difference between the thicknesses measured by these instruments could be due to a more sophisticated algorithm used in FD OCT for defining RNFL boundaries. In another study, sensitivity of FD OCT was shown to be significantly higher than TD in differentiating glaucomatous eyes from normal ones [123]. Another study found the opposite results [124].Gonzales-Garcia *et al.* also reported that, despite great reproducibility of the two instruments, RNFL measurements by FD OCT were thicker than TD OCT outcomes [125].

The majority of studies have compared retinal thickness (rather than RNFL) measurements between the two types of OCT. Findings of the study done by Kiernan *et al.* showed that FD readings are about 43 μm greater than TD. This is potentially related to a different calculation of reference band and therefore retinal thickness in new generation of OCTs [126]. Similar findings were reported by Legarreta and co-workers where they found FD retinal measurements on average 50 μm greater than TD [127].These differences between the measurements were also confirmed by a recent

study where good reproducibility between the two OCTs was reported; however the thickness measured by TD OCT was 60 μm greater in the macula area [128].

The instrument used in the current thesis (RTVue) is the first United States Food and Drug Addministration (FDA) approved FD OCT. The normative database employed in the instrument for both RNFL and macular measurements compromise information from over 1000 people with their age, ethnicity and optic disc size have been taken intoaccount [107]. Although some studies suggest that the measurements produced by TD OCT and FD OCT are not entirely interchangable [125], FD generation preceds other generations of OCT by its capablility in detecting early retinal pathologies in the absence of visual function loss [129].

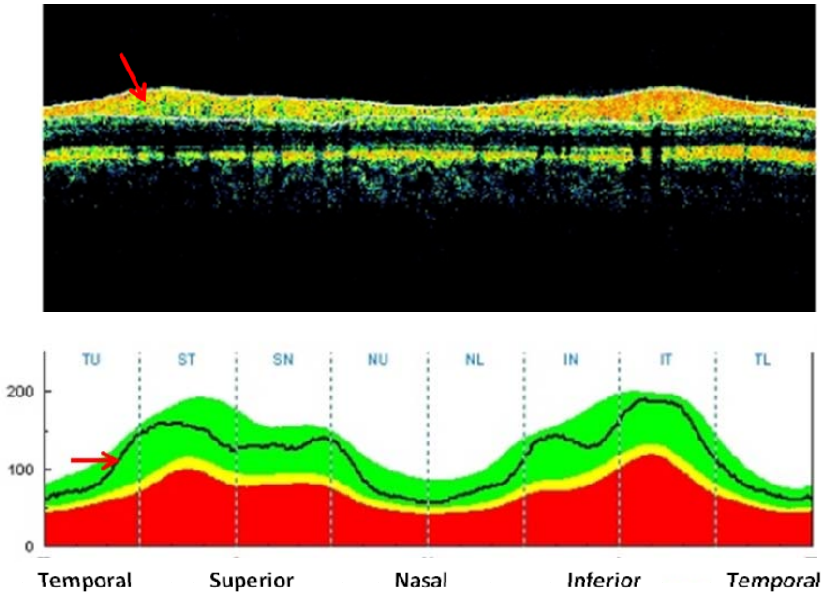


Figure9.A cross sectional optical coherence tomography image showing the double-hump pattern.The arrows indicate retinal nerve fibre layer. The colour-coded map below compares the captured thickness (black line) with the normative database (green: normal >99% reference limits, yellow: borderline 95-99% reference limit and red: abnormal <95% reference limit).

2.8 Evaluation of Retinal Nerve Fibre Layer in Diabetes

Evaluation of retinal nerve fibre layer(RNFL) is a useful method for recognizing likely axonal loss earlier than any evidence of visual field abnormalities [79].The RNFL has been the main focus in glaucoma studies given that changes in the features of the layer including thickness can be a vital factor in diagnosis of the pathology [130]. Many studies on retinal structure and visual function in diabetes have prominently focused on the retinal vascular abnormalities [131, 132]. However, some other studies have shown that structural damage and functional impairment occur when no ophthalmic sign of retinopathy is visible [133-138]. Although a potential relationship between the presence of retinal microvasculopathy in diabetes and severity of DPN has been suggested [26]; there is no clear link between retinopathy and neuropathy, and only a limited number of studies have investigated this relationship [26, 139].Given that neural defects in diabetes can be caused by a variety of mechanisms including polyol pathway changes, hypoxia and oxidative stress [36], it is reasonable to question whether such mechanisms can also damage the RFNL and, as such, whether RNFL changes can predict peripheral neuropathy elsewhere in the body.

Chihara*et al.*[134] measured RNFL thickness in 137 patients with type 2 diabetes using black and white negatives photographs and classified retinopathy into four groups with level 1 showing minimal retinopathy. Their results demonstrated that 20% of the study cohort, classified as level 1 retinopathy, has evidence of RNFL defects. They suggested that cotton-wool spots, as occur in more advanced stages of retinopathy, are likely to cause retinal nerve fibre layer defects. They also found a positive correlation between RNFL defect and systolic blood pressure, which can cause an asymptomatic vascular

lesion and hence a localized RNFL defect. In this study, participants with level 1 retinopathy who had diabetes less than 10 years were excluded from the analysis as the major aim was assessment of RNFL thickness in association with retinopathy.

Lopes de-Faria and colleagues [140] conducted the first quantitative assessment of RNFL thickness in a small sample of patients with type 1 diabetes using scanning laser ophthalmoscopy. Their results showed a significant reduction of mean RNFL thickness in the superior quadrant for a cohort with diabetes who had no ophthalmoscopically evident retinopathy. They also suggested that involvement of retinal neural components happens along with vascular complications of the retina and the detected thinner RNFL areas (in their study superior quadrant) have more susceptibility for future vascular damage. Another study by Skarf and associates [141] confirmed the results.

Sugimoto *et al.*[135] were one the early groups who evaluated RNFL thickness in 32 people with type 2 diabetes by means of OCT. Their results showed a general reduction in RNFL thickness in all quadrants for a diabetic cohort without evident retinopathy, but the reduction was more significant superiorly, in agreement with earlier SLP findings [140].

In contrast, a recent OCT study failed to find significant difference in RNFL thickness between diabetic group without retinopathy and controls; although those with proliferative diabetic retinopathy demonstrated reduced RNFL thickness globally and in all quadrants [142]. Their findings also showed that RNFL thickness reduction is more evident in proliferative stages of diabetic retinopathy and correlates with a longer duration of diabetes. They suggested that RNFL damage caused by diabetes maybe

more sensitive and prone to faster progress in men than in women but did not provide any further information with this regard.

The studies discussed above have all considered changes to the retinal nerve fibre layer thickness in the absence of vascular complications. Although these are different with regards to theirstudy cohort, types of diabetes and means of assessing RNFL thickness, the final outcome showedthinning of RNFL prior to clinically evident diabetic retinopathy. However, none of these researches have considered the effect of diabetic neuropathy on retinal neural structure. Findings from these studies are supporting evidencethat reduced RNFL thickness may substantially be caused by neuropathic status of the participants. The work in the current thesis is aiming to address this question.

2.9 Diabetes Induced Retinal Pathophysiology

The concept of neurodegeneration as a component of diabetic retinopathy has been a subject to debate for many years. Retinal neuro-degeneration as the primary reason for vasculature changes in diabetes was first suggested and examined by Wolter [143], where they noticed pkynosis (degeneration of cell nucleus)of retinal neural cells in post-mortem diabetic retinae. A further study by Bloodworth [144] on 295 post-mortem human retinas showed apoptosis of retinal ganglion cells. Apoptosis involves a series of biochemical changes, which lead to cell shrinkage and eventual regulated cell death [145] and is known to happen in retinal degenerative diseases such as glaucoma [146]. Barber and associates [63] were one of the early groups who reported a diabetes-induced increase in apoptosis of retinal neural cells in rats and humans. They observed that the majority of apoptotic cells in streptozocin-induced diabetic rats were ganglion cells. Given that the ganglion cell bodies and dendrites (IPL), as two major components of

ganglion cell complex, can degenerate in diabetes, it can be assumed that the damage caused from apoptosis may include axons of the cells (RNFL) consequently [63].

Neurodegenration of the retinal glial cells has also been shown to occur due to high metabolic stressin diabetes [147]. Müller cells are the major glial cells of the retina, which provide support and nutrition for the neurons. They radiate from the soma in the inner nuclear layer to the inner border of the retina, adjacent to the vitreous. Some branches of the main trunk of Müller cells form the sheath that surrounds neural cell bodies, dendrites. The sheath also surrounds axons of ganglion cells in the optic nerve [59]. One of the main functions of these cells is to support endothelial cells biochemically to form a blood-retinal-barrier. Apoptosis of retinal glial cells includingMüller cells may also contribute to microangiopathy and barrier impairments [63]. Additionally, these cells play the important role of up-taking glucose from the retinal circulation and transferring energy to neurons in the retina. High concentration of glucose in neural parenchyma as a consequence of a hyperglycaemic condition, which happens in increased permeability of the blood-retinal level, leads to dysfunction of glial and neural cells [148]. Furthermore, Müller cells act as a transporter to remove glutamate, which is very toxic to the retinal neurons, from the extracellular space and there is likelihood that impaired function of these cells in diabetic retinae can cause oxidative stress – a potential factor in pathogenesis of DPN [149].

Other possible diabetes-induced retinal pathophysiology includes the role of Vascular Endothelial Growth Factor (VEGF) secretion in the neural retina. VEGF is thought to have a contradictory effect on retinal vascular permeability in the hypoxic areas as well as reducing retinal neural apoptosis [150]. VEGF has also been proposed as a factor in pathogenesis of diabetic peripheral neuropathy [36]. Additionally, increased

phosphorylation of neurofilament proteins that creates focal swelling in larger axons such as retinal nerve fibres, has beenshown to be associated with neurodegenerationof neural cells and impaired axonal transport [151, 152].Similar increases in phosphorylation ofneurofilaments have been identified in peripheralnerves of diabetic rats and humans [153].

In conclusion, diabetic neuropathy has been suggested to affect both central and peripheral nervous systems. This histological body of evidence suggest thatpathophysiology of the retinal neural components, structurally a part of CNS, is associated with diabetes. Although none of these studies have considered the effect of neuropathy in creating such changes to neural components of the retina, their findings still suggest that neuropathy may potentially contribute to such damage.

2.10 Evaluation of Visual Function in Diabetes

Current standard diagnosis factors of retinopathy as provided by Early Treatment for Diabetic Retinopathy Study (ETDRS) [132]are based on the progression of retinal micro-vascular complications.; however, several studies have shown evidence of visual function deficits in diabetes in eyes with normal visual acuity and minimal presence of diabetic retinopathy[154-156]. It has been argued that diabetic retinopathy should not be assessed solely as a vascular complication and the same argument applies to diabetic neuropathy as not being considered as exclusively a neural disease [157]. Studies of anatomy and physiology of diabetic retinae can provide evidence of a potential relationship between early pathology of retinopathy and interruption in retinal neural component. A number of these studies and their methods of assessing visual function are described below.

2.10.1 Electroretinogram

Electrical activity of the retina in association with diabetes-induced neural deficiency has been investigated in a number of research studies. Electroretinogram (ERG) detects functional and biochemical changes at the retinal level and there is good evidence that it is impaired in diabetes before the onset of retinopathy [158, 159]. Di Leo *et al.* [160] and Caputo et al. [161] found reduced pattern ERG (PERG) amplitudes in diabetic patients without retinopathy. Multifocal ERG (mfERG) evaluates small areas of retina individually and is valuable for assessing diabetic pathologies such as microaneurysm and cotton-wool spots that may affect visual function in spatially localised patches [162]. Multifocal ERG (mfERG) evaluates small areas of the retina individually, which can be particularly important in assessing diabetic retinal pathologies including microaneurysms, cotton-wool spots and other pathologies that occur in local patches [162]. The technique has also been used to study early functional changes of the retina in diabetes, which may precede retinopathy. Significant reductions in the direct response amplitude and implicit times in diabetic patients with no evidence of retinopathy have been reported [163-165]. Other studies have shown that the onset of oscillatory potentials (OP) is delayed in diabetes in the absence of retinopathy [166, 167]. OP wave components are believed to originate from inner retinal layers through light-induced interactions between amacrine, bipolar and ganglion cells [168, 169].

2.10.2 Visual evoked potential

Studies of visual evoked potential (VEP), the P100 and P300 latencies have also been conducted in people with diabetes. VEP is known to provide objective information about visual function and is useful in detection of neuro-sensory disorders of visual pathways

[170, 171]. Most studies have shown significant increase in P100 latency in people with diabetes compared with controls [172-174]. Interestingly VEP (P100) gained attention as an approach to possible neuropathy of the central nervous system in individuals with diabetes [175]. Two studies have shown positive relationships between peripheral nerve conduction and P100 latency in the absence of retinopathy suggesting a potential effect of neuropathy on optic pathways [176, 177]. One study on P300 have also found prolonged latencies related to diabetes in people with normal cognitive function and no retinopathy [178].

2.10.3 Standard automated perimetry

Very few studies have investigated the ability of commercially available standard automated visual field tests to detect contrast sensitivity changes in diabetic individuals.A number of studies have compared the efficacy of short-wavelength automated perimetry (SWAP) and other forms of perimetry for the detection of early psychophysical abnormalities in diabetes [179-181].However more investigation is required before efficacy of any of these techniques can be ascertained. The earliest investigations relied on manual perimetry techniques. Roth *et al.*were one of the early groups to investigate the effect of diabetes on visual function using a custom-designed scotometer [182, 183].They assessed the central 20 degrees of visual field and reported an occurrence of scotomain people who did not have retinopathy. They also suggested that existence of a scotoma can be a pre-retinopathic indicator. Wisznia and colleagues investigated visual field defects at various stages of diabetic retinopathy using Goldman perimetry [184]. Their results showed a partial constriction of the central isopter of the visual field in diabetic patient with non-proliferative diabetic retinopathy. However, there is evidence that manual perimetry does not always detect visual field deficits, even

in presence of substantial loss of neural cells [185].The evolution of static automated perimetry(SAP) enabled quantitative analysis of contrast sensitivity for a well defined grid of test points, improving the potential for visual field analysis techniques to detect earlier, spatially specific changes in visual sensitivity [185].Trick *et al.* used the method of automated perimetry to examine the extent of visual field sensitivity in both type 1 and type 2 diabetic patients who had no, minimal or mild retinopathy [186]. Their results showed a significant higher pattern deviation and lower mean deviation values for diabetic patients compared to the controls. Their analysis of the sub-groups revealed that the mean deviation in both type 1 and type 2 diabetic patients was dependent on the level of retinopathy. Bell and Feldonfound isolated loss of sensitivity in the central 15 degrees of visual field in participants with normal retinal perfusion and suggested that the loss may have been caused by retinal microangiopathy which may serve as an index for retinal glial deficits [187].

2.10.4 Flicker sensitivity

Flicker threshold is the ability of an observer to detect intermittent light and dark alternations of a visual stimulus. Most studies suggest that flickering stimuli are perceived by magnocellular pathways [188, 189].This pathway is characterized by fast conduction velocity, sensitivity to transitory changes in retinal stimulation and ability to detect movement [190].Flicker electrtoretinogram has been used to demonstrate impaired visual sensitivity in diabetes [138].Lobefalo*et al.* investigated flicker sensitivity in a group of children with type 1 diabetes who did not have any clinical signs of diabetic retinopathy [191]. They divided their participants into two groups according to their metabolic control (poor and good). They found significantly lower mean flicker fusion frequency values for both groups compared to their age-matched control

participants and outcomes were also highly related to degree of metabolic control. The authors suggested that flicker sensitivity impairment in the absence of clinically detectable diabetic retinopathy and media opacities can be a result of nerve layer abnormalities in people with diabetes.

Stavrou and Wood evaluated flicker sensitivity as a function of central visual field in a cohort with type 2 diabetes and compared their findings with the results obtained for static perimetry. Their results revealed that the majority of the defects found by flicker perimetry occurred in the central 6 degrees, while defects shown by static perimetry were more towards the periphery. Their hill-of-vision analysis for flicker perimetry also showed a significant depression in the central 6 degrees [192]. In a recent study, Zele*et al.* found loss of sensitivity in people with diabetes for both red-on-white and white-on-white flicker and static perimetry across the entire retinal field (central and periphery) when compared to the age-matched control group [193]. However, they indicated that red-on white perimetry is more capable of detecting deeper field defects than standard white-on-white.

The metabolic condition in diabetes has been suggested as a potential reason for reduced flicker sensitivity in various pathologies including diabetes and age-related macular degeneration [191, 194]. It has been shown that a flicker stimulus increases capillary blood flow by 30%, indicating that microvasculature and metabolic demand of the retina are tightly linked [195]. Mandecka*et al.* [196]suggested that flickering light increases the diameter of retinal vessels in a healthy retina. They showed that normal flickering-induced vasodilatation of retinal microvasculature was diminished in participants with type 1 diabetes without clinically manifested retinopathy.

2.10.5 Frequency doubling technology

Frequency doubling technology (FDT) has been proposed as a useful predictor of glaucoma at early stages [197, 198]. Frequency doubling describes a phenomenon where alternating light and dark bars appear to have twice the actual number of bars. This happens when a low spatial frequency sinusoidal grating undergoes high temporal frequency counterphase flicker [198]. The perception of this phenomenon is known to be mediated by magnocellular retinal ganglion cells [199]. Parikh and associates examined the ability of FDT to detect visual field defects [200]. Their results showed that the screening programme of FDT (20-1) is capable of detecting visual field defects in retinopathy; however it fails to detect macular oedema in people with mild and moderate non-proliferative retinopathy. Parravano*et al.* [201] also examined the role of FDT in diagnosing field defects at an early stage in people with type 1 diabetes. The authors suggested that reductions in retinal sensitivity in people with diabetes may be related to dysfunction of magnocellular pathway-related retinal components, as these are more likely to be damaged under hyperglycaemic conditions. They further suggested that these visual function changes may be a result of neural loss, implying that neuropathy rather than vasculopathy is the primary underlying mechanism.

2.10.6 Colour vision

Impaired colour vision has been reported to be one of the early signs of visual dysfunction in diabetes [202, 203]. Acquired blue-yellow losses as measured by FM-100 test in the diabetic population have been reported to occur before the onset of retinopathy [204]. Hardy *et al.* found abnormal colour vision in 57% of their study cohort with no evidence of retinopathy [137]. Roy *et al*. also reported colour vision

losses in an insulin-dependent diabetic sample with minimal retinopathy [205]. These findings suggest that colour discrimination losses in diabetes may not be of vascular aetiology.

2.10.7 Contrast sensitivity

Contrast sensitivity measurements can revealvisual function defects that are not detected by visual acuity charts. Changes in contrast sensitivity have been demonstrated in children and adults with diabetes. Several studies have found a reduction in contrast sensitivity at different spatial frequencies in diabetic patients [136, 155, 206, 207]. A few studies have compared contrast sensitivity in participants with well-controlled diabetes opposed to those with poorly controlled disease. Della Sala*et al.* showed contrast sensitivity more than two standard deviations below normal values in a diabetic cohort when compared with age-matched controls [154]. Ghafour*et al.* reported increased thresholds at high spatial frequencies in diabetic patients without retinopathy [207]. Mackie *et al.* also reported a significant loss in contrast sensitivity thresholds obtained by Pelli-Robson chart in patients with no retinopathy as compared with those with background retinopathy [208]. Another group also found similar results [209]. A number of studies have investigated the impact of metabolic control of diabetes on contrast sensitivity. Di Leo *et al.* suggested that hypoglycaemia in patients with type 1 diabetes may contribute more to neuronal damage than a hyperglycaemic condition [160]. Ewing *et al.* also found contrast sensitivity deterioration during hypoglycaemia in type 1 diabetic participants who had no evidence of retinopathy [210].

2.10.8 Other measurements of visual function

A number of other psychophysical measurements such as dark adaptation response have also been investigated in diabetes [133, 211, 212]with a number of groups focusingon post-photocoagulation outcomes [213, 214]. Some findings have suggested longer adaptation time occurs in diabetes and that the final light threshold is higher than in age matched norms [212]. Another group reported that loss of adaptation is related to progression of retinopathy at different stages but that changes were also observed before the onset of vasculopathy [215].

Thesefindings provide important insight to considering mechanisms other than diabetes-induced retinal vasculopathy, in this case diabetic neuropathy, as potential reasons for loss of visual function.

2.11 Overall Rationale and Research Questions

Diabetic peripheral neuropathy is one of the major complications of diabetes. More than 50% of individuals with diagnosed diabetes will suffer from consequences of peripheral neuropathy including foot ulcerations and amputations [48].A high percentage of these endpoint complications can be prevented if they are diagnosed at early stages. Therefore effort in establishing early, less invasive and comfortable diagnostic tools should be a priority aim of public health associations.

Studies outlined in this chapter have reported significant association between reduced retinal nerve fibre layer thickness and diabetes in the absence of retinal vascular complications. Similar findings have been reported for visual function in people with

diabetes. Much of this research, however, is based on diabetes-induced retinal vascular deficits. As such, there is little direct research relating to the potential role of diabetic peripheral neuropathy in retinal anatomy and visual function changes.

The primary aim of the current thesis was to evaluate retinal nerve fibre layer thickness and its association with the severity of diabetic peripheral neuropathy using two different techniques of assessing neuropathy (Neuropathy Disability Score and Quantitative Sensory Testing). The secondary aim of the current work was to investigate two measures of visual function (contrast sensitivity and flicker sensitivity as derived from automated perimetry techniques) and their associations with severity of diabetic peripheral neuropathy.

The following research questions are addressed in this thesis:

Q1: Is retinal nerve fibre layer thickness associated with peripheral neuropathy in type 2 diabetes?

Q2: Is there an association between contrast sensitivity (using visual field measures) in and the severity of diabetic peripheral neuropathy in type 2 diabetes?

Q3: Are retinal nerve fibre layer thickness and/or contrast sensitivity measures potential new ophthalmic markers for assisting with the diagnosis of peripheral neuropathy in type 2 diabetes?

Hypotheses specific to these research questions are presented in the relevantchapters.

3 Methodology

The contents of this chapter outline the common methods and recruitment procedures employed throughout the constituent chapters. The methodologies pertinent to the outcome measures for each component are described in more detail in the relevant chapters.

3.1 Study design

This project was as a case-controlled, cross-sectional study and was carried out as the baseline examination of a five-year longitudinal study on patients with type 1 and type 2 diabetes. However, the data collected for individuals with type 2 diabetes are incorporated for analysis in this thesis. All medical and ophthalmic examinations took place at Anterior Eye Lab, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.

3.2 Ethical approval

The projected granted ethics approval from Queensland University of Technology (QUT) Human Research Ethics Committeeand Princess Alexandra Hospital Human (PAH) Research Ethics Committee, Brisbane and the investigation was conducted in accordance with thetenets of the Declaration of Helsinki.

3.3 Participant recruitment

Participant recruitment commenced after the project achieved ethics approval. The PA hospital patient database was searched for individuals with type 2 diabetes and recruitment began in January 2009. The database contained 1265 patients with type 2 diabetes amongst which 410 eligible patients were identified to be included according to the inclusion-exclusion criteria. Letters of invitation and study information brochures (Appendix 1) were sent to the identified individuals. Follow up telephone calls were made after two weeks if no response was received from the invitees. Details regarding response rate are shown in Table 5.

Table 5.Participant responses to invitation to participate in the study.

Action	Number(percent)
Invitation letters mailed	410
Patient-initiated bookings	12 (3%)
Follow-up phone call initiated bookings	88 (21%)
Not eligible	19 (5%)
Unable to contact	289 (71%)

Individuals who passed the pre-screening questionnaire conducted on the telephone and were interested in participating were sent a study package. Each package contained the Information and Consent Form, a personalized letter with a brief explanation of the procedure including the date, time and location of the study and a map to assist them with locating the facility at QUT. To minimize inconvenience, transport in the form of taxi vouchers was provided to individuals and parking was provided for those who chose

to drive. Recruitment ceased in August 2009 with 105 participants being enrolled in the study. Efforts were made to have equal representation of males and females. There was no gender bias with respect to enrolment.

3.4 Eligibility

A number of inclusion and exclusion criteria were established prior to participant recruitment to ensure that the study cohort was consistent with the research hypotheses.

3.5 Inclusion criteria

- Age between 18 to 75 years
- Type 2 diabetes
- Capable of complying with the study protocol
- Signed written consent

3.5.1 Ophthalmic exclusion criteria and rationale

- History of ocular trauma or surgery including previous retinal laser photocoagulation for diabetic retinopathy, glaucoma surgery, laser therapy for vitreous detachment.

- History of retinal disease including age-related macular degeneration (ARMD),or glaucoma as these conditions affect the visual sensitivity.

- Concurrent ocular infection or inflammation.

- Visual acuity ≤ 6/9 to allow inclusion of participant with minor retinopathy changes and minimal macular oedema [192].

- Spectacle prescription less than ±6.00 DS/ ±2.5 DC [193] as it reduces quality of scans and photographs.

- Intra ocular pressure (IOP) ≤21 mmHg in the test eye as it may be a risk for glaucoma.

- Diabetic retinopathy not greater than 'moderate' according to Australian National Health and Medical Research Council (NHMRC) grading scales [216].

3.5.2 Medical exclusion criteria and rationale

History of the following systemic conditions:

- Treatment for psychiatric disorders such as bi-polar or schizophrenia , as patients with such conditions may have difficulty coping with informed consent and/or other aspects of the protocol.

- Pancreatic or renal transplant or currently on dialysis therapy, as it regenerates the peripheral nerve fibres [28].

- Viral hepatitis (B and C) due to risk of infection

- Diabetic foot ulcers (not previous healed ulcers).

- History of neuropathy due to a non-diabetic cause for example alcoholism, amyloidosis,

- autoimmune disorders, chronic kidney failure, connective tissue disease, infectious disease (e.g. HIV/AIDS, hepatitis B, leprosy), liver failure, radiculopathy, vitamin deficiencies (e.g. pernicious anaemia)so as to exclude causes of observed neuropathy other than diabetes.

- Participation in any other interventional research studies, to avoid any conflicts of interest.

3.6 Statistical Considerations

3.6.1 Sample Size Analysis

To address hypotheses 1 and 2 of this thesis (i.e. are retinal nerve fibre layer thickness and contrast sensitivity associated with peripheral neuropathy in type 2 diabetes) the following sample size formula for correlation between two continuous variables was used:

$$n = 3 + \frac{4C}{\left[\ln\left(\frac{1+r}{1-r} x \frac{1-r_0}{1+r_0}\right)\right]^2}$$

[217]where ***n*** is the number of individuals needed to show that a postulated (positive) correlation coefficient ***r*** is different from a specified r_0; ***C*** is a constant that depends on the values chosen for α and β (C=10.50 for 90% power and C=7.84 for 80% power). For α = 0.05 and 1-β = 0.80 and a postulated correlation between RNFL/Contrast sensitivity and several measures of neuropathy of 0.45, a sample of 36 is required. For α = 0.05 and 1-β = 0.90 and a postulated correlation between RNFL/Contrast Sensitivity and QST of 0.45, a sample of 48 is required.

For comparisons between groups the following formula was applied to calculate sample:

$$n = \frac{I^2 s^2}{d^2}$$

where***I*** is a constant that depends on the values chosen for α and β (C=3.24 for 90% power and C=2.80 for 80% power), ***s*** is the standard deviation and ***d*** is the estimated clinically meaningful difference between groups. In this instance d=10 microns and s= 15 microns for RNFL. Sample estimates have been addressed in each subsequent study.

Since recruitment of the same number (n=45) for the control group could not be achieved (24 recruited), it was decided to increase the number of diabetes participants. Recruitment of 45 individuals per neuropathy group (see *section 3.7.2.2*) was not successful due to time constrains of the study. Hence it was attempted to recruit as many participants with Type 2 diabetes as possible. A total of 105 diabetic participants were recruited but only 82 met the eligibility criteria.

3.6.2 Statistical Analysis

Descriptive statistics (including mean, standard deviation, range and median) were calculated for all variables. Normally distributed continuous data were summarised using mean and standard deviation (SD) statistics. Continuous data not normally distributed were summarised using median and range statistics.

Specific statistical analyses undertaken for each data set are detailed in the relevant chapters. All linear regressions were assessed for model fit and residuals were examined to confirm the model assumptions for normality, linearity and homoscedasticity.

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) (version 18.0, SPSS, Chicago, IL) and Microsoft Excel Version 2007 (Microsoft Corporation, 1997). The significance criterion was set at $p < 0.05$.

3.7 Examination procedure

3.7.1 Information and consent form

Written informed consent was obtained from each individual (Appendix 2). Recruited individuals had the opportunity of reading the information sheet and consent documents prior to their appointment, and their signature in the presence of a witness was obtained after further explanation of the study procedures was conducted on the day of testing.

3.7.2 Medical examination

After obtaining informed consent, participants were asked to answer explicit questions about their general systemic conditions. A case report form sample is provided in Appendix 3.

3.7.2.1 Quantitative sensory testing

Quantitative sensory testing (QST) in the assessment of neuropathy, as Dyck describes it, is analogous to testing visual acuity in eye examinations [26].QST has shown to be a relatively sensitive, reliable and accurate method of assessing these modalities [218]. It is widely used in clinical trials as a non-invasive method for investigation of sensory neuropathies [18]. QST has a range of sensation modalities to assess the function of various nerve fibers. Small nerve fibers of the peripheral nervous are responsible for mediating the sensation of warmth, and pain while the larger fibers mediate the sensation of cold and vibration [36].

Thermal testing instrument

The Neurosensory Analyzer Model TSA-II (Medoc Ltd**.,** Ramat Yishai 30095, Israel) is a computerized device specifically designed for quantitative assessment of peripheral nerve fibres functionality (Figure 10). The system measures thresholds for cold, warmth, cold-induced pain and warm-induced pain using a method of limits (*see below*). The instrument allows comparison of the results with normal population values that are based on age, gender and anatomical site to differentiate those with and without peripheral nerve diseases [219].

Vibration testing instrument

The Vibratory Sensory AnalyzerVSA-3000 is a device used to quantify the perception of vibration in hands or feet as the function of large A-beta fibres. The test is performed in 5-10 minutes and the acquired results are presented in comparison with the age-matched normative database values. The stimulator is a platform to support the foot or hand with a button on the top where the hallux (big toe) or ball of hand rests.

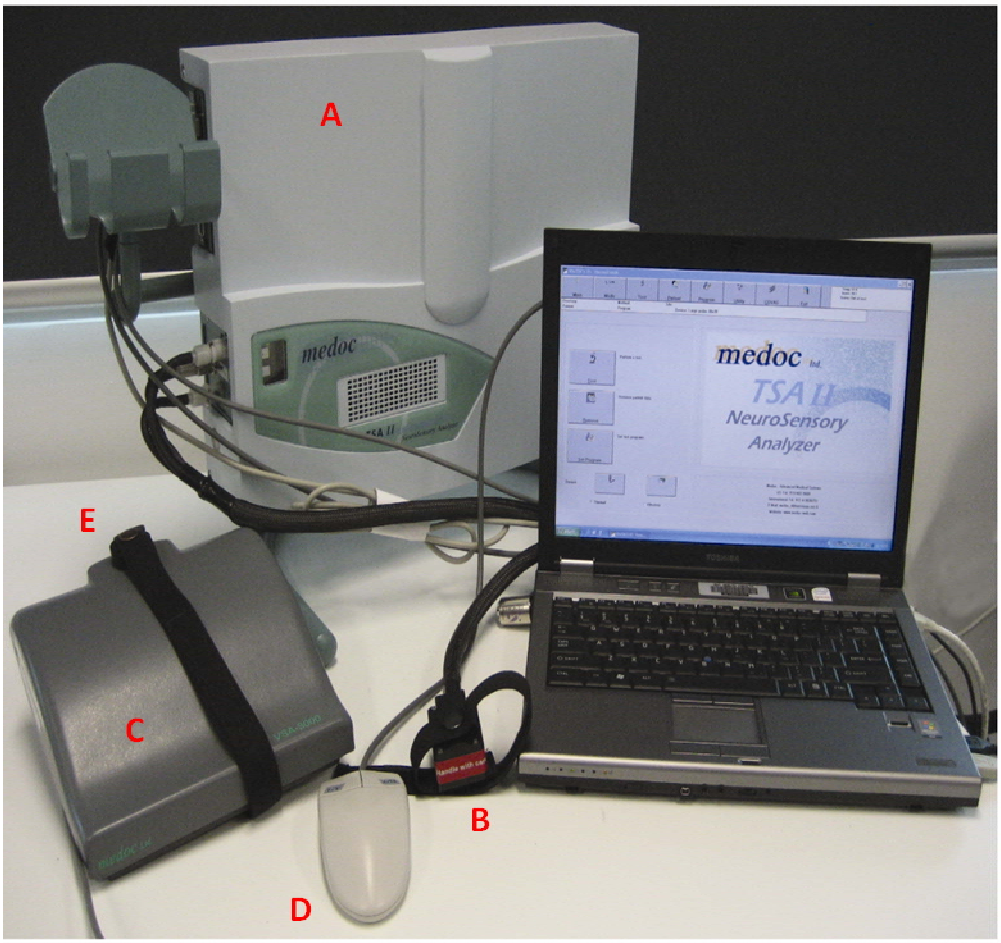


Figure 10.Quantitative sensory testing apparatus (Medoc Ltd).Components include neurosensory analyzer (A), thermode (B), vibratory sensory analyzer (C),patient response button (D), and vibration stimulator (E).

Method of threshold determination

Several algorithms have been developed for the purpose of sensory threshold detection to minimize the subjective influences on the results. Medoc TSA-II and VSA-3000 have provided an overview of the threshold determination in the instruments manual. All the paradigms mentioned below are applicable to both TSA and VSA components (Figure11).

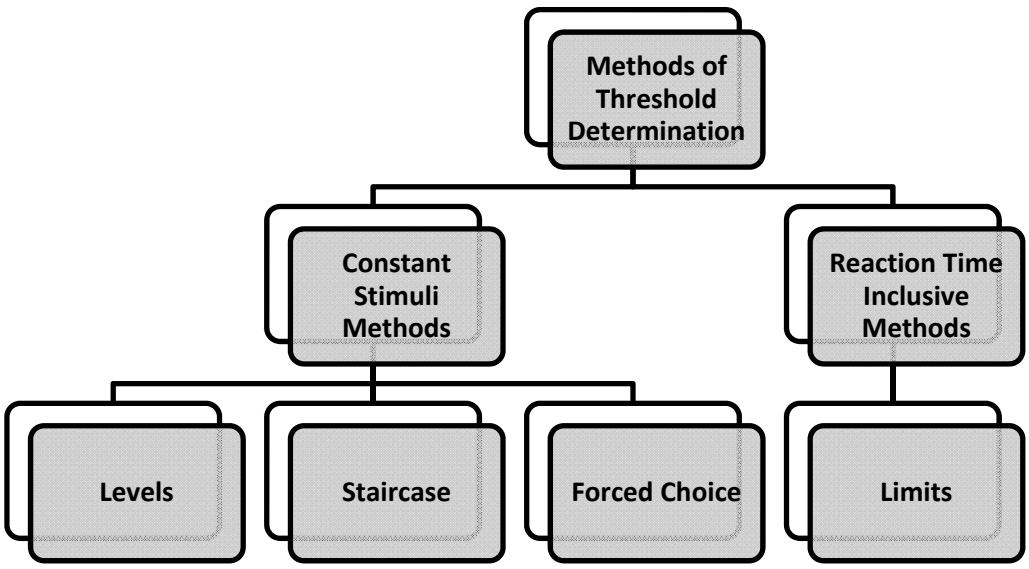


Figure11.Methods of threshold determination using Medoc TSA II and VSA – 3000 instruments.

Method of Limits

The "Method of Limits" was selected for the purpose of threshold determination in the entire QST experiments of the current thesis. In this method the subject is exposed to a stimulus of decreasing or increasing intensity and is asked to express the first onset of sensation. Once the stimulus is perceived, the test will be halted by the participant. Reaction time artefact can increase the value of the threshold specifically when measuring thermal sensation [220]. However this can be diminished by applying a slower rate of temperature change. The method of limits is the most commonly used since it takes less amount of test time. Additionally, pain threshold measurements as well as non-painful thermal threshold are assessable using this method.

Repeatability and reproducibility of quantitative sensory testing

In clinical settings, 'method of limits' is the most often adopted strategy for QST measurements [221], which by comparison to other methods such as 'method of levels', is rather quick and easy to perform. However, in previous studies thismethod has shown a varying degree of reproducibility and variability[222, 223]. Such variations occur as the result of differences in methodological strategies and statistical analyses. Hence, a direct comparison between studies is somewhat difficult. Table 6 represents a number studies that have investigated the reproducibility and repeatability of QST outcomes by means of different QST instruments.

Table 6.Reproducibility studies on quantitative sensory testing

Ref	Subjects	Interval	Modality	Equipment	Methods	Measure of reproducibility	Conclusion
Meier et al. [224]	101 HI, age 6-17 years	2 – 4 weeks	Warm Cold vibration	Medoc TSA 2001 (Medoc, Ramat, Yishai, Israel)	**Warm, cold thresholds:** a. Method of Limits. Skin adaptation temperature 32ºC, average of 4 readings, b. Method of Levels. Yes or no response. Initial temperature step of 3ºC. **Vibration:** a. Method of Limits. Linearly increasing train of 4 stimuli. Intensity starting at 0 µm amplitude and increased at 0.1 µm/s. Threshold = average of 4 consecutive determinations	Wilcoxon signed-ranks test with a Bonferroni correction for multiple comparisons. Hand and foot. p>0.05	No significant differences between two session for any of the measurements
Yarnitsky et al. [225]	72-76 HI, age 20-59 years	2 weeks	Heat-pain	Medoc TSA-2001 (Medoc, Ramat, Yishai, Israel	Method of Limits. Skin adaptation temperature 32ºC, rate of temperature change 2ºC/s, average of 3 readings, 20 s inter-stimulus interval.	**Correlation coefficient 'r' value:** Thenar 5.85** Foot 4.47 **significant intersession bias	Poor repeatability for thenar but sufficient for foot
Yarnitsky et al. [222]	72-76 HI, age 20-59 years	2 weeks	Warm Cold	Medoc TSA-2001 (Medoc, Ramat, Yishai, Israel	**a. Method of Limits.** Skin adaptation temperature 32ºC, rate of temperature change 1ºC/s, cold and warm perception thresholds, average of 3 readings **b. Method of Levels**. Yes or no response, initial step of 4º0C, reduced by half until step size reached 0.2ºC. **c. Method of Levels.** Staircase algorithm (Fowler 1987), yes or no response, initial temperature step of 4ºC, subsequent steps at 1ºC, then 0.2ºC, test terminates after 4 "no" responses.	**Correlation coefficient 'r' value:** a. Thenar cold 1.964** Warm 1.587** Foot cold 3.778 warm 4.298 b. Thenar cold 1.040 warm 0.572 Foot cold 3.016 warm 3.758 c. Thenar cold 1.144 warm 0.720 ** significant intersession bias	Poor Good Good Good Good

Examination procedure

The participant was seated on a comfortable upright chair in a quiet room to avoid any distraction. The test room was kept at a temperature in a range of 18 – 22˚C. It was mandatory that the participant had not taken any tranquilizers or stimulants in the preceding of 12 hours as well as not taken more than one hot drink prior to the test. As recommended by the manufacturer (Medoc Ltd**.,** Ramat Yishai 30095, Israel), the foot skin surface should be in the range of 30 - 35˚C; therefore the foot temperature was measured using an infrared thermometer. If the foot temperature was below the required temperature, a heating pad or a hot water bottle was used to warm up the foot. Participants were positioned in a way that they could not see the test monitor at all times. The test foot was always chosen according to hand dominancy and exceptions were made in cases of amputation and/or active ulcers. The order of the tests was as follows:

Thermal sensation assessment

The TSA-II model is equipped with a thermode (30 × 30 mm) to heat or cool the skin. The thermode was attached to the dorso-lateral site of the test foot in a flat manner where it had the most contact with the skin. The initial temperature of the thermode is between 30 - 32˚C. Therefore, soon after the contact between the thermode and skin, participants felt neither cold nor warm. As described earlier, the Method of Limits was chosen to assess cold sensation (CS), warm sensation (WS), heat-induced pain (HIP) and cold-induced pain (CIP). Temperature sensation measurement was performed in a randomised order. Minimum participant details (ID code, gender, date of birth) were

entered on the system and the correct anatomical site was chosen. A response button was given to the participant and the following instructions were given:

Thermal sensation instruction

"We wish to determine if you can detect these temperature changes. The test will involve either raising or lowering the temperature in the probe attached to your foot from a baseline temperature, which is the current temperature of the probe. It is important that you press the button at the first moment that you detect a temperature change, as such; keep your finger on the buttons so that you can respond quickly. However, do not press the button until you are confident that you have felt a change in temperature. After pressing the button, please indicate to me if it was a warm or cool sensation that you felt. Pressing the button will turn the thermal device off. We will repeat this procedure several times to obtain consistent readings. Following each response from you, the probe will return to baseline temperature, and then begin again. The computer will make a key stroke sound to indicate that the probe has returned to baseline temperature and the next procedure."

Thermal pain instruction

"The test has now changed. The probe will heat up or cool down slightly faster. Let the probe temperature change from the initial baseline temperature, and allow the temperature to get warm or cold without pressing the button. Wait until you feel some degree of discomfort or pain. Again, keep your finger on either of these two buttons so that you can respond quickly. Press either of the two buttons the instant you feel discomfort or pain. This is not a test of how long you can endure pain. Rather, we want

to know the instant you decide the sensation is painful. Please stay alert and concentrate throughout the test. Pressing the button will turn the thermal device off. The instrument cannot burn you because it has an automatic cut-out to prevent this. We will repeat this procedure several times to obtain consistent readings."

A short training session was performed before the onset of the actual test. Three clusters of stimuli were given in each sub-test and the rate of temperature change was set between 0.3 - 0.4˚C/sec. The final threshold was recorded as the average of the three readings. The participant was asked to indicate the perceived temperature (hot or cold); it was noted when this was incorrect. An example of TSA-II 'Method of Limits' output is shown inFigure12.

Vibration sensation assessment

Assessment of vibration was performed using the VSA-3000 device. The test was performed on the first metatarsal head (pulp of the big toe) as the toe was consistently pressing the vibrating pin at 50 grams. An approximate estimated pressure of 50 grams was achieved by comfortably positioning the foot and encouraging the participants to relax the toes. The 'Method of Limits'was applied such that the intensity of the stimulus (or the amplitude) increases until the vibration sensation is felt. The correct anatomical site of the body was also selected on the system and the participant was instructed as follows:

"We wish to determine if you can detect these vibrations at levels appropriate to your age. The button that your big toe is resting on will gradually begin vibrating. The test will involve an increase in the amount that the pin begins vibrating. At the first moment

that you can feel this vibration, immediately press the button. Please note that vibration is not necessarily felt in the toes and it can be felt in other parts of your foot as well. It is important that you press the button at the first moment that you detect any vibration, as such, keep your finger on the buttons so that you can respond quickly. However, do not press the button until you are confident that you have felt a vibration. Please stay alert and concentrate throughout the test. Pressing the button will turn the vibration device off. We will repeat this procedure several times to obtain consistent readings. Following each response from you, the probe will stop vibrating. The computer will make a small sound to indicate that the probe has returned to baseline and the next procedure is beginning."

The range of stimuli was between 0-130 Hz and the rate of vibratory change was determined between 0.1-4.0 Hz/sec. The recorded threshold was an average of four readings. An example of vibration threshold determination using the Method of Limitsis shown inFigure12.

Repeatability of Quantitative Sensory Testing

Given that the nature of QST is highly subjective and there were two examiners who were assigned for taking these measurements, it was important to determine the reliability of these measurements.The inter-observer intra-class correlation coefficients(ICC)were calculated for quantitative sensory testing and neuropathy disability score measurements in 14 healthy individuals using two-way mixed models with absolute agreement criteria. Measurements were taken by two examiners separately on two different days.

The inter-observer reliability was found to be very good heat-induced pain and vibration perception threshold (0.90 and 0.84; respectively) but not as high for the

remaining measurements (ICC ≤ 0.71 for all). Similarly, the intra-observe reliability for heat-induced pain was the highest of all (0.88) followed by vibration perception threshold (0.82). The outcome shows a possible learning effect from cold sensation threshold to vibration threshold perception. This might indicate that randomization of these measurements may improve the reliability of output. The ICC outcomes are outlined in Table 7.

Table 7.Intra-class correlation (ICC) for QST measurements.

Inter-observer ICC	QST CST	QST WST	QST CIP	QST HIP	QST VPT
Observe 1-Observer 2 Day 1	0.33 (p=018)	0.42 (p=0.10)	0.66 (p=0.01)	0.83 (p=0.001)	0.96 (p<0.0001)
Cronbach's Alpha (α)	0.47	0.58	0.81	0.91	0.97
Observe 1-Observer 2 Day 2	0.28 (p=0.21)	0.94 (p<0.0001)	0.76 (p=0.003)	0.06 (p<0.0001)	0.72 (p=0.005)
Cronbach's Alpha(α)	0.41	0.96	0.86	0.98	0.85
Intra-observer ICC	**QST CST**	**QST WST**	**QST CIP**	**QST HIP**	**QST VPT**
Observer 1 (day1-day2)	0.10 (p=0.36)	0.68 (p=0.004)	0.49 (p=0.07)	0.90 (p<0.0001)	0.79 (p=0.002)
Cronbach's Alpha (α)	0.21	0.85	0.64	0.95	0.88
Observer 2 (day1-day2)	0.18 (p=0.27)	0.06 (p=0.42)	0.36 (p=0.12)	0.86 (p<0.0001)	0.81 (p=0.003)
Cronbach's Alpha (α)	0.33	0.13	0.55	0.93	0.91

CST: cold sensation threshold, WST: warm sensation threshold, CIP: cold induced pain, HIP: heat induced pain, VPT: vibration perception threshold. Cronbach's alpha: coefficient of reliability

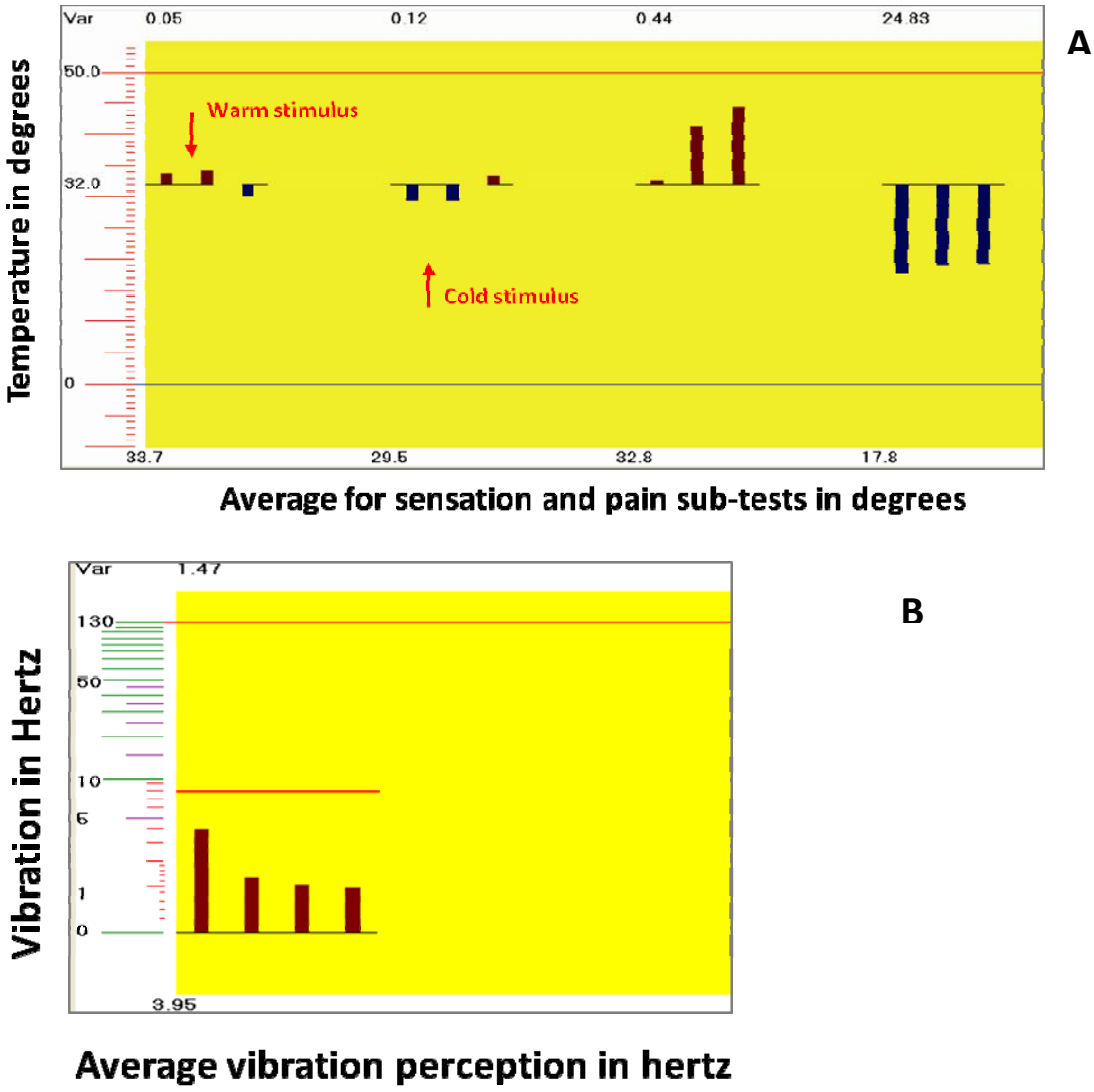


Figure12.Medoc QST outputs for thermal and vibration sensation. Warm and cold sensation in a randomised order (2 sets on the left) and cold-induced pain thresholds output (2 sets on the right) (A). The red colour bars indicate warm temperature and the direction is upward, starting at a baseline temperature of 32 degrees. The blue bars represent decrease in temperature again starting at a base line temperate of 32 degrees. Vibration sensation threshold output starting at a baseline of zero up to 130 Hertz (B). Individuals' final threshold was determined by the point that they have pressed the response button i.e. where the red/blue bars for each stimulus had stopped.

3.7.2.2 Neuropathy Disability Score

Neuropathy disability score (NDS) is a clinical scoring system used for simple neurological examinations to define the abnormalities of various modalities including vibration sensation, pin prick perception, hot/cold sensation and assessment of Achilles tendon reflex. The following procedure was performed on each participant.

The participant was asked to lie down for the first three tests. Pin prick perception was performed on the plantar surface of each big toe by means of a Neuropen and two Neurotips (Owen Mumford, Ltd, Woodstock, England) devices; the sharp end in one Neuropen and blunt end in another. The Neurotip was placed within one Neuropen to ensure that a force of 40 grams was safely applied to the skin without risk of penetration and consequent infection. The participants were instructed to indicate whether the sharp stimulus occurred at presentation '1' or '2'. Vibration perception was assessed using a 128 Hz tuning fork. Participants were again instructed to determine whether the vibration occurred at presentation '1' or period '2'. For assessment of hot/cold perception two metal rods were left in hot and cold water for 30 seconds. The rods were pressed against the dorsum of the feet and the participants were instructed to determine whether cold sensation occurred at presentation '1' or '2'. The participants were given a demonstration of differentiating stimuli on the forearm before each test. They were asked to close their eyes to avoid visual cues. All tests were repeated three times and the order of presenting the stimuli were randomised. Results for each foot were recorded as 0 for normal or 1 for abnormal (≥2/3 correct responses = normal). In order to investigate the presence or absence of Achilles tendon reflex, participants were instructed to kneel on a chair. The plantar surface of the foot was held with one hand so that the Achilles tendon was under moderate tension and the tendon hammer was left to fall under its own

weight on to the Achilles tendon. A reflex movement in the foot and a contraction of the gastrocnemius muscle was observed. If absent, the participant was asked to pull their hands together in the reinforcement position just prior to hammer strike. The result was recorded as follows: 0 for normal, 1 for present with reinforcement, or 2 for absent reflex (Figure 13).

NDS Scoring

The test was performed bilaterally. The results for both feet were added up. If the participant was missing a toe or a leg because of amputation, the results for the other foot were doubled [45]. Neuropathy was classified into four groups of none, mild, moderate and severe according to the scoring system outlined in Table 8.

Table 8.Neuropathy severity group based on neuropathy disability score (NDS).

NDS group	None	Mild	Moderate	Severe
Score	0 – 2	3 - 5	6 - 8	9 - 10

Figure 13. Neuropathy disability score test equipments. Tendon reflex hammer (A), metal rods (B), 128 Hz tuning fork (C), Neurotip (D), Neuropen (E).

Repeatability of neuropathy disability score test

Similar to assessment of repeatability for quantitative sensory outcomes, intraclass correlation was assessed for NDS measurements on 14 healthy individuals by two observers. These outcomes are presented in Table 9. Measurements were only highly repeatable intra-observers on the second day (p=0.004).

Table 9.Intraclass correlation (ICC) for NDS measurements

Inter-Observer ICC		Intra-Observer ICC	
Observe 1-Observer 2 Day 1	**Observe 1-Observer 2 Day 2**	**Observer 1 day1-day2**	**Observer 2 day1-day2**
0.19 (p=0.24) α= 0.38	0.70 p=0.004 α=0.86	-0.03* (p=0.53) α=-0.05	0.44 (p=0.07) α=0.53

The minus value occurs due to high number of zero measurements, α: Cronbach's alpha indicating reliability covariate

3.7.3 Ophthalmic Examination

The ophthalmic examination was conducted following the medical investigation. Participants were given a break and provided with some refreshments in the interim.

3.7.3.1 Screening procedure

All participants underwent a brief ocular screening assessment to confirm eligibility including ocular history, visual acuity, slit-lamp biomicroscopy and intraocular pressure measurement. The ocular history assessed any previous ocular history that may have resulted in any vision loss, for example glaucoma or aged macular degeneration. The anterior eye segment was assessed for corneal and other media opacities. Any lenticular opacification was graded according to the LOCS III scale [226]. Participants with mild, early cataracts on clinical examination were included in the study. Habitual spectacle prescriptions (for both distance and near vision) were measured and recorded for the purpose of visual field correction requirements. The procedure below was conducted on each participant.

Visual acuity

Monocular visual acuities were measured using distance refractive correction for each participant where applicable. A Bailey-Loviehighcontrast letter chart was positioned at 6 metres. The chart consists of lines of five high-contrastletters (95% Weber contrast), with each consecutive line decreasing inangular size by 0.1 log units in minimum angle of resolution (logMAR) [227].

Participants were directed to begin reading theletters at the top of the chart and to continue reading down the chart until atleast three of the five letters on a line were called

incorrectly. Visual acuitywas recorded as the total number of letters read correctly. If acuity on targeted test eye was worse than 6/9.5, it was checked with pinhole. If best achieved acuity remained worse than 6/9.5, the other eye was considered for test. If neither eye achieved 6/9.5, the participant was excluded.

Slit lamp examination

Binocular white light examination was performed to assess the general health of eyelids, conjunctiva, entire cornea, lens, and anterior chamber. Any white-light epithelial disruption, red eye or other ocular conditions were noted. In case of bacterial or viral conjunctivitis, the appointment for the participant was postponed until the condition was completely resolved. All participants had nuclear, posterior sub-capsular or cortical cataracts grade III or less than that. The depth of the anterior chamber was also assessed for the purpose of dilation.

Intraocular pressure

Intra-ocular pressure (IOP) was measured using an iCare-Tonometer ™;an average of the three measures of IOP were recorded in mmHg. In cases of high IOP (≥21 mmHG), the pressure was re-checked with a Perkins ™ applanation tonometer. If the high pressure reading was confirmed, the participant was excluded.

Pupil dilation

Participants had the pupil of their test eye dilated if considered safe to do so according to the criteria of a Van Herrick estimate of anterior chamber angles >0.3 and/or recent

history of uncomplicated dilation. This was undertaken at least 20 minutes prior to image acquisition. One drop of 1% tropicamide was administered as standard, with an additional one drop of 2.5% phenylephrine only if required was applied to one eyes.

Fundus photography

In order to check for the cup-to-disc ratio and screen for presence of retinopathy, 45 degrees field fundus photographs were obtained by anon-mydriatic digital camera (Visucam, Carl Zeiss Meditec Ltd, Germany). The photographs included macula and optic nerve head areas. Cases of suspicious optic nerve head appearance, regardless of IOP, were ineligible due to concerns about glaucoma and professional care was arranged for these individuals.

3.7.3.2 Diabetic retinopathy grading

The Australian National Health and Medical Research Centre (NHMRC) guidelines were used for classification of diabetic retinopathy [216]. This grading is a simplified version of The Early Treatment of Diabetic Retinopathy Study (ETDRS) modification of Airlie House Study for classification of retinopathy [132]. The detailed grading is outlined in Table 10. In the current study, four grades of retinopathy including none, minimal mild, moderate and severe was employed in agreement with ETDRS level 10 – 53 E.

Table 10.Airlie House Study for classification of retinopathy.

Level	Severity	Definition
10	No retinopathy	Diabetic retinopathy absent
20	Very mild NPDR	Micro-aneurysms only
35	Mild NPDR	Hard exudates, cotton-wool spots, and/or mild retinal haemorrhages
43	Moderate NPDR	43A Retinal haemorrhages moderate (>photograph If) in four quadrants or severe (≥photograph 2A) in one quadrant 43B Mild IRMA (<photograph 8A) in one to three quadrants
47 A -D	Moderate NPDR	47A Both level 43 characteristics 47B Mild IRMA in four quadrants 47C Severe retinal haemorrhages in two to three quadrants 47D Venous beading in one quadrant
53 A-D	Severe NPDR	53A ≥2level 47 characteristics 53B Severe retinal haemorrhages in four quadrants 53C Moderate to severe IRMA (≥photograph 8A) in at least one quadrant 53D Venous beading in at least two quadrants
53E	Very Severe NPDR	≥2 level 53A-D characteristics
61	Mild PDR	NVE<0.5 disc area in one or more quadrants
65	Moderate PDR	65A NVE≥0.5 disc area in one or more quadrants 65B NVD <photograph 10A (<0.25-0.33 disc area)
71, 75	High-Risk PDR	NVD ≥photograph 10A, or NVD <photograph 10A or NVE<0.5 disc area plus VH or PRH, or VH or PRH obscuring ≥1 disc area
81, 85	Advanced PDR	Fundus partially obscured by VH and either new vessels upgradable or retina detached at the centre of the macula

***NPDR**: non-proliferative diabetic retinopathy; **PDR**: proliferative diabetic retinopathy; **IRMA:** intra-retinal microvascular abnormalities; **NVE**: new vessels elsewhere; **NVD**: new vessels on or within 1-disc diameter of the optic disc; **PRH:** pre-retinal haemorrhage; **VH**: vitreous haemorrhage [228]*

3.7.3.3 Retinal nerve fibre layer assessment

Optical Coherence Tomography (OCT) is a reliable, reproducible and non-invasive optical imaging technique that has been used for almost two decades for *in vivo* assessment of retinal tissue structure [106]. Tomographic real-time imaging of the retinal microstructure with high resolution properties, as performed by OCT, has been widely used for diagnosis of critical retinal pathologies including glaucoma [229].

3.7.3.4 Optical coherence tomography

The OCT instrument used in this project was an RTVue RT-100, Version 4.0 (Optovue, Fremont California, USA) (Figure 14). RTVue is the new generation of OCT based on the Fourier domain optical property which is superior in rapid image acquisition and has higher resolution than the older generation instruments. RTVue is an ultra-high speed and high resolution OCT which captures 26,000 A-scans per second. The broad-band light source in the system provide up to 5 μm resolution which is 2 times higher than conventional OCT resolution and it is known to be 65 times faster than Time-domain OCT. The scan depth in the tissue ranges between 2 and 2.3 mm.

3.7.3.4.1 Optical principal

Optical coherence tomography employs the technique of low-coherence interferometry to measure the rapid light echo time delay. Imaging is performed by measurements of interference between the backscattered light from the sample and a reference mirror [230]. In a standard OCT where Time-domain property is employed the delay time travelled by light and the reference mirror position are scanned mechanically and sequentially to acquire an A-scan (measuring light echoes versus depth of travel). The beam of light is then scanned laterally to create a 2-dimensional image in false colours

(B-scan). The mechanical movement of the scanning device in this technology limits the speed rate of image acquisition.

In Fourier-domain technology (as in case with RTVue 100), light echoes are detected by a spectrometer and charge-coupled high speed cameras to determine the interference spectrum. Fourier-domain employs the mathematical operation of Fourier transforms where the frequency of a signal is the representation of time. In Fourier-domain technology, the interference spectrum consists of oscillations whose frequencies are proportional to light time delays. Therefore by applying the Fourier transform operation, the axial scan details can be measured. The echo time delay in Fourier-domain is measured simultaneously hence the sensitivity and speed of acquisition are extremely improved. For more detail regarding retinal nerve fibre layer assessment using these two technologies refer to *Chapter 2*.

The normative data-base values stored in RNFL measuring instruments (in this case Optovue, RTVue optical coherence tomography) covers most of the variation in RNFL measurements caused by determinants discussed in *Chapter 2 (section* 0*)*. For this purpose, a number of precise inclusion and exclusion criteria have been outlined for the sampled normative database. In brief, participants were eligible if they were over 18 years of age, had no history of ocular pathology with intra-ocular pressure (IOP) less than 22 mm Hg, and a normal visual field test based on the Humphrey 24-2 white on white test. The appearance of the optic disc, however, has not been used as an exclusion criterion because it could introduce bias in the database. As such, all measurements have been corrected for the normal age and optic disc size effects due to significant associations between RNFL measurements, age and ONH size [231].

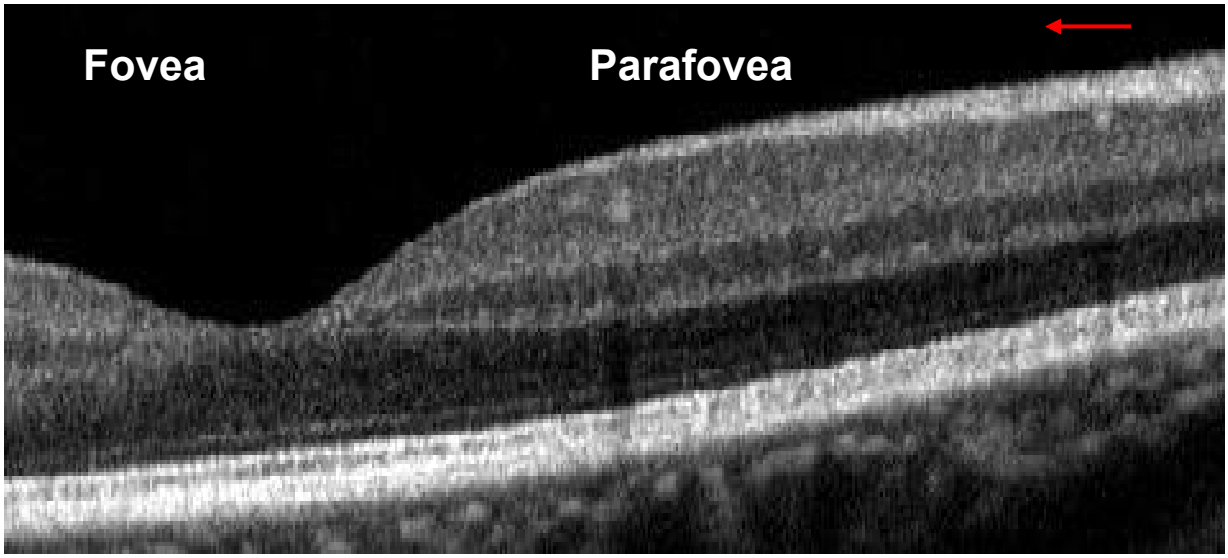


Figure 14.RTVue Optical coherence tomographer. Retinal nerve fibre layer thickness in an OCT scan is defined as the innermost highly reflective layer (arrow).

3.7.3.4.2 Optical coherence tomography examination procedure

The scans were acquired in a darkened room with adilated pupil. Participants were seated on a comfortable chair and asked to fixate on an internal target while resting the chin in the chin-cup. Measurements were taken from one eye only using the Optic Nerve Head (ONH) protocol. This protocol along with 3D Disc protocol provides details quantifying several important features of disc morphology: disc and cup areas, cup/disc ratio, RNFL 3.45 and NFL thickness map from 2 mm radius from the centre of the disc. The TSNIT histogram in this protocol provides thickness of the RNFL calculated at 3.45 mm diameter around the centre of the optic disc and not the centre of the scan. Compared with a simple RNFL 3.45 scan (Figure15), this automatic re-sampling of ONH protocol improves the accuracy of the measurements, therefore de-centering of the disc related to the scan beam will not affect the readings (Figure16).

3.7.3.4.3 Normative database in RTVue 100 instrument

The database in software version 4.0 of RTVue 100 contains more than 1600 people of various ethnicities and is so far the largest database of normal values for OCT. The database is sectioned according to three different factors: age, optic disc size and ethnicity. The system uses colour coding tables to show where the results of each individual fall, in comparison with the normal values (Figure17).

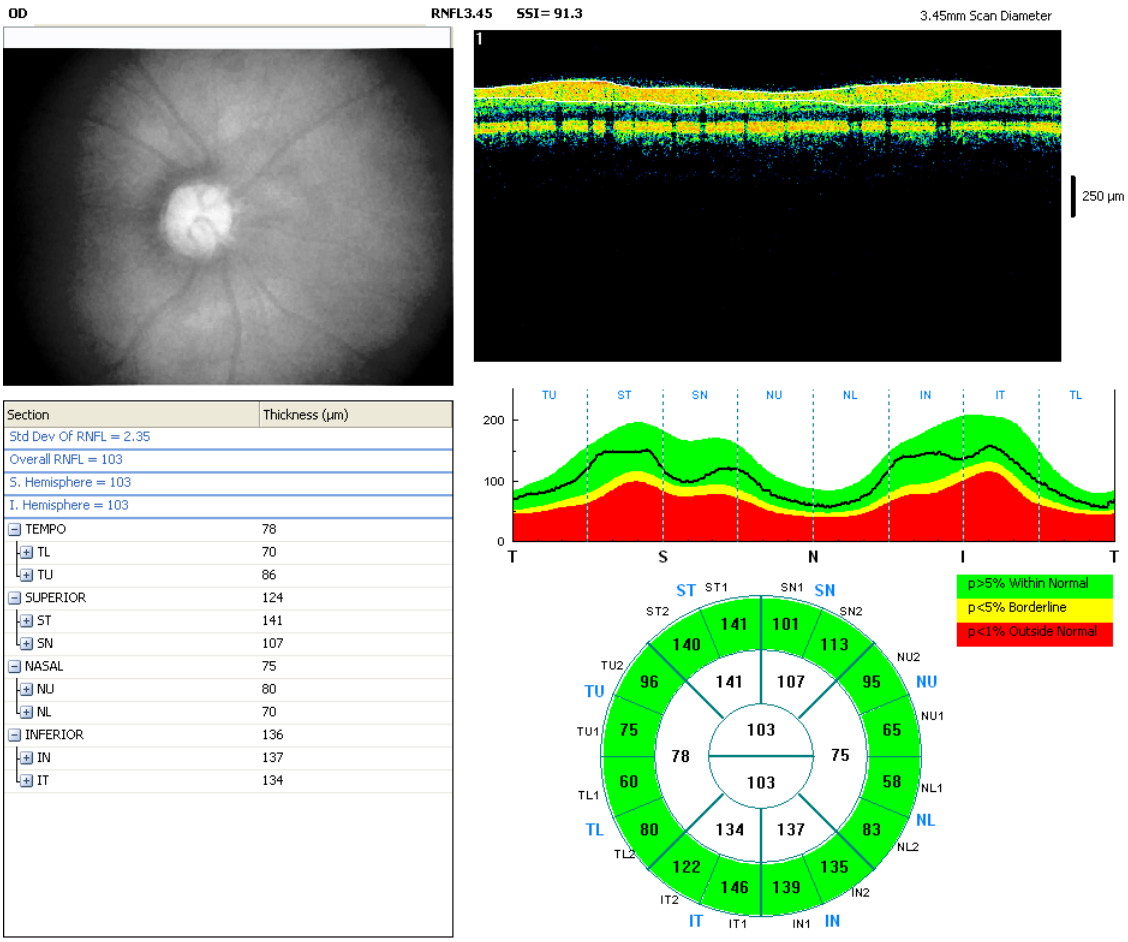
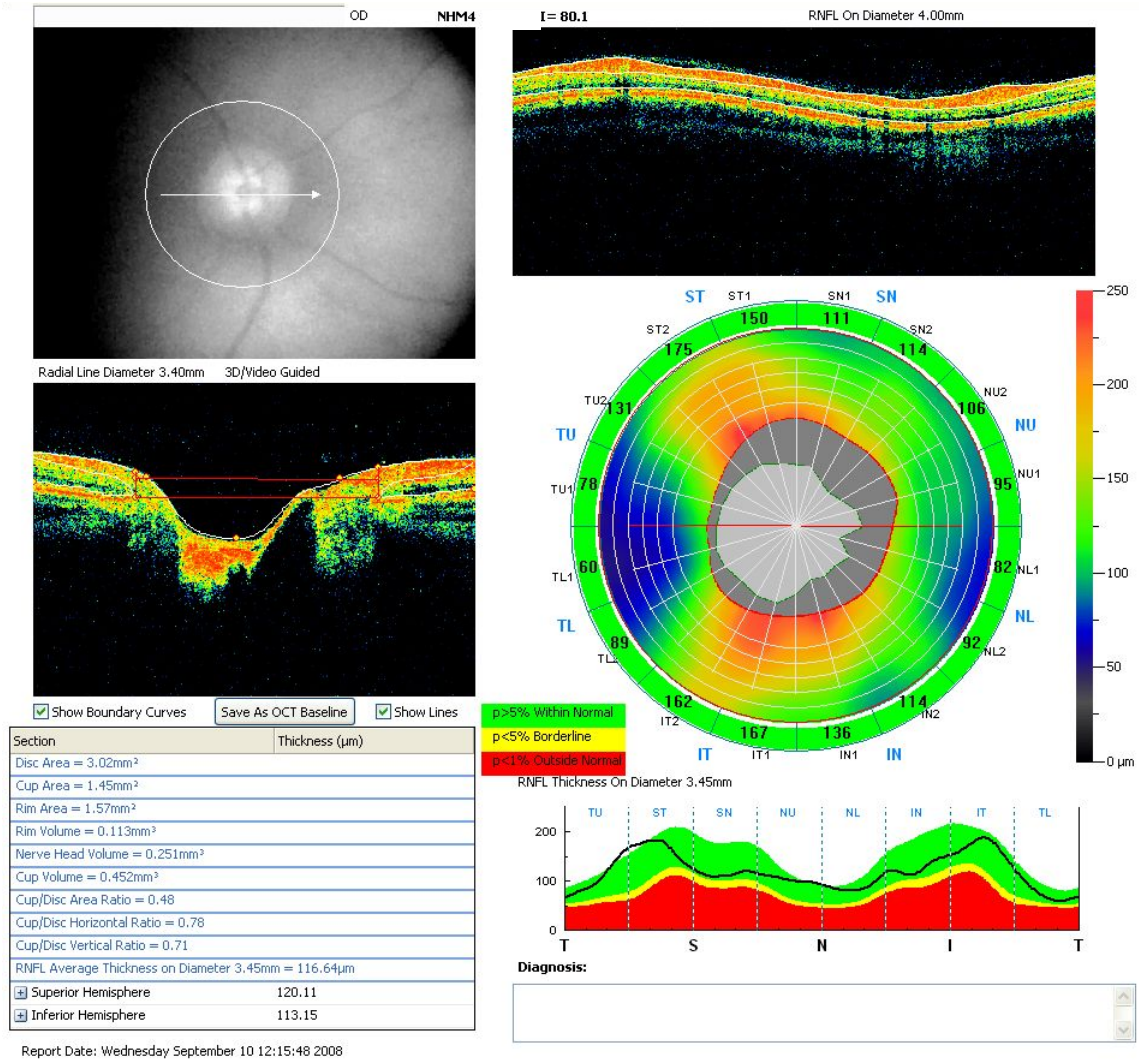


Figure15.RNFL 3.45 protocol output for the right eye of a 23-year old healthy individual. All measurements are in units of micrometers.



Section	Thickness (µm)
Disc Area = 3.02mm²	
Cup Area = 1.45mm²	
Rim Area = 1.57mm²	
Rim Volume = 0.113mm³	
Nerve Head Volume = 0.251mm³	
Cup Volume = 0.452mm³	
Cup/Disc Area Ratio = 0.48	
Cup/Disc Horizontal Ratio = 0.78	
Cup/Disc Vertical Ratio = 0.71	
RNFL Average Thickness on Diameter 3.45mm = 116.64µm	
Superior Hemisphere	120.11
Inferior Hemisphere	113.15

Figure16.Optic nerve head (ONH) protocol output for the right eye of a 30-year old healthy individual. Optic nerve head characteristics are additionally measured through this scan (table left-down)

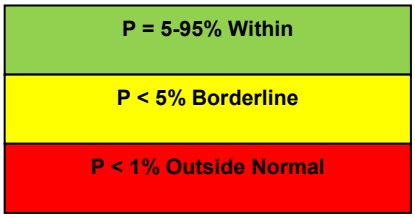


Figure17. Colour legend for RNFL thickness measurements.

3.7.3.5 Monocular visual field assessment

Visual field assessment has proved to be a reliable clinical technique in diagnosing visual and neurological pathologies. Visual perception is achieved via different subtypes of retinal and neural cells through specific visual pathways. As such two methods of visual sensitivity assessment were used in this research to explore visual function in general as well as helping to selectively highlight responses from specific cell types.

3.7.3.6 Medmont Field Analyzer

Medmont M700 (Medmont Pty Ltd, Camberwell, Victoria, Australia) was used to assess visual function (Figure18). The M700 is an Australian manufactured visual field analyser that works through Medmont Studio software [232].

3.7.3.6.1 Optical principal

The Medmont M700 is a part hemispherical bowl with radius of 30cm and integrated diffusing surface. The instrument includes a rear projection light emitting diode where 100 pale green colour testing points, with wavelength of 565 nm, are produced in the central 30 degrees protocol. Each test point equals 0.43 degrees at viewing distance of 37 cm that is equivalent to Goldman size III [232]. The stimuli are situated in a radial fashion along 8 concentric rings at1, 3, 6, 10, 15, 22, 30, 40, 50 and 80 degrees of eccentricity around fixation spot (Figure18).The back ground illumination which incorporatesrear projection light-emitting diodes is automatically kept at 10 apostilbs (asb) (Medmont Pty Ltd 2009).A staircase procedure is employed for threshold determination and response from the participant determines the intensity of the next stimulus to be presented. The initial change occurs at 6 dB steps until the first reversal

happens and this is followed by 3 dB changes. The stimulus intensity varies between 0.3 to 1000 asb.

A yellow fixation light (LED, wavelength 583 nm) in the centre is used to control patient fixation as well as a built-in camera which automatically tracks the pupil and informs the practitioner of any loss of fixation (Figure18).

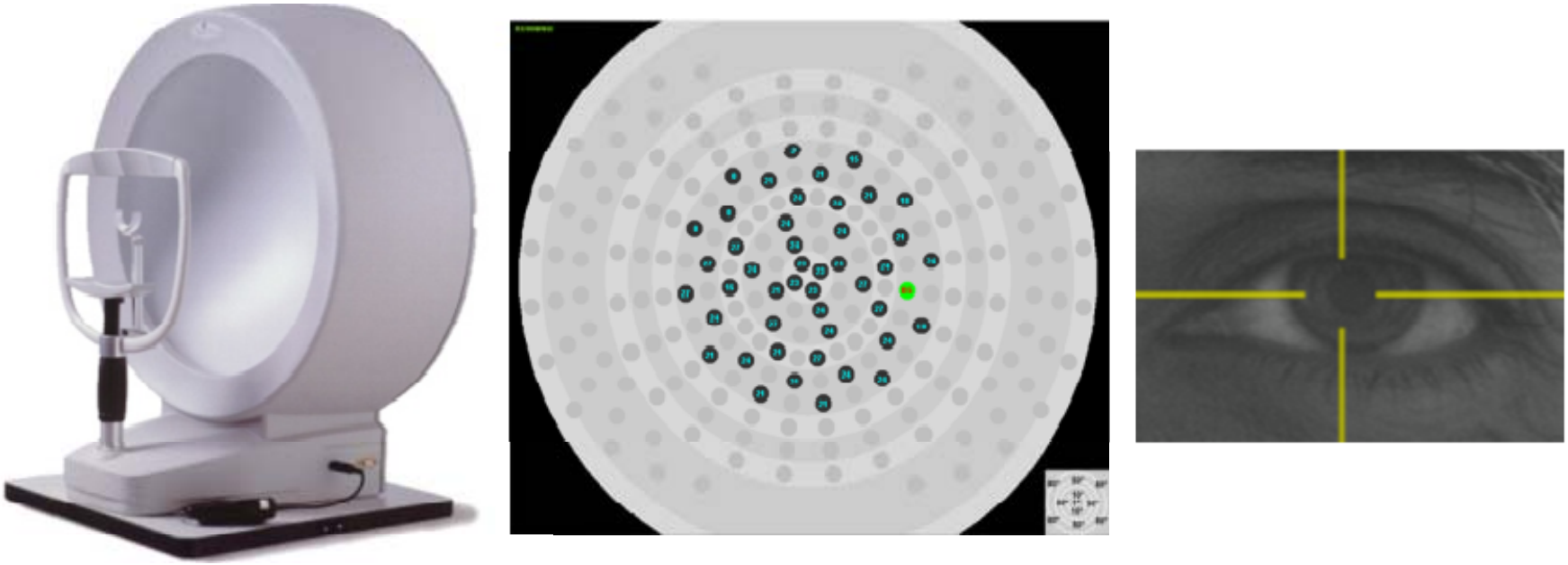


Figure18.Medmont M700 visual field analyser.The Medmont instrument (left), test screen illustrating eight concentric rings in light and dark grey (middle), pupil video tracking in M700 instrument

3.7.3.6.2 Visual field examination procedure

Participants were seated on a comfortable chair at the instrument with the chin placed in the chin rest and head against the forehead rest. The tests were performed on the dilated eye only and each test was performed once. The participants were instructed for each test separately and they were given breaks within and between the procedures as required.

3.7.3.6.3 Standard automatedperimetry

Standard achromatic automatic perimetry (SAP) or white-on-white perimetry is a commonly used technique in ophthalmic examination. A central 30 degree field was tested, comprising of 100 test points. A few additional points were added to cover the macula area. The participants were instructed to press the response button once the green flash was perceived while looking at the central fixation light. In all cases, the optimal lens correctionfor the working distance of the perimeter was placed before the tested eye. This was previously measured from the current prescription of each participant during the screening procedure. The test duration was six minutes generally; however if additional breaks or instructions were required it could extend as long as 10 minutes. Reliability of individual resultswere determined according to the visual field indices as follows :

Fixation losses < 33%– To assess the peripheral visual sensitivity, it is very important that the patient keep the eye being tested focused straight ahead on the fixation light. In practice it is difficult to maintain this eye position for very long, since the natural tendency is to look to the side, towards the flashing light. Given that such movements may cause unreliability, the machine records the number of times that the participant

moves the eye off centre. In some individuals, the natural optic nerve head size appeared to be larger than usual; hence care was taken in not to count these as fixation loss errors[233].

False positive errors < 33% - This error happens when the participant pushes the response button indicating he/she has seen a flash when in fact no flash has been shown. This misinformation obviously seriously detracts from the test ability in determination of what has actually been observed [233].

False Negative Errors < 33% - Visual field test is designed to repeat flashes at the same location with different levels of intensity. False negative error happens when the individual reports seeing a flash at a certain location, but does not report seeing the same intensity flash at the same spot the second time. People with glaucoma may have normal fluctuations at the edge of their visual field loss, so not all of these type of errors represent a true complication or field loss [233].

Overall Defect (OD)is a measure of differences between the outcome sensitivities and age-adjusted normal sensitivities, therefore describing a general depression or elevation of the visual field. **Pattern Defect (PD)** indicates irregularity of visual field sensitivity. This measure rules out the effect of diffuse loss that might have been caused by cataract and therefore, shows abnormalities that are mostly disease-related[234]. Overall Defect and PD were both recorded for all individuals. An example of SAP printout is shown in Appendix 4.

3.7.3.6.4 Flicker sensitivity

Flicker perception is thought to be mediated by magno-cellular pathways. The flickering stimulus that has been used in M700 has a fixed flickering rate and contrast; however the luminance changes to determine the threshold. The test presents two types of stimuli - one that looks like a normal SAP stimuli and a flickering stimulus. The flicker frequency of stimuli is dependent on eccentricity with rates varying from 18 Hz at 1°eccentricity to 9 Hz at 22° eccentricity.Participants were instructed to respond to the flickering stimuli only. The same reliability determinants were applied to flicker perimetry results. Overall Defect and PD were also recorded. An example of flicker test printout is shown in Appendix 5.

3.8 Summary

In summary, this chapter described the recruitment procedures and commonmethods used throughout the thesis. Characteristics of the participants will be presented in the subsequent chapters. Thefollowing six chapters outline the findings from the cross-sectional studies.

4 Investigating the Association between Quantitative Sensory Testing and Neuropathy Disability Score

4.1 Introduction

Clinical assessment of peripheral neuropathy in diabetes involves a combination of symptoms evaluation and neurological examination of clinical signs. Several methods are commonly used to screen and investigate diabetic peripheral neuropathy (DPN) ; these include superficial pain assessment, reflex testing, light touch and vibration perception. Quantitative sensory testing (QST) is a relatively reliable and reproducible method of detecting small fibre neuropathy. The test has been shown to be capable of predicting progression to foot ulceration in patients with DPN who are already identified by skin biopsy or nerve conduction studies [235]. QST has the capability to differentiate small and large axon neuropathy through a variety of sensory modalities [236]. Additionally, the test is designated as safe and effective as a diagnostic tool [48]; however, it is subjective, time-consuming and requires patients cooperation to achieve reliable results [48].

Neuropathy disability score (NDS) has been established for rapid assessment of DPN. This test provides a score system based on evaluation of sensation and reflexes. The test is capable of predicting risk of foot ulceration, is available for clinical routine examination and has been recommended for clinical screening [45].NDS has been

validated against QST and a relatively strong association between the measurements of the two techniques has been demonstrated [237].

The main focus of this chapter is to compare results obtained from QST and NDS to investigate their similarities and differences in predicting neuropathy. These two tests - QST and NDS - are the principle means employed for assessment of peripheral neuropathy in this thesis; hence the following comparison was performed to justify the inclusion of both tests are reasonable, but not identical, measures of DPN in following chapters.

4.2 Aims and hypotheses

The aim of this study was to:

1. Investigate the association between NDS and QST in participants with type 2 diabetes and healthy controls.

This study tested the following specific hypotheses:

1. NDS and QST results are significantly different between:

    a. Individuals with type 2 diabetes and healthy controls

    b. NDS groups (none-mild-moderate and severe)

2. QST results are significantly associated with NDS outcomes in people with type 2 diabetes.

4.3 Methods

4.3.1 Participants

This study was cross-sectional in design. Ninety three participants with type 2 diabetes and 24 healthy control participants consented to the study. Participants ranged from 45 to 77 years of age (mean ± SD: 61.1 ± 6.8 years). The differences in age of the participants in the two groups was close to significance ($t = 1.8$, $p = 0.07$). When stratifying participants according to their neuropathy disability score (*see section 4.3.3*), again differences in age among the NDS groups and the control group did not reach statistical significance ($F = 1.78$, $p = 0.15$). Additionally, NDS groups did not have a statistically significant difference in duration of diabetes ($F = 2.27$, $p = 0.08$) (Table 11). All tests were performed in one session at Institute of Health and Biomedical Innovation (IHBI), Queensland University of Technology (QUT). Sample characteristics are presented in Table 11.

To address the hypothesis regarding association between QST variables a sample of 48 will provide 90% power ($\alpha=0.05$) that an association between QST and NDS where one exists. The sample is inadequate to address the hypothesis regarding differences between groups, as a sample of 53 per group is necessary to reveal a clinically meaningful difference of 5 hertz between diabetes groups and controls for VPT when standard deviation of 13 hertz is applied (values from [18]). These data will be considered pilot data from our lab.

4.3.2 Quantitative Sensory Testing

Quantitative sensory testing was performed by attaching a thermode on the dorso-lateral side of the foot on the hand-dominant side of each participant to test for participants ability to detect temperature change (cold sensation threshold and warm sensation threshold), pain thresholds (cold induced pain and heat induced pain) as well as their ability to detect vibration (vibration perception threshold). QST protocol was explained to the participants and a few trials were performed to familiarise them with the technique. For a comprehensive description of QST please see *Chapter 3*.

4.3.3 Neuropathy Disability Score

The neuropathy disability score (NDS) was performed to grade the severity of neuropathy in participants. NDS was derived from responses to sharp and blunt stimuli, vibrating and non-vibrating tuning fork on the plantar metatarsal head (pulp of the great toe), hot and cold stimuli on the dorso-lateral part of foot and the presence or absence of Achilles tendon reflexes. Based on the NDS score (0-10), participants were divided into four groups: no neuropathy (0-2), mild neuropathy (3-5), moderate neuropathy (6-8) and severe neuropathy (9-10). Detail description regarding this test has been provided in *Chapter 3*.

Table 11.Characteristics of the study cohort.The data are presented in mean ± standard deviation (SD). Note that grouping of the participants is based on NDS score (*see section 4.3.3*). ANOVA F statistics show the comparison between the control participants and four NDS groups.

Parameter	Control	No Neuropathy	Mild Neuropathy	Moderate Neuropathy	Severe Neuropathy	F =	P =
N	24	27	33	17	16	-	-
Age (years)	58.0 ± 6.7	59.2 ± 6.8	62.6 ± 6.0	63.2 ± 7.9	62.5 ± 6.8	2.19	0.07
DD† (years)	n/a	10.7 ± 8.9	13.8 ± 8.6	15.5 ± 9.2	17.6 ± 9.2	2.27	0.08
Gender (M/F)	8/16	14/13	25/8	11/6	12/4	-	-

† *Duration of diabetes*

4.3.4 Statistical Analysis

The data were checked for normality of distribution histograms as well as normality statistics are reported in Figure 19 and Table 12; respectively. Given that none of the test variables were normally distributed, the choice of statistical analysis was based on non-parametric samples. To address the first part of the first hypothesis, Man-Whitney u-test was used in order to compare the outcomes between the control groups and the entire participants with type 2 diabetes. The second part of the primary hypothesis was investigated using Kolmogorov-Smirnov test for comparison between groups of NDS for participants with type 2 diabetes. To address the second hypothesis, the associations between the variables were calculated using spearman's correlation statistics. A p-value less than 0.05 was considered statistically significant. Statistical package for social sciences (SPSS) version 18 was used for all statistical analyses.

Table 12. Test of normality of distribution of QST variables and neuropathy disability score test

Test of Normality		
Parameter	**Kolmogorv-Smirnov statistics**	**P-value**
Neuropathy disability score	0.23	< 0.001
Cold sensation	0.08	< 0.001
Warm sensation	0.28	= 0.049
Cold-induced pain	0.35	< 0.001
Heat-induced pain	0.26	< 0.001
Vibration perception	0.16	< 0.001

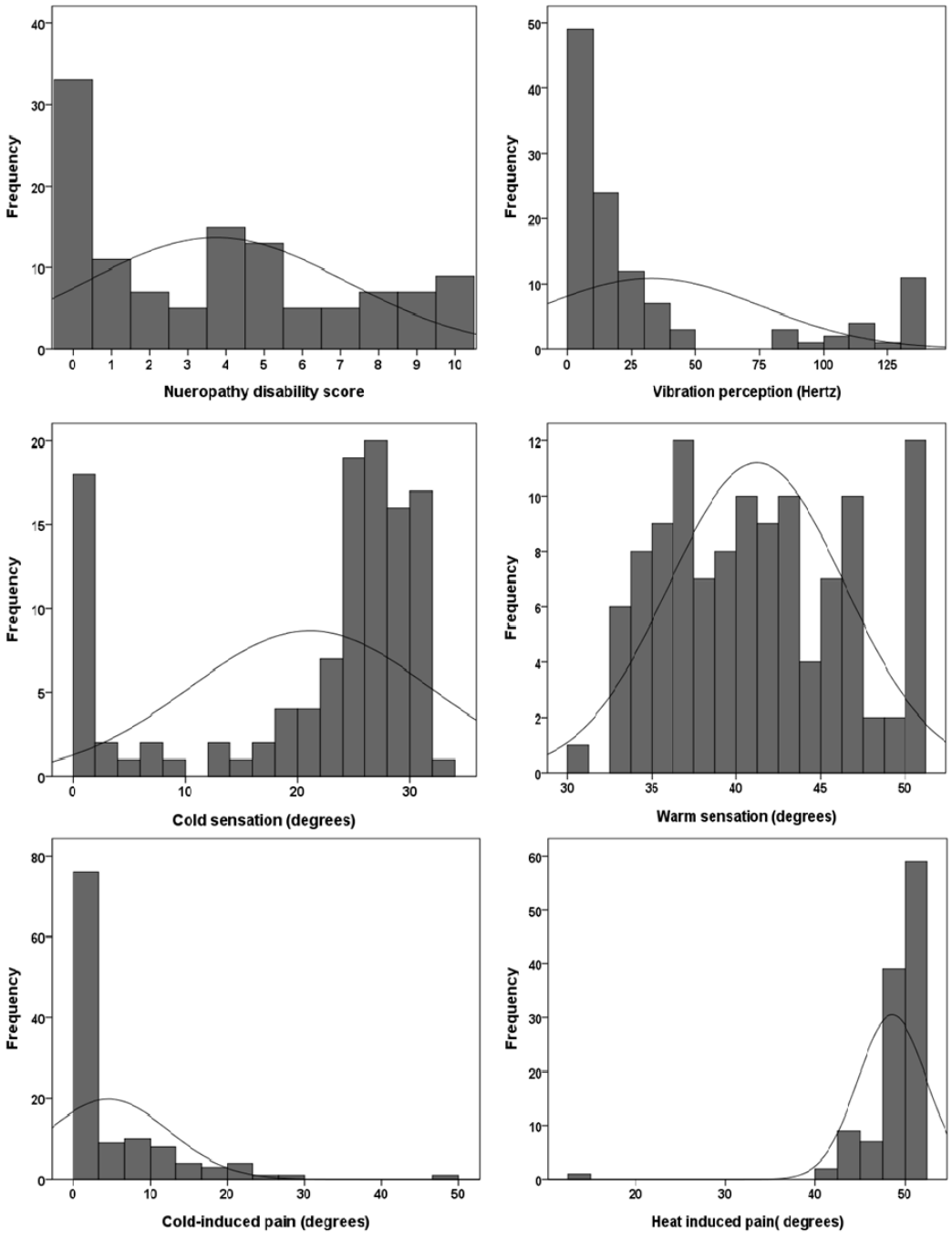


Figure 19. Histograms showing distribution of NDS and QST variables

4.4 Results

Control participants were found to have significantly different NDS and QST outcomes compared with participants with type 2 diabetes (Table 14). Additionally QST outcomes were significantly different between the four NDS groups (Table 14). Pair-wise comparisons revealed that cold sensation and warm sensation for mild, moderate and severe neuropathy groups were significantly different from each other (detailed statistics in Table 14). Groups with no neuropathy and mild neuropathy did not have statistically significant QST measurement differences ($p > 0.78$ for all paired-comparisons). Similar results were found for groups with moderate and severe neuropathy ($p > 0.90$ for all paired-comparisons).

The associations between QST outcomes and NDS in individuals with type 2 diabetes were found to be significant for all the variables ($p < 0.001$), with vibration perception and NDS score showing the highest correlation coefficient value ($r = 0.66$). Detailed statistics are presented in Table 15. Associations between QST outcomes and NDS are also presented in Figure 20. When same associations were investigated for the control participants, no significant correlations were observed between any of QST parameters and NDS (
Table 16).

Table 13.Mann-Whitney U test results for QST measurements and neuropathy disability score comparisons between control participants and individuals with type 2 diabetes.

QST parameter	Status	N	Median	U test	P =
Cold sensation (°C)	**Control**	24	28.25	- 4.20	<0.0001**
	Type 2	93	24.30		
Warm sensation (°C)	**Control**	24	38.95	2.03	= 0.042*
	Type 2	93	41.30		
Cold-induced pain (°C)	**Control**	24	9.35	- 4.32	<0.0001**
	Type 2	93	0.00		
Heat induced pain (°C)	**Control**	24	48.60	3.67	<0.0001**
	Type 2	93	50.00		
Vibration perception (Hz)	**Control**	24	5.14	4.73	<0.0001**
	Type 2	93	17.40		
Neuropathy disability score	**Control**	24	0.00	5.76	<0.0001**
	Type 2	93	4.00		

** Indicates significance at 0.05 level, ** Indicates significance at < 0.0001 level*

Table 14.Median descriptive and Kruskal – Wallis test outcomes for comparison of quantitative sensory testing parameters across the NDS groups.

Parameter	NDS group	Median	Range (Min – Max)	Kruskal-Wallis$_{(df)}$	P =
Cold sensation(°C)	**None**	26.2#	0.0 – 31.0	$22.78_{(3)}$	<0.0001**
	Mild	22.5†	0.0 – 30.9		
	Moderate	4.30†#	0.0 – 31.0		
	Severe	7.1†#	0.0 – 28.2		
Warm sensation (°C)	**None**	41.1#	33.3 – 50.0	$22.66_{(3)}$	<0.0001**
	Mild	39.6†	33.3 – 50.0		
	Moderate	46.4†#	36.6 – 50.0		
	Severe	47.1†#	33.7 – 50.0		
Cold-induced pain (°C)	**None**	0.3†#	0.0 – 25.1	$17.49_{(3)}$	=0.001*
	Mild	0.6	0.0 – 27.30		
	Moderate	0.0 †#	0.0 – 0.0		
	Severe	0.0	0.0 – 15.9		
Heat-induced pain (°C)	**None**	49.5#	42.6 - 50	$20.68_{(3)}$	<0.0001**
	Mild	49.7†	47.3 - 50		
	Moderate	50.0†#	49.5 - 50		
	Severe	50†#	48.9 - 50		
Vibration perception (Hz)	**None**	6.9#	2.1 – 119.5	$33.25_{(3)}$	<0.0001**
	Mild	14.2†	3.1 – 130.0		
	Moderate	89.8†#	5.1 – 130.0		
	Severe	96.7†#	8.0 - 130.0		

*# and † indicate groups that are significantly different from each other,df: degrees of freedom,*significance at 0.05 level, ** significance at < 0.0001 level*

Table 15. Spearman correlation coefficients showing associations between QST variables and NDS in participants with type 2 diabetes (N = 93)

Parameter	Correlation Coefficient	NDS
cold sensation(°C)	r =	-0.58
	Sig. (2-tailed) p =	< 0.001*
warm sensation(°C)	r =	0.45
	Sig. (2-tailed) p =	< 0.001*
cold-induced pain(°C)	r =	-0.44
	Sig. (2-tailed) p =	< 0.001*
heat induced pain(°C)	r =	0.48
	Sig. (2-tailed) p =	< 0.001*
vibration perception(Hz)	r =	0.66
	Sig. (2-tailed) p =	< 0.001*

** Indicates significance, NDS: neuropathy disability score*

Table 16. Correlation coefficients between QST outcomes and NDS for control participants (N = 24)

Parameter	Correlation Coefficient	NDS
cold sensation(°C)	r =	-0.29
	Sig. (2-tailed) p =	0.15
warm sensation(°C)	r =	0.13
	Sig. (2-tailed) p =	0.52
cold-induced pain(°C)	r =	0.06
	Sig. (2-tailed) p =	0.79
heat induced pain(°C)	r =	- 0.26
	Sig. (2-tailed) p =	0.20
vibration perception(Hz)	r =	-0.26
	Sig. (2-tailed) p =	0.24

NDS: neuropathy disability score

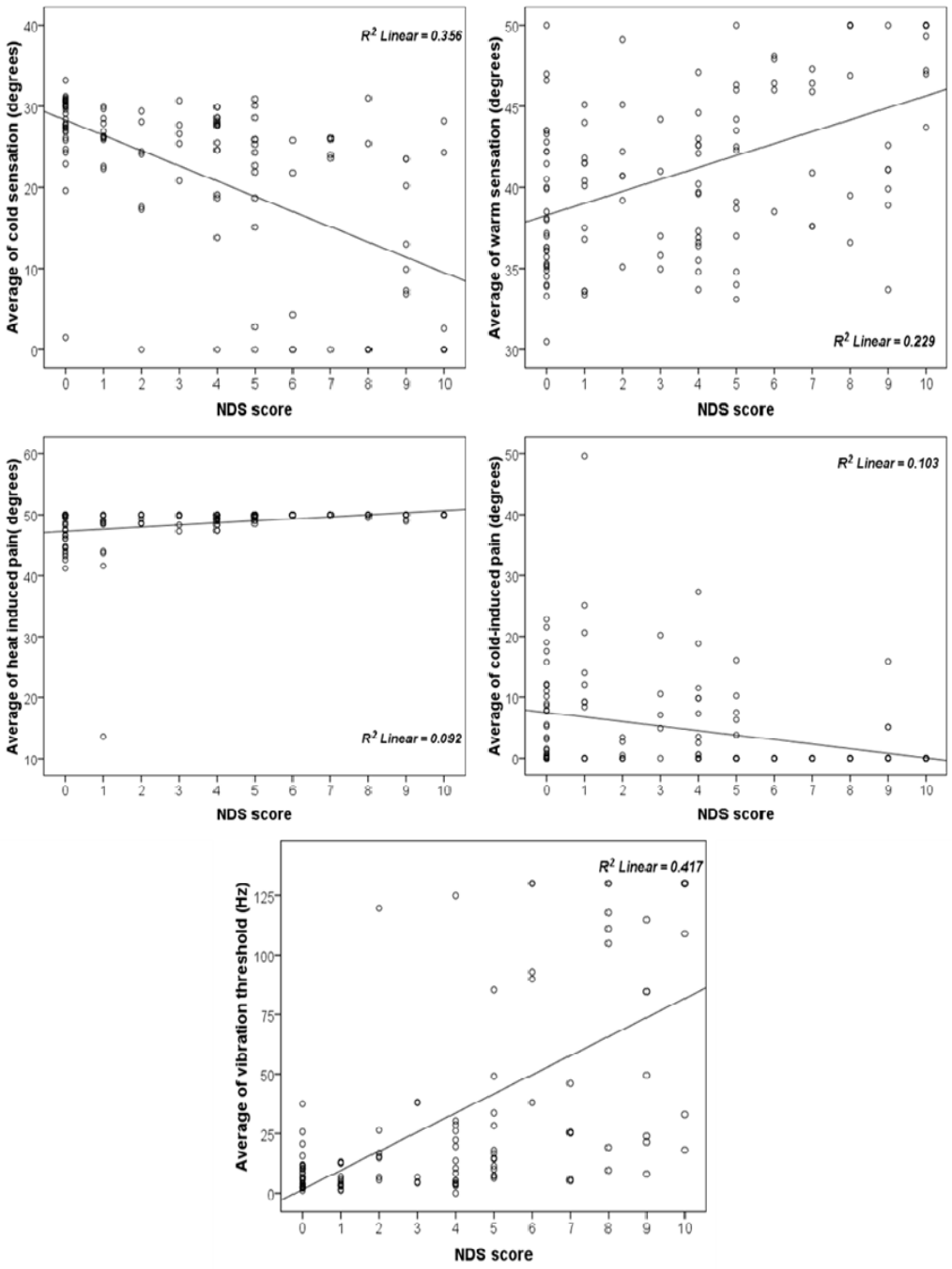


Figure 20. Scatterplot showing the association between quantitative sensory testing and neuropathy disability score test. Decreased cold sensation, cold-induced pain thresholds are associated with increasing NDS score indicating loss of sensation in feet. Decreased warm sensation is the opposite. Note that the scales on y-axis are different for each graph. Increased warm sensation threshold, heat-induced pain thresholds and vibration perception thresholds are also association with increased severity of neuropathy as measured by NDS.

4.5 Discussion

This study investigated the association between neuropathy disability score and quantitative sensory testing as two means for evaluating peripheral neuropathy in diabetes. It was hypothesized that tests results for healthy participants would be significantly different from individuals with diabetes as well as between the NDS groups. Additionally, increase or decrease in QST sub-tests or in other terms, loss of sensitivity for each QST parameter, was predicted to be associated with higher NDS scores.

Peripheral neuropathy in diabetes affects a variety of nerve fibre types throughout the body, therefore detection of the condition requires a range of diagnostic techniques [24]. Both NDS and QST are considered to be clinically practical screening tests for DPN. QST is capable of identifying progressive loss of sensation [36]. The advantages of this technique compared to a simpler screening approach include its ability to perform a wide range of sub-tests over a range of intensities as well as using a psychophysical procedure to obtain more precise sensitivity [48]. QST modalities are designed to stimulate specific neuronal pathways including both large and smaller axons. The large myelinated A-fibres that are sub-classified to alpha, beta and delta, mediate motion perception, vibration and cold sensation while the smaller unmyelinated C-fibres mediate slow pain perception and warm sensation. Given that conventional nerve conduction studies (NCS) only assess larger nerve fibres, the capacity for QST to assess different types of fibres can be considered as an advantage in the broad assessment of neuropathy [48]. However, unlike NCS and NDS, the subjective nature of QST can highly influence its outcomes. QST results, like any other psychophysical procedures (including visual field

assessments – addressed in later chapters) are influenced by fatigue and distraction levels as well as by learning effects. Even though QST has been shown to be a relatively sensitive method for detecting DPN in patients who had no other signs of peripheral neuropathy[218]; QST results in neuropathy assessment should always be considered in combination with other means of diagnosis [48]. Reports on reproducibility of QST sensory testing have shown variation in the outcomes; nonetheless such variation has been explained as a natural part of threshold measurement [218, 238-240].

Neuropathy disability score, compared with QST, is a simpler technique of assessing peripheral neuropathy. Instruction for responses to most of the NDS sub-tests only require a simple 'one' or 'two', with the Achilles tendon reflex being a relatively objective observation. Previous reports have suggested that NDS is a good measure of peripheral neuropathy by itself [45] and high associations have been found for NDS and clinical measurements such as glycemic levels and evidence of micro-vascular complications [237].

The current study demonstrated significant differences between all outcomes for control healthy group and those with type 2 diabetes. This was in agreement with finding from previous studies [237]. Additionally, this experiment showed no association between QST and NDS outcomes in the control group; however the outcomes demonstrated a relatively moderate association between QST and NDS in participants with type 2 diabetes. These findings suggest that these two techniques, although non-identical, can both detect presence of nerve damage in diabetes; hence their results are more comparable when neuropathy is present. Other studies have reported significant association between QST vibration perception and NDS [237]. However, classification

of NDS groups in their type 1 diabetes study cohort was different to what have been used in the current research.

In conclusion, our study demonstrated a moderate association between NDS and QST outcomes in individuals with type 2 diabetes. NDS and QST are demonstrably different measures of DPN. However, understanding the association between the two is important as the retinal nerve fibre layer outcomes as well as visual function measurements in the subsequent chapters will be analyzed against each of these techniques separately. It could be argued that it would be more accurate again to investigate associations between retinal nerve fibre layer thickness and visual function with alternate and arguably methods of quantifying peripheral neuropathy (such as NCS and skin or nerve biopsy); however this is beyond the scope of this thesis.

5 Reduced Retinal Nerve Fibre Layer Thickness isAssociated with Increasing Severity of Diabetic Peripheral Neuropathy

5.1 Introduction

Foot ulceration and consequent lower extremity amputation are two critical endpoints of diabetic peripheral neuropathy (DPN) and can affect up to 50% of people with diabetes [18]. Early diagnosis of DPN is currently most accurately achieved using methods such as skin and nerve biopsy; however the procedures are unpleasant, not available for routine clinical evaluation, and can create infection risks for the patients. Corneal confocal microscopy has introduced the prospect of a new and relatively precise ophthalmic marker for diagnosing DPN at early stages [50].However, the technique of corneal confocal microscopy requires contact with the cornea under anaesthesia. Additionally, there is increasing evidence to support a role for other anatomical ophthalmic indicators, such as retinal nerve fibre layer (RNFL) thickness, fulfilling a similar function in a more comfortable and non-invasive manner.

The RNFL makes up the innermost neural layer of the retina and is composed of the large unmyelinated axons of ganglion cells. These fibres originate from various locations of the retina and converge together in a unique pattern to form the optic nerve. Optical coherence tomography (OCT) is a non-invasive, non-contact imaging technique which is able to capture axial images of the retina *in vivo*, allowing measurement of

RNFL thickness[241]. This method provides detailed anatomical information and has strong repeatability, facilitating diagnosis and monitoring of retinal pathologies such as glaucoma and age-related macular degeneration (AMD). The technique is rapid and is capable of producing quality images even in presence of media opacities or with small pupil sizes [242, 243]. Additionally, reproducibility of this imaging technique in both healthy and diseased eyes has been demonstrated [125].

Much research on ophthalmic complications in diabetes has focused on vascular related aspects of the condition.[132, 244]. However, some studies have found evidence of structural [135, 140, 141] and functional [160, 192] changes prior to clinically detectable vascular complications. Lopes de-Faria*et al.*[140] and Skarf*et al.*[141] assessed RNFL thickness in early stages of retinopathy and found that reduced thickness prior to clinically visible vasculopathy was evident in cohorts with diabetes in comparison with normal healthy groups. Similarly, Sugimoto *et al.*[135] found decreased RNFL thickness, using OCT, in people with type 2 diabetes without retinopathy. This evidence supports the prospect that RNFL thinning may develop independent to micro-angiopathy and that RNFL can be considered as another potential ophthalmic marker of DPN.

The current study aimed to investigate the capability of retinal nerve fibre layer thickness in predicting DPN at early stages. Specifically, we predicted that RNFL thickness would be significantly reduced in individuals who were at risk of foot ulceration.

5.2 Aims and hypothesis

The aim of this study was to:

1. Investigate the association between peripheral neuropathy and RNFL thickness; to date this has not been attempted previously.

This study tested the following specific hypotheses:

1. RNFL thickness is significantly different between:

    a. Healthy individuals and those with type 2 diabetes without DPN

    b. Participants with type 2 diabetes with/without DPN

2. RNFL thickness is significantly inversely related to the severity of diabetic peripheral neuropathy in people with type 2 diabetes.

3. Retinal nerve fibre layer thickness is significantly reduced in individuals who are at risk of foot ulceration.

5.3 Methods

5.3.1 Participants

The study was conducted between January and July 2009 at Institute of Health and Biomedical Innovation, Brisbane, Australia. All volunteers with type 2 diabetes were

recruited from the Department of Diabetes and Endocrinology, Princess Alexandra Hospital. Participants were in the age range of 45-77 years. Eighty-two individuals with type 2 diabetes and 24 healthy controls consented to the study. Individuals with diabetes were classified into sub-groups of none, mild, moderate or severe neuropathy. A full description of the neuropathy classification method is provided below ("*Assessment of Neuropathy"*). No significant difference between the ages of the five groups (four with diabetes and one control) was observed (mean ± standard deviation of the mean: 61± 6 vs 59.1 ± 7.2 for all participants with type 2 diabetes and healthy controls; respectively, F= 1.3, p=0.25). The demographics of the study cohort are shown in Table 17.

To address the hypothesis regarding association between RNFL thickness and severity of neuropathy a sample of 48 will provide 90% power (α=0.05) that an association between RNFL and NDS where one exists. The sample is inadequate to address the hypothesis regarding differences between groups, as a sample of 45 per group is necessary to reveal a clinically meaningful difference of 5 microns between diabetes groups and controls for RNFL when standard deviation of 12 microns is applied (values from unpublished pilot data in our lab). These data will be considered further pilot data from our lab.

The majority of published work on RNFL variations in normal healthy individuals or diseased cohort have shown that gender has no significant effect on retinal nerve fibre layer thickness in healthy eyes[79]. Based on this, gender has not been fitted as a factor in any of the regression models.

Table 17.Demographics and characteristics of participants (mean ± standard deviation),

		Type 2 diabetes			
Parameter	**Control**	**No DPN**	**Mild DPN**	**Moderate DPN**	**Severe DPN**
N	24	23	32	16	11
Age (yrs)	59.1 ± 7.2	56.2 ± 6.1	62.1 ± 6.0	62.1 ± 7.8	62.5 ± 6.0
Gender (M/F)	13/11	12/11	22/9	10/6	8/3
Duration of diabetes (yrs)	0	11.8 ± 9.5	13.0 ± 8.2	14.8 ± 9.3	17.2 ± 10.3

DPN: diabetic peripheral neuropathy

5.3.2 Ophthalmic examination

One eye of each participant was selected for examination based on the hand-dominant side, unless this eye did not meet eligibility criteria. All participants underwent a screening examination and eyes with visual acuity worse than 6/9, history of intra ocular pressure greater than 21 mmHg, advanced media opacity or any history of retinal disease, including AMD or glaucoma, were excluded from the study. Fundus photographs (45 degrees) were obtained using a non-mydriatic camera (Visucam Pro, Carl Zeiss MeditecInc, Dublin, CA, USA) to assess diabetic retinopathy. All photographs were graded by two observers according to NHMRC grading scales guidelines[216].

Retinal nerve fibre layer thickness was measured using optical coherence tomography (RTVue, Optovue, Fort mount, CA, USA). OCT employs the principle of low-coherence interferometry to produce two-dimensional images by optical back-scattering with an axial resolution of up to 5 μm [245]. Aside from employment of light instead of

sound, OCT is comparable to ultra-sonic echo imaging technique [110]. Participants were seated comfortably in a darkened room and were asked to fix on an internal target. The "Optic Nerve Head" (ONH) protocol was used to generate an RNFL thickness map by centring a circle 3.45 mm in diameter on the optic nerve head. The software algorithm attains 24 radial scans in 0.37 seconds and records thickness at 3.45 mm in diameter for each scan. The average RNFL thickness is summarized into temporal, superior, nasal, and inferior (TSNI) quadrants as well as a global average.

5.3.3 Assessment of neuropathy

A modified neuropathy disability score (NDS) was used to evaluate the level of peripheral neuropathy [18, 45]. A comprehensive description of the test and classification of NDS groups have been explained in *Chapter 3*. NDS score of six or greater has been shown to indicate increased risk of ulceration [45]. This is discussed further in the next section.

5.3.4 Statistical Analysis

Normality of distribution for each RNFL measurement was assessed and the average RNFL thickness was found to be approximately normally distributed in the study sample (Kolmogorov-Smirnov statistics = 0.06, p = 0.20) (Figure 21).Comparison between four NDS-derived groups of participants with type 2 diabetes was performed using analysis of variance (ANOVA), and a Student t-test was used for the comparison between the no neuropathy group and healthy controls. Univariate regression analysis was performed to assess the effect of NDS scores, age, and duration of diabetes on RNFL thickness measurements. NDS score of six was used as the cut-off point for risk of ulceration and an independent t-test was used for this additional analysis. Statistical Package for the Social Sciences (SPSS) version 18 was used for all statistical analyses.

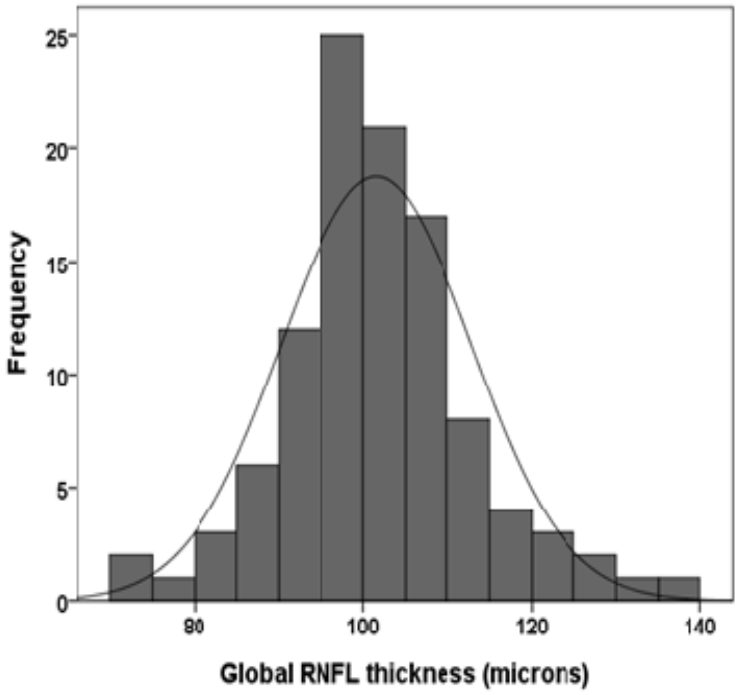


Figure 21. Retinal nerve fibre layer thickness distribution plot in the cohort with diabetes

5.4 Results

Retinal nerve fibre layer thickness was not significantly different between healthy control participants and individuals with diabetes without neuropathy (Table 18). Difference in the nasal RNFL thickness between these two groups was close to significant but not quite ($p = 0.07$).

A weak non-significant trend towards RNFL thinning with increasing levels of DPN (from mild to severe) was observed globally and for each quadrant (the superior quadrant outcome was not linear). However, mean differences between the four groups with diabetes were found not to be statistically significant (Table 19). When the two no-

neuropathy groups (those with diabetes and controls) were added, the mean values showed a mildly curved shape globally and in two quadrants (Figure 22).As such, both linear and quadratic regression models were applied to the data. These models explained essentially the same amount of variance, so a linear model was used for these analyses.

A significant reduction of 1.46 microns for every unit increase in severity of neuropathy was found for the inferior RNFL quadrant (p= 0.03). Age, level of diabetic retinopathy and duration of diabetes (DD) did not show any significant association with inferior RNFL thickness (Table 20). The same model applied to all other quadrants and to the global measurements produced no statistically significant outcomes (Table 20). Similarly, age, duration of diabetes and level of diabetic retinopathy did not show significant associations with RNFL measurements for any of the regression models (Table 20).

Table 18.RNFL thickness comparisons between controls and individuals with diabetes without DPN.

RNFL thickness (µm)	Control	No neuropathy	t =	p =
Global	102.8 ± 11.2	101.8 ± 13.1	- 0.28	0.78
Temporal	80.7 ± 8.7	76.6 ± 16.8	- 1.07	0.28
Superior	127.4 ± 17.3	117.2 ± 20.4	-1.76	0.07
Nasal	72.4 ± 11.7	77.1 ± 15.2	1.17	0.24
Inferior	131.7 ± 21.2	132.5 ± 20.5	0.11	0.90

t: independent samples student t test statistics value

Table 19.Global and quadrant mean (± standard deviation) RNFL thicknesses for four NDS groups with type 2 diabetes with increasing levels of neuropathy. Degrees of freedom are (3,78)

RNFL (µm)	None	Mild	Moderate	Severe	F =	P =
Global	101.8 ± 13.1	102.9 ± 9.2	101.2 ± 10.4	94.7 ± 13.1	1.49	0.22
Temporal	76.6 ± 16.8	76.5 ± 12.9	72.5 ± 14.1	68.8 ± 13.5	0.85	0.47
Superior	117.2 ± 20.4	121.9 ± 13.5	127.2 ± 21.0	112.7 ± 17.4	1.81	0.15
Nasal	77.1 ± 15.2	80.9 ± 12.5	78.1 ± 13.6	75.8 ± 16.9	0.52	0.66
Inferior	132.5 ± 20.5	132.9 ± 18.2	125.3 ± 7.7	119.6 ± 14.4	2.27	0.08

F: ANOVA statistics value

Table 20. Regression analysis for associations between RNFL measurements, NDS, age, duration of diabetes (DD) and level of diabetic retinopathy (DR)

	NDS		Age (yrs)		DD (yrs)		DR		Adj R^2
	B =	p =	B =	p =	B =	p =	B =	p =	
Global	-0.20	0.63	-0.25	0.24	0.13	0.40	-3.04	0.07	0.02
Temporal	-0.07	0.87	-0.59	0.03*	0.35	0.07	-3.30	0.12	0.05
Superior	0.37	0.51	-0.22	0.61	0.10	0.69	-3.40	0.22	-0.03
Nasal	0.03	0.95	0.06	0.80	-0.10	0.61	-0.44	0.83	-0.04
Inferior	1.46	0.03*	0.09	0.76	0.03	0.86	-0.35	0.89	0.02

B : regression coefficient, p : significance

Effect of diabetic retinopathy

The effect of diabetic retinopathy (DR) was additionally analysed in a separate model to assess its influence on RNFL thickness relative to the effect of neuropathy. Seventy eight fundus photos were graded to determine the level of DR with four photos excluded because of insufficient quality. Two eliminated photos were from the group with mild neuropathy, one from the moderate and another from the severe neuropathy group. Diabetic retinopathy was graded according to Australian National Health and Medical Research Council (NHMRC) guidelines [216]and individuals were stratified into four groups of none (n = 36), minimal (n = 16), mild (n = 25) and moderate (n = 1). None of the participants had severe non-proliferative or proliferative retinopathy. ANOVA demonstrated that neither DR group nor the interaction between DR and NDS had a significant effect on RNFL measurements (all p-values > 0.49 for main effect and > 0.13 for interaction).

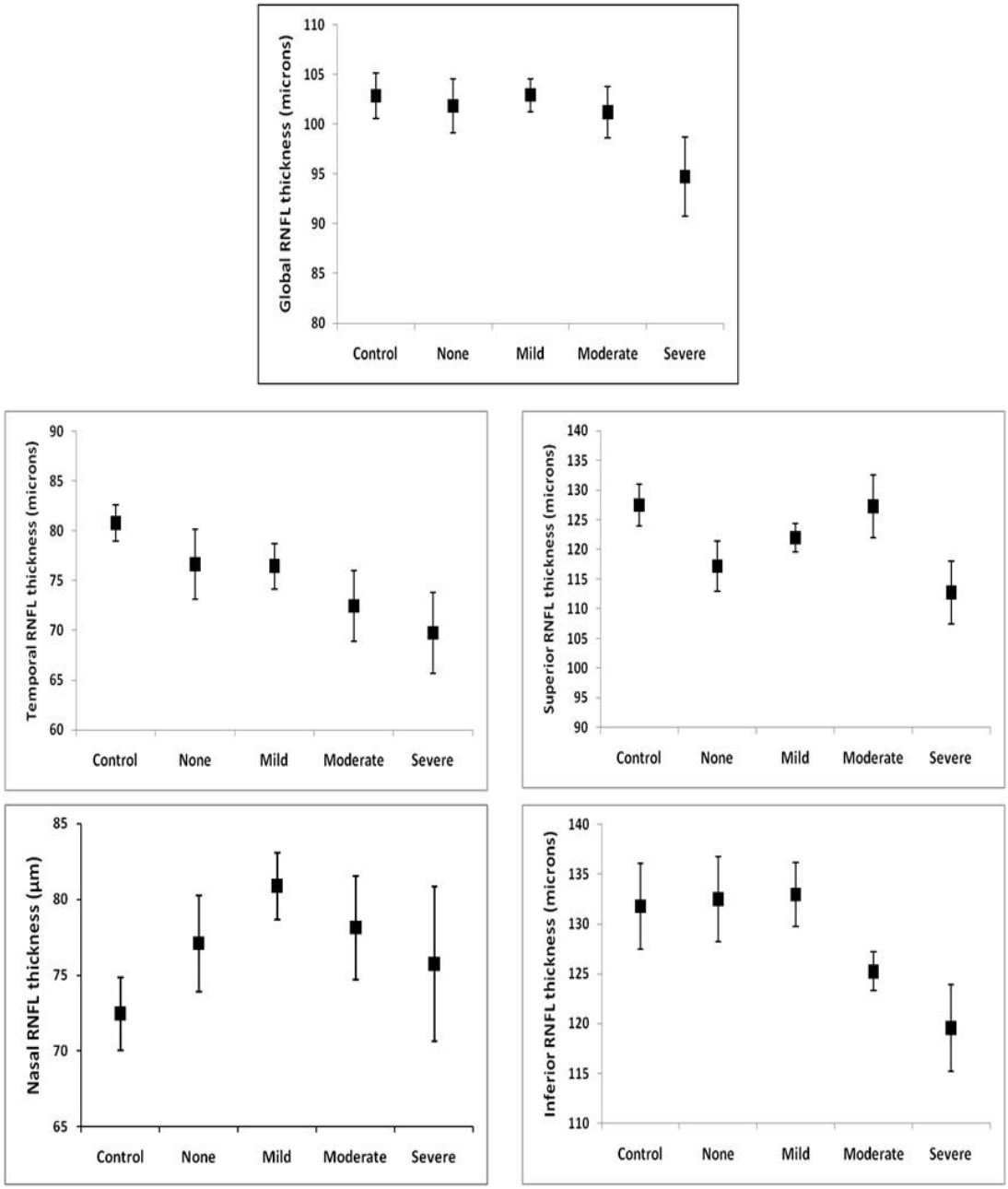


Figure 22.Mean RNFL thicknesses (± standard error) for global and quadrant outcomes.Note that the y-axis scales for each graph do not match.

Assessment of risk of ulceration

An NDS cut-off point of six was used to assess the association between RNFL thickness and risk of ulceration in participants with diabetes. No significant differences were found between the ages of the two groups (age ± SD: 61 ± 6 years vs 62 ± 7 years for lower and higher risk; respectively, $t = -0.93$, $p = 0.35$). The group who were considered at risk of ulceration [45] (NDS ≥ 6; n = 27, 18 males) demonstrated lower RNFL thicknesses globally and in each quadrant, except superiorly, than those not at risk (n = 55, 34 males). A significant difference was found for the mean inferior RNFL thickness comparison ($t=2.9$, $p<0.005$); however not for the remaining quadrants (temporal $t = 1.5$, $p = 0.12$; superior $t = -0.3$, $p = 0.75$; nasal $t = 0.65$, $p = 0.51$), nor global RNFL thickness ($t = 1.4$, $p = 0.14$) (Figure 23).

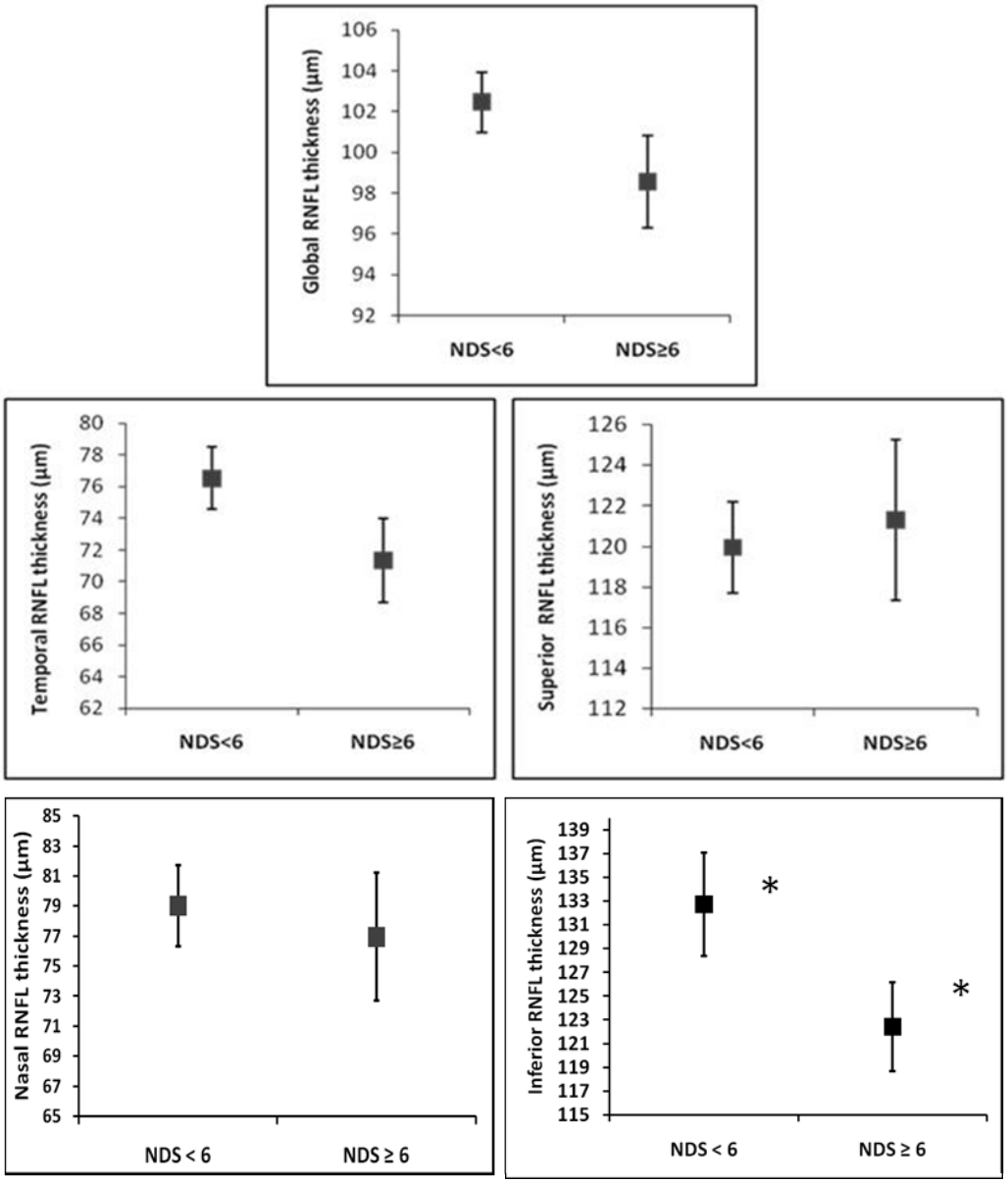


Figure 23. Comparison of RNFL thickness measurements grouped according to NDS cut-off point of six, which indicates risk of ulceration in people with diabetes. $p < 0.005$. Note that the y-axis scales for each graph do not match

5.5 Discussion

This experiment aimed to investigate the association between diabetic peripheral neuropathy and retinal nerve fibre layer thickness. Results showed an overall tendency towards thinning of RNFL as the severity of peripheral neuropathy increased. However, the characteristics of this association were not necessarily consistent for each quadrant; inferior region demonstrated the highest levels of relative loss as NDS score increased (Figure 5.2). Previous work has reported an average reduction of 2.7 μm in thickness of global RNFL per increasing decade of age [68]. By comparison, our finding of 1.46 μm reduction in the inferior quadrant for each NDS score unit can potentially be considered to be clinically meaningful. In addition, this finding was independent of the effect of age, duration of diabetes and diabetic retinopathy; hence, RNFL changes in this sample can be confidently explained by neuropathy status of the cohort. To further comment on this point, age-induced RNFL 2.7 microns thickness reduction occurs on average, over 10 years while neuropathy-induced reduction changes may occur in a much shorter time period and compound the effect attributable to age. A longitudinal study will be required to demonstrate the actual time-frames associated with these cross-sectional derived models.

Neuropathy in diabetes is thought to be characterised by neural ischemia, and a close link between diabetes progression with both neural and vascular dysfunction has been established [246]. Therefore, microvascular abnormalities such as basement membrane thickening (which also occurs in retinopathy) can lead to neuralischemia and hypoxia. However, the occurrence of neural damage prior to micro-vasculopathy has also been argued [247]. In addition, apoptosis of retinal neural cells has been reported in post-

mortem human studies and in animal models of diabetes [63, 144].These studies provide support for a putative model of primary neuropathic damage in the retina, unrelated to clinically detectable vascular change.

Two research groups who investigated the association between diabetes and RNFL thickness reported thickness reduction to be most evident in the superior quadrant[135, 141].Others have found no significant quadrant or hemisphere differences [248, 249]. None of these studies, however, stratified their diabetic cohorts according to peripheral neuropathy status. In the current study, inferior RNFL thickness was reduced more than the other quadrants with increasing levels of DPN.

One way to rationalise the findings of this experiment is to contrast them with the pathophysiology of glaucoma. In glaucoma, changes to the inferior neural rim and RNFL thickness associates most closely with progression of the disease [75]. This has been explained by the thicker inferior nerve fibre layer having higher sensitivity to oxygen deprivation in comparison with the superior quadrant - the second thickest RNFL sector [250]. In a normal retina, inferior RNFL is the thickest of all quadrants, hence it requires greater oxygen and blood supplies than the other three [251]. In a diabetes model, the high inner-retinal metabolic demand (including RNFL) may limit the ability of the inner retina to adapt to the increased metabolic stress created by the disease. Given that the blood flow per retinal nerve fibre tissue volume in normal human retina has been reported as lower for the inferior sector than for others [252], the effect of metabolic stress caused by diabetes may be amplified for this region.

Other investigated factors did not substantially account for the reported RNFL thickness changes in the current study. Duration of diabetes has been demonstrated as an

important risk factor for proliferative retinopathy[253].However, using our regression model, neither disease duration nor age independently explained global or regional RNFL thickness changes. Almost all participants had no greater than mild levels of retinopathy and many had none at all (only one had moderate) so, again, retinopathy is unlikely to be responsible for these findings. There were no significant RNFL thickness differences (globally or in any quadrant) between healthy controls and participants who had diabetes but no neuropathy; this suggests that diabetes per se is less important than neuropathy status in explaining the outcomes.

The findings of this study are important - firstly because retinal nerve fibre layer thickness reduction could underlie subtle and unrecognised impairments of visual function in people with diabetic peripheral neuropathy. This would need to be investigated by testing a range of visual function measures in this population. Secondly, the significant association between RNFL thickness and DPN, although only demonstrated in the inferior retina, raises the prospect of RNFL thickness as a potential surrogate marker possibly in conjunction with other ocular markers. Optical coherence tomography has the advantage of being non-invasive and relatively cost-effective. In particular, the current study showed the capacity to identify those at risk of foot ulceration, which may ultimately help to reduce the incidence of lower limb amputation associated with neuropathy. These are promising although very early findings - nonetheless they justify further investigation of the role of the retina in diabetic peripheral neuropathy.

6 Relationship between Retinal Nerve Fibre Layer Thickness and severity of Diabetic Peripheral Neuropathy as determined by Quantitative Sensory Testing

6.1 Introduction

Diabetic neuropathy is known to affect different divisions of the peripheral nervous system through a variety of pathologic processes. These procedures have been shown to also appear indiabetes-induced cerebral functional and structural complications [254-256]. For instance, impaired cognitive functioning and increased visual evoked potential (VEP) wave latency in individuals with diabetes are good examples of neuro-physiological manifestation of central nervous system (CNS) and higher brain dysfunction associated with diabetes[257, 258]. As such, diabetic neuropathy should not be considered merely as a complication of the PNS.

Retinal nerve fibre layer (RNFL), structurally a part of the CNS, has been shown to be thinner in people with diabetes [134, 135, 259]. Histological studies of neural components of the retina have also shown that diabetes-induced biochemical mechanisms can potentially cause neural cell degeneration [152, 260]. Such findings consequently support the prospect of structural damage to the CNS linked with diabetes. However,the underlying reasons for RNFL thinning or retinal neural degeneration are as yet unknown. The major focus of research studies addressing this question has been on structural changes in the absence ofretinal vascular complications. This evidence

supports the concept of RNFL thickness reduction in people with diabetes having a neuropathic origin.

Optical coherence tomography (OCT) is a relatively new non-invasive optical imaging technique and is employed for capturing cross sectional images of the retinal layers, *in vivo*[261]. The technique has been described in detail in Chapter 2. Quantitative sensory testing (QST) is an arguably reliable method of detecting small fibre neuropathies and predicting foot ulceration in patients with DPN[235]. QST is a highly subjective method of evaluating responses to vibrating and thermal stimuli and for determining sensation and pain thresholds; it can be applied at a number of anatomical sites but is commonly used on the feet in people with suspected diabetic peripheral neuropathy[48]. The advantage of QST compared with other methods of assessing neuropathy such as NDS and monofilament is that the stimulus intensity is much better controlled[238]. However the outcome can be influenced by factors like age of participants, smoking and alcohol intake as well as their cooperation while performing the test [36].

Previously it was demonstrated that retinal nerve fibre layer, more particularly in the inferior quadrant, becomes thinner as the severity of neuropathy increases (higher neuropathy disability score) (*Chapter 5*). Therefore, it was concluded that RNFL thickness has as association with diabetic neuropathy assessed by means of NDS andprimarilyis capable of predicting increased risk of foot ulceration. In this chapter the association between QST and RNFL was investigated as QST is arguably a more precise method of assessing DPN in comparison with NDS due to its capability of controlling the stimulus intensity.

6.2 Aims and hypothesis

The aim of this study was to:

Investigate the association between RNFL thickness and QST in individuals with type 2 diabetes.

This experiment specifically investigates the following hypothesis:

1. RNFL thickness is significantly reduced with increased severity of diabetic peripheral neuropathy (loss of sensitivity) as measured by quantitative sensory testing.

6.3 Methods

6.3.1 Participants

Characteristics of the participants with type 2 diabetes have been described in *Chapter 5, section 5.3.1*. Ethics approval for this study was obtained from QUT and Princess Alexandra Hospital Research Ethics Committees. Inclusion and exclusion criteria are explained in *Chapter 3, section 3.4*. Participants were in the age range of 45 - 77 years. One hundred and five individuals with type 2 diabetes consented to the study of which 82 eligible participants (52 males, 30 females) formed the study group. It is noteworthy that the number of male participants is %22 more than females. Given that gender is known not be influential on RNFL outcomes [79], it has not been fitted in the models. To address the hypothesis regarding association between RNFL thickness and severity

of neuropathy a sample of 48 will provide 90% power (α=0.05) that an association between RNFL and QST variables where one exists.

6.3.2 Assessment of retinal nerve fibre layer thickness

Retinal nerve fibre layer scans were captured using Fourier domain optical coherence tomography (Optovue, RTVue, Ltd, Fremont, USA). Optic nerve head (ONH) protocol was employed to acquire radial scans. The ONH protocol covers an area of 3.75 mm in diameter centred on the optic nerve head. The output is a measure of global as well as temporal, superior, nasal and inferior (TSNI) quadrant RNFL thicknesses. A comprehensive explanation of the test is outlined in *Chapter 3, section 3.7.3.4.*

6.3.3 Assessment of neuropathy by quantitative sensory testing

Quantitative sensory testing (QST) was performed on the dorso-lateral side of the foot on the hand-dominant side of each participant, in order to test for participants' ability to detect temperature change (cold sensation threshold and warm sensation threshold), pain thresholds (cold induced pain and heat induced pain) as well as the ability to detect vibration (vibration perception threshold). The QST protocol was explained to the participants and a trial was performed to familiarise the participants with the technique. For more details on QST please see *Chapter 3, section 3.7.2.1*.

6.3.4 Statistical Analysis

Descriptive statistics are reported, including mean ± standard deviation (SD).Univariate regression analysis was used to assess the association between QST measurements and RNFL thickness globally and in all quadrants. All models reported the effect of each

QST subtest (cold, warm, cold induced pain, heat-induced pain and vibration) separately as well as main effects for age, duration of diabetes and level of diabetic retinopathy. Analyses of pain thresholds (both cold-induced and heat-induced) have been performed using two approaches that will be explained in the appropriate section (*section 6.5*). A p-value less than 0.05 was considered as statistically significant. Statistical Package for Social Sciences (SPSS) version18 was used to analyse the collected data.

6.4 Results

Non-significant results were found for the association between QST measurements and RNFL thicknesses globally and in all quadrants. These outcomes have been summarized in Table 21. Associations between RNFL thickness measurements and QST cold sensation, warm sensation and vibration perception are shown in Figure 24 - Figure30.

Table 21.Associations between quantitative sensory testing (QST) sub-tests and retinal nerve fibre layer thickness outcomes.

QST	Covariate	Retinal nerve fibre layer thickness region													
		Global			Temporal			Superior			Nasal			Inferior		
		B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2
CS	Main€	-0.16	0.17	0.01	0.04	0.75	0.04	-0.34	0.08	-0.01	-0.27	0.08	-0.01	< -0.01	0.99	-0.03
	Age	-0.25	0.20		-0.47	0.06		-0.29	0.42		-0.16	0.52		-0.03	0.90	
	DD	0.05	0.69		0.34	0.07		-0.07	0.76		-0.14	0.43		0.08	0.72	
	DR	-2.90	0.07		-3.17	0.10		-0.74	0.76		-0.42	0.82		-3.55	0.14	
WS	Main	0.31	0.17	< -0.01	-0.06	0.85	0.03	0.69	0.12	-0.02	0.33	0.34	-0.05	-0.14	0.73	-0.03
	Age	-0.23	0.25		-0.48	0.05		-0.17	0.58		-0.08	0.72		-0.01	0.97	
	DD	0.05	0.72		0.34	0.07		-0.09	0.71		-0.14	0.46		0.09	0.68	
	DR	-2.92	0.07		-3.19	0.10		-0.84	0.73		-0.28	0.88		-3.39	0.17	
CIP	Main	0.01	0.95	-0.01	0.16	0.52	0.04	-0.07	0.82	-0.05	-0.13	0.60	-0.05	0.51	0.10	<-0.01
	Age	-0.18	0.37		-0.48	0.05		-0.06	0.84		-0.04	0.86		<0.01	0.99	
	DD	0.08	0.52		0.34	0.06		-0.03	0.90		-0.11	0.53		0.11	0.63	
	DR	-2.59	0.10		-3.13	0.10		-0.18	0.94		-0.04	0.98		-3.14	0.15	
HIP	Main	0.36	0.68	< -0.01	1.24	0.25	0.05	0.99	0.48	0.04	0.46	0.67	-0.05	-0.63	0.64	-0.03
	Age	-0.18	0.34		-0.53	0.03*		-0.09	0.77		-0.04	0.85		-0.01	0.96	
	DD	0.07	0.62		0.3	0.10		-0.04	0.75		0.12	0.53		0.09	0.68	
	DR	-2.68	0.09		-3.56	0.06		-0.06	0.88		-0.04	0.98		-3.40	0.16	
VP	Main	0.03	0.39	< -0.01	<0.01	0.89	0.03	0.06	0.30	-0.04	0.05	0.26	-0.04	-0.05	0.30	-0.02
	Age	-0.21	0.33		-0.53	0.05		-0.12	0.71		-0.12	0.64		0.11	0.73	
	DD	0.05	0.72		0.33	0.08		-0.07	0.75		-0.15	0.42		0.12	0.59	
	DR	-2.98	0.07		-3.36	0.10		-0.87	0.74		-0.63	0.75		-2.69	0.29	

€: refer to main effect of the QST parameter, CS: cold sensation, WS: warm sensation, CIP: cold-induced pain, HIP: heat-induced pain, VP: vibration perception, DD: duration of diabetes, DR: diabetic retinopathy, B: regression coefficient, Adj R^2: adjusted R^2

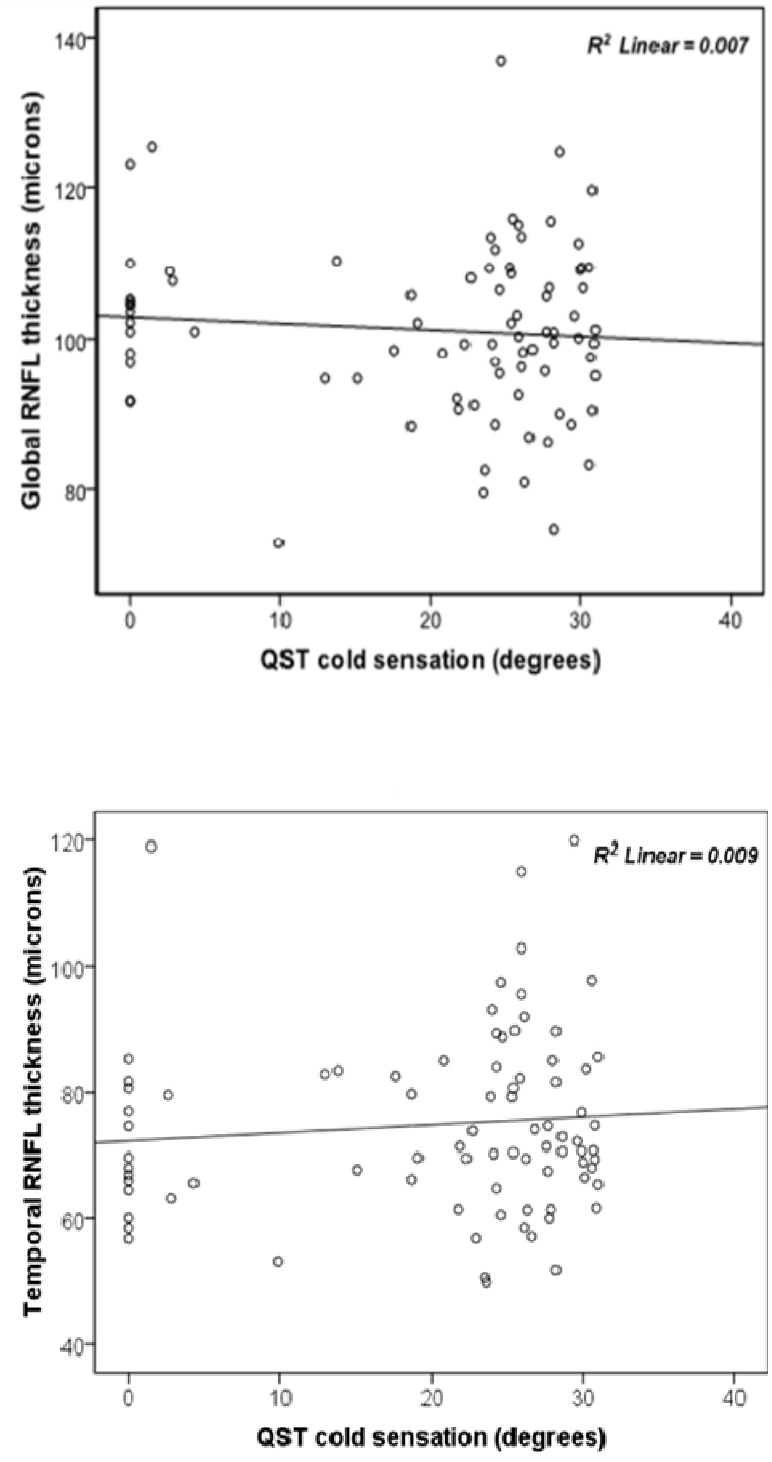


Figure 24. Scatter plots for global and temporal RNFL measurements and QST cold sensation threshold. Note the y-axis scale differs for each plot. Decrease in sensitivity is to the left on the x-axis for each panel.

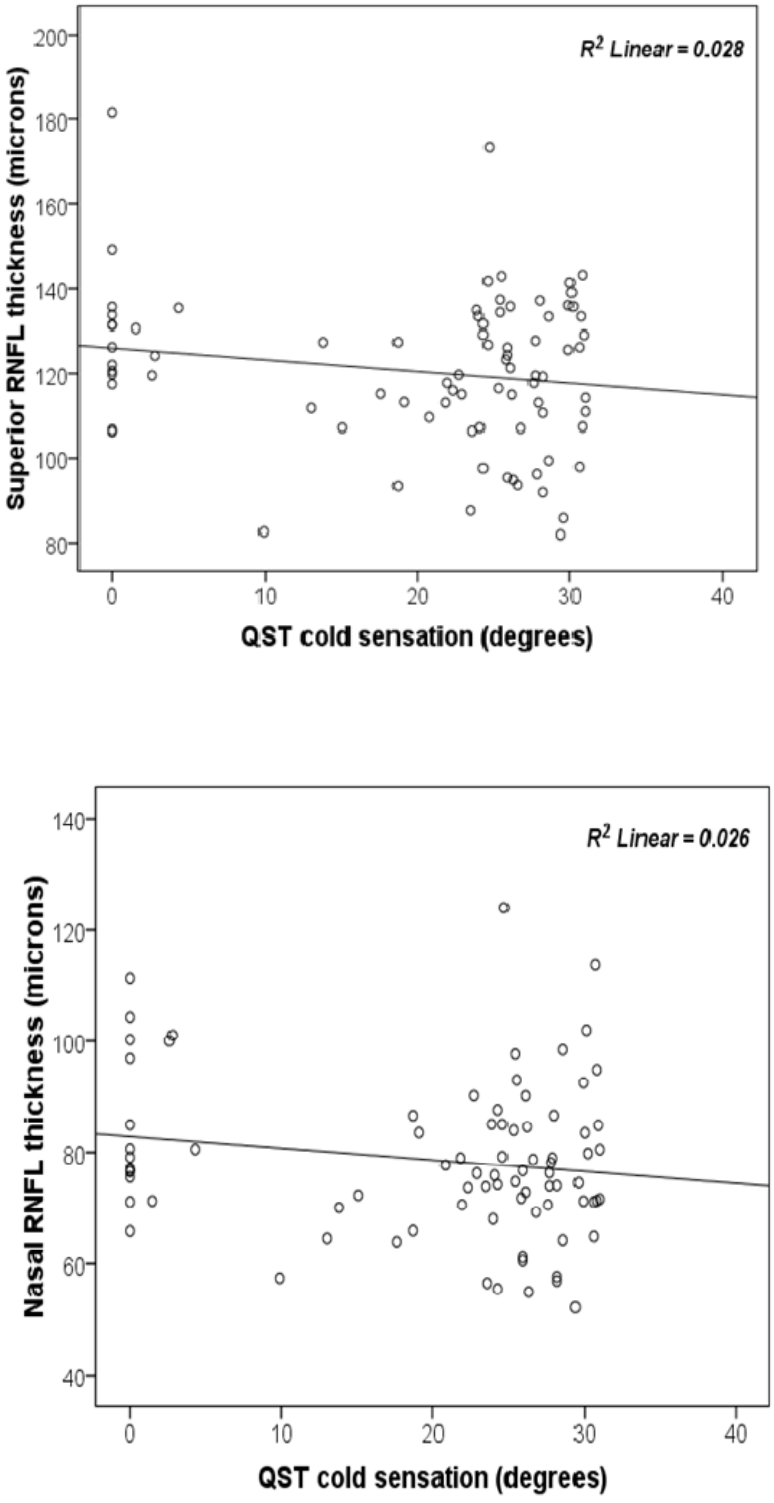


Figure 25. Scatter plots for superior and nasal RNFL measurements and QST cold sensation threshold. Note the y-axis scale differs for each plot. Decrease in sensitivity is to the left on the x-axis for each panel.

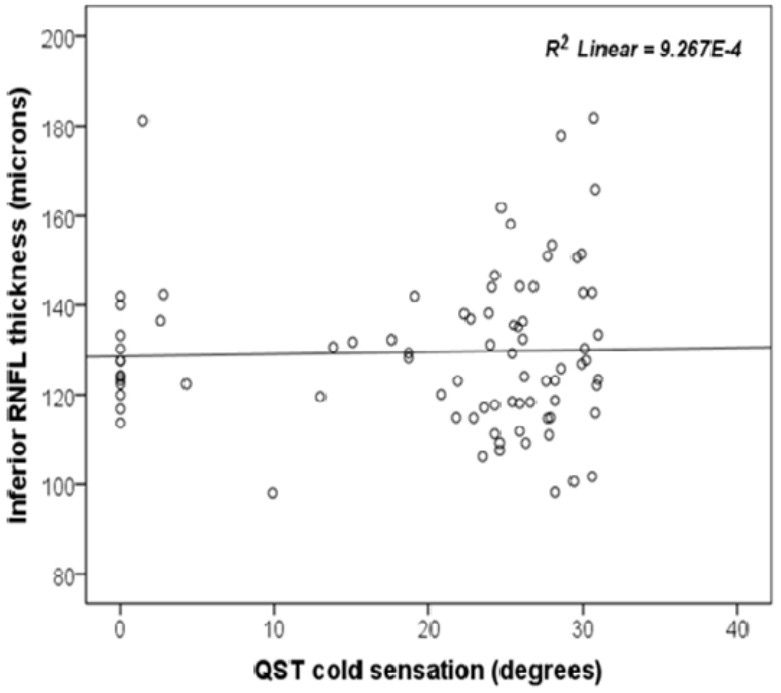


Figure 26. Scatter plots for inferior RNFL measurement and QST cold sensation threshold. Decrease in sensitivity is to the left on the x-axis for the panel.

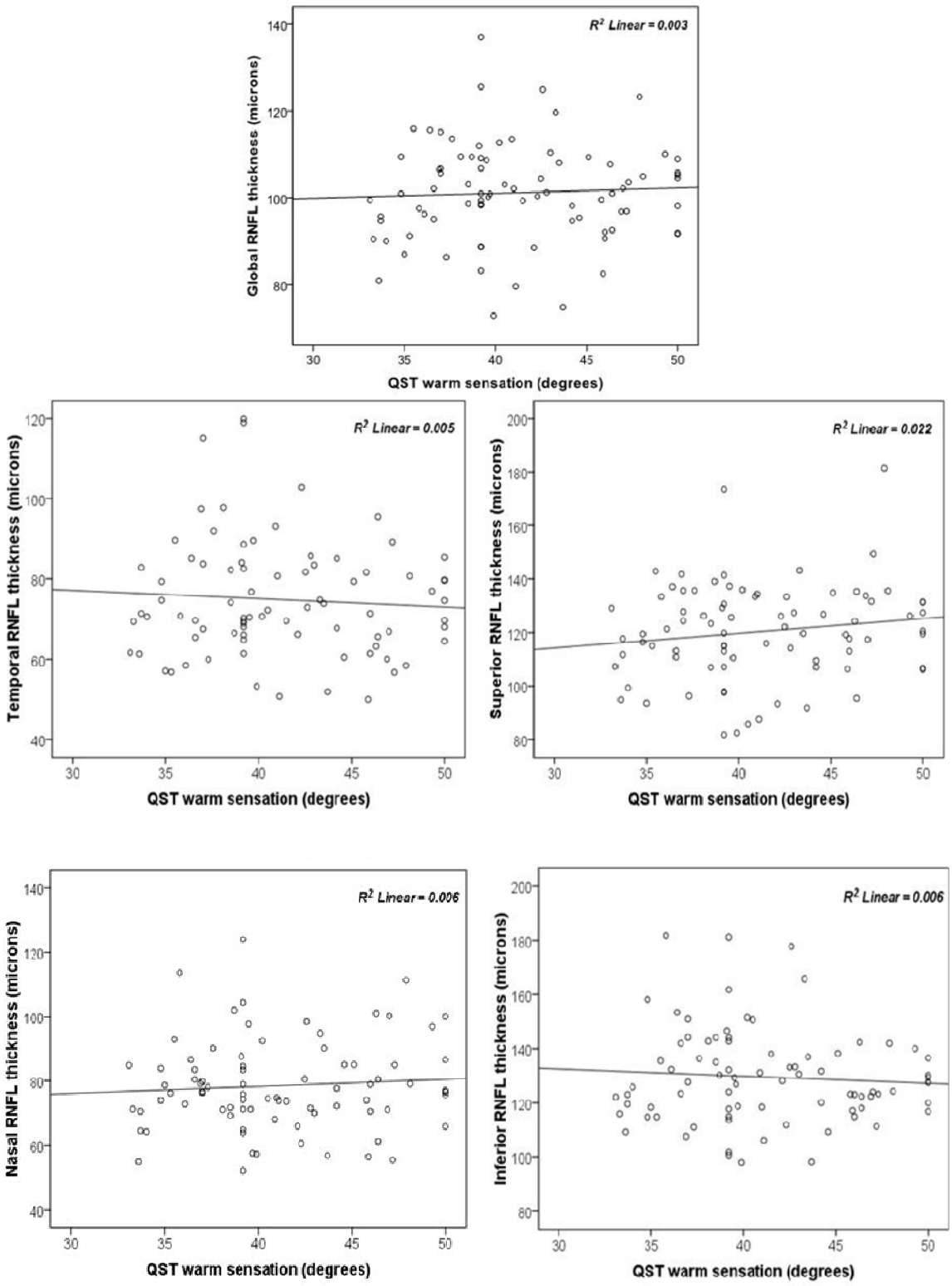
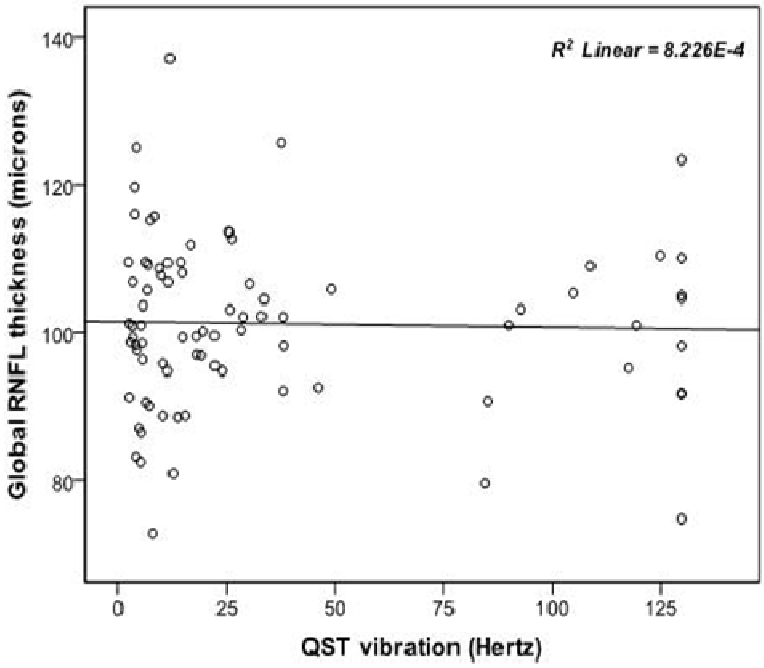


Figure27. Scatter plots for RNFL measurements and QST warm sensation threshold. Note the y-axis scale differs for each plot. Sensitivity decreases to the right on the x-axis for each panel.



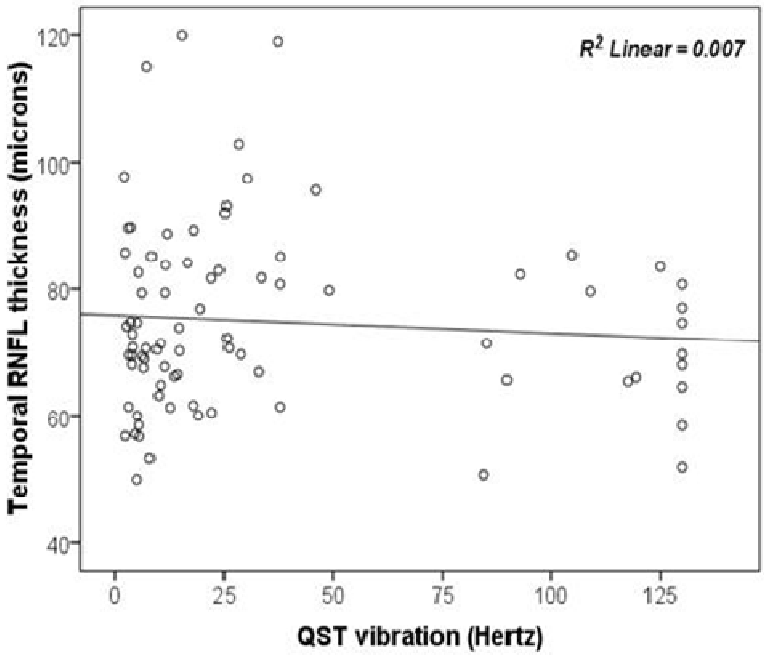
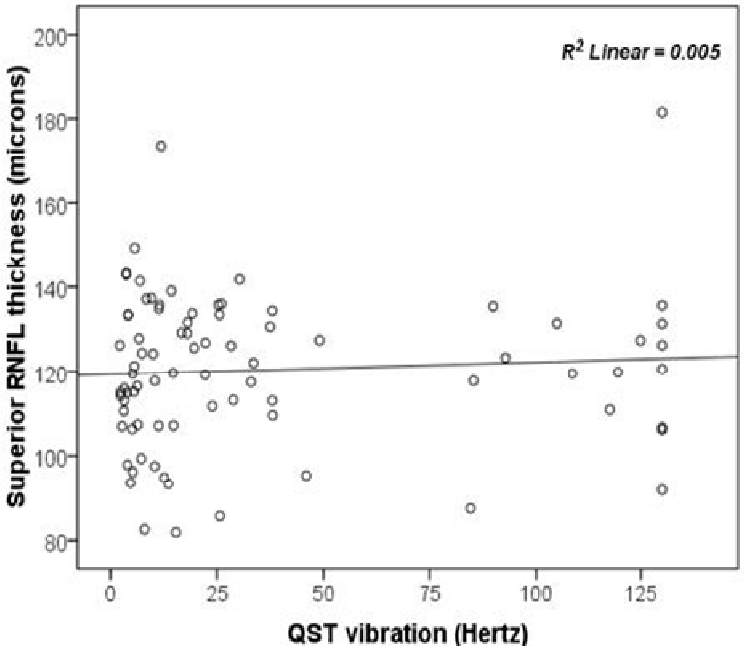


Figure28. Scatter plots for global and temporal RNFL measurements and QST vibration perception threshold.Note the y-axis scale differs for each plot. Sensitivity decreases to the right on the x-axis for each panel.



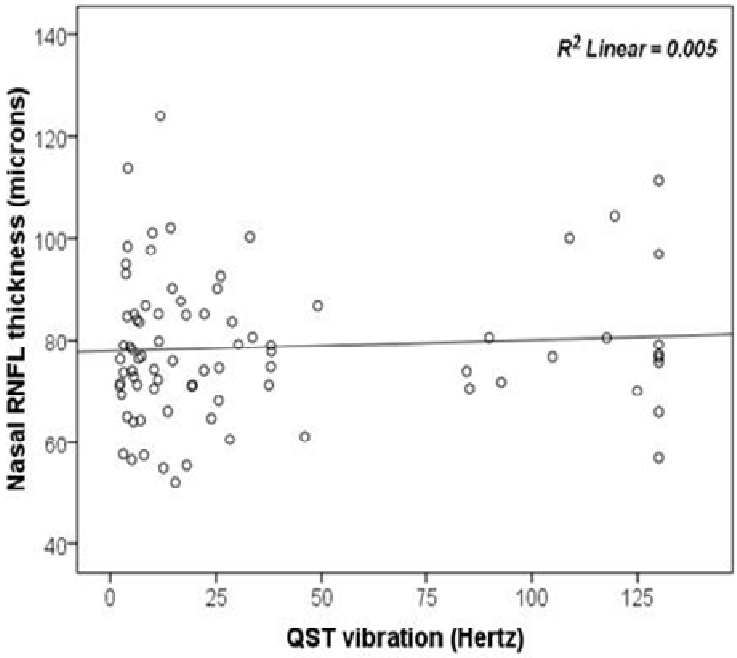


Figure29. Scatter plots for superior and nasal RNFL measurements and QST vibration perception threshold.Note the y-axis scale differs for each plot. Vibration sensitivity decreases to the right on the x-axis for each panel.

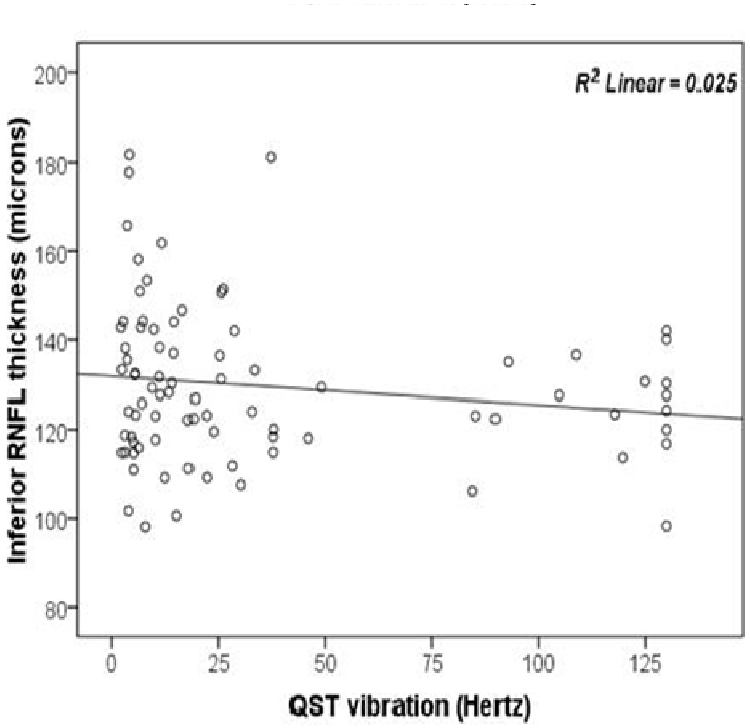


Figure30. Scatter plot for inferior RNFL measurement and QST vibration perception.

6.5 Pain thresholds

The cold and warm pain threshold tests of the QST (TSA-3000, Medoc. Ltd, Israel) has a lower and upper limit of 0 and 50 degrees respectively as a safety procedure to avoid skin burns. A number of participant's thresholds were recorded at the floor (0°C) or ceiling (50°C) values and these values are not expected to reflect the true cold or heat pain thresholds. Therefore, analysis of temperature induced pain was performed using two separate approaches. For cold-pain analysis one analysis included the floor values and another eliminated the floor values. Similarly, for heat-induced pain analysis the ceiling values were first included and then eliminated in the second approach.

6.5.1 Cold –induced pain

The first approach included the floor values. Sixty one precent of participants (50/82) had a value of zero degrees as their cold pain threshold. Median cold pain threshold was zero and values ranged between 0 and 27.3°C. There was no significant association between cold pain threshold and RNFL thickness globally and in all quadrants. Table 21 represents the regression coefficient values. Figure31 to Figure33shows the association between global and quadrant RNFL thicknesses and cold pain threshold.

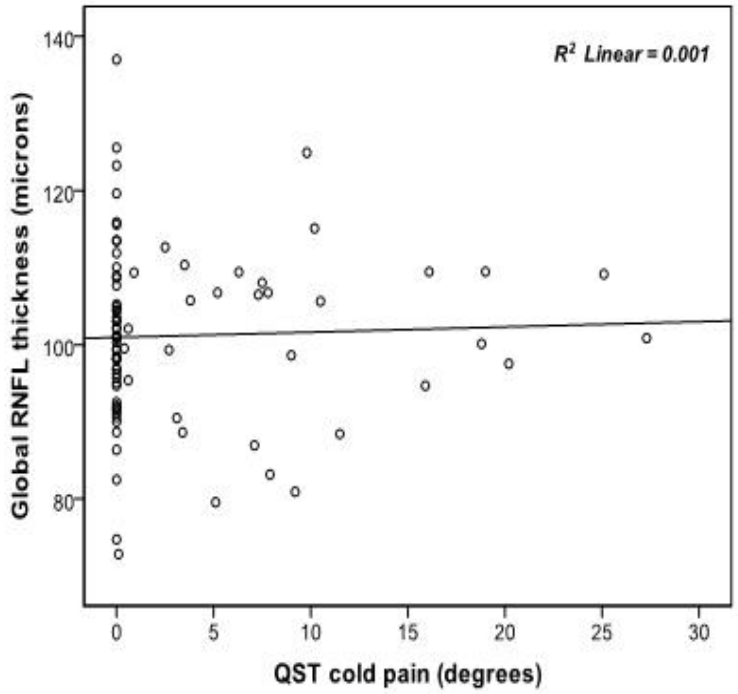


Figure31.Association between global RNFL measurement and cold pain sensation threshold. Sensitivity decreases to the left on the x-axis for each panel.

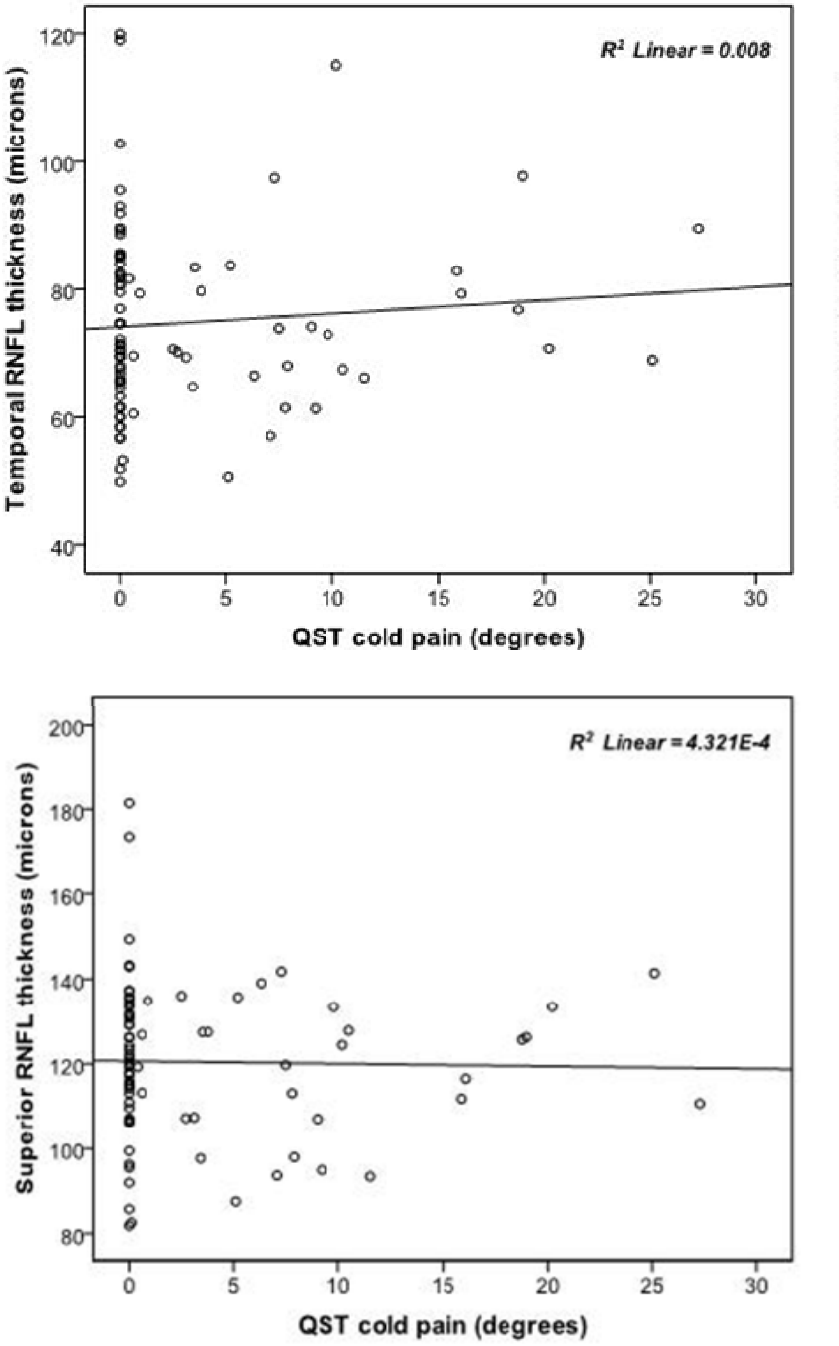
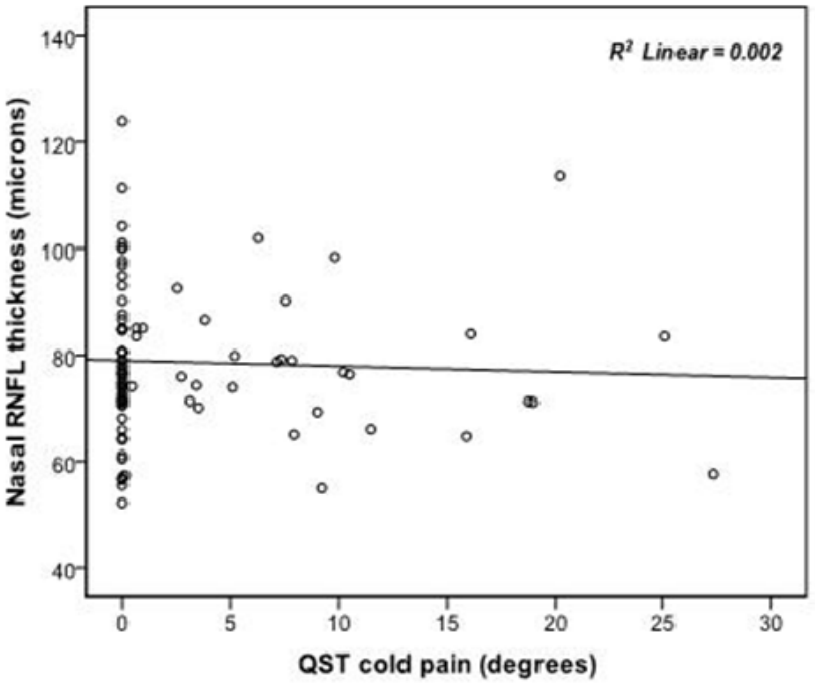


Figure32.Association between temporal and superior RNFL measurements and cold pain sensation threshold. Sensitivity decreases to the left on the x-axis for each panel.



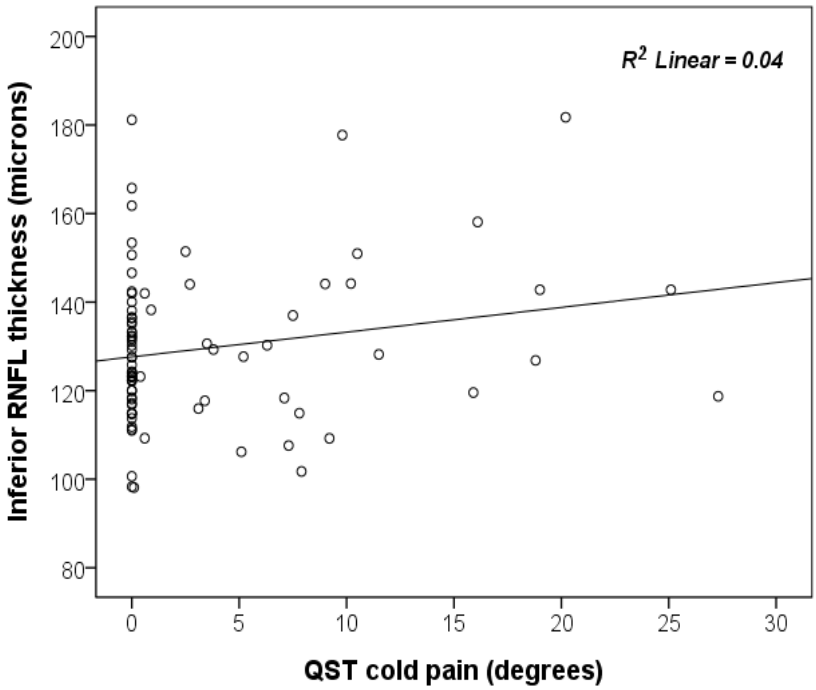


Figure33.Association between nasal and inferior RNFL measurements and cold pain sensation threshold. Sensitivity decreases to the left on the x-axis for each panel.

The second approach eliminated the floor values. It was known from NDS classification of the participants (described in *Chapter 5*) that of 50 participants (61% of total cohort) who had cold pain threshold of 0°C, 46% of these were classified as having moderate and severe neuropathy. The group with moderate neuropathy was eliminated entirely on the basis of these floor values and the remaining numbers of participants per each NDS group were as follows: no neuropathy (n = 11), mild neuropathy (n = 17) and severe neuropathy (n = 4). It is important to emphasise that a smaller sample of those with higher levels of neuropathy were included in this part of the analysis. Please note that there was no group comparisons in this section as QST heat-induced pain is a continuous variable. The neuropathy groups and numbers mentioned above are just to explain the severity of neuropathy based on NDS in the participants included in this analysis.

Median cold induced pain in 32 remaining participants (20 males) was 7.4 degrees ranging from 0.1 - 27.3 degrees. The median age of these participants was 60 years ranging 45 – 70 years.Using regression analysis, cold pain threshold did not appear to be a good predictor for RNFL thickness globally or in any of the four quadrants. Statistical reports are outlined inTable 22. Scatter plots for global and quadrant RNFL thickness and cold pain threshold are shown in Figure 34 to Figure36. There was a tendency towards RNFL thinning globally and in temporal, superior and inferior RNFL along with lower cold pain thresholds; with temporal quadrant showing the most obvious trends; however still not quite statistically significant (p = 0.05).

Table 22. Regression coefficient for RNFL measurements and cold pain when excluding floor values (0°C)

	CIP		Age (yrs)		DD (yrs)		DR		
	B =	p =	B =	p =	B =	p =	B =	p =	Adj R^2
Global	0.30	0.30	-0.05	0.86	0.16	0.57	-3.40	0.06	-0.03
Temporal	0.61	0.05	-0.37	0.16	0.31	0.31	-5.36	0.04	0.14
Superior	0.44	0.32	-0.02	0.96	0.33	0.44	-1.19	0.73	-0.08
Nasal	-0.09	0.79	0.11	0.76	-0.05	0.87	1.17	0.54	-0.12
Inferior	0.78	0.12	0.52	0.34	-0.23	0.64	-2.42	0.57	0.01

CIP: cold-induced pain, DD: duration of diabetes, DR: diabetic retinopathy, Adj R^2: adjusted R^2, B: regression coefficient, indicates significance at 0.05 level

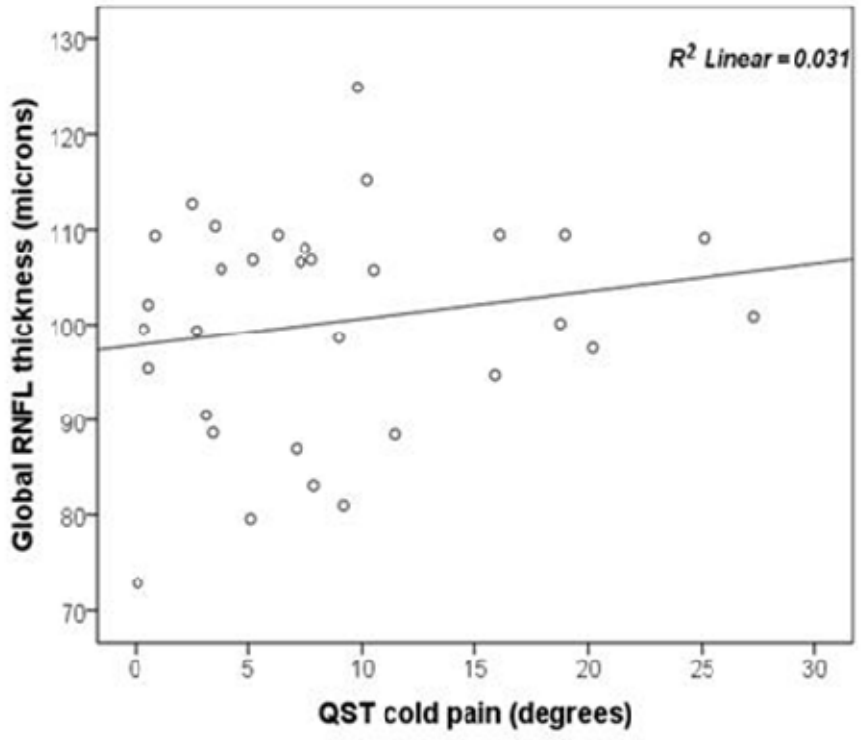


Figure 34.Association between global RNFL measurement and cold-induced pain sensation after elimination of the floor values. Sensitivity decreases to the left on the x-axis for each panel.

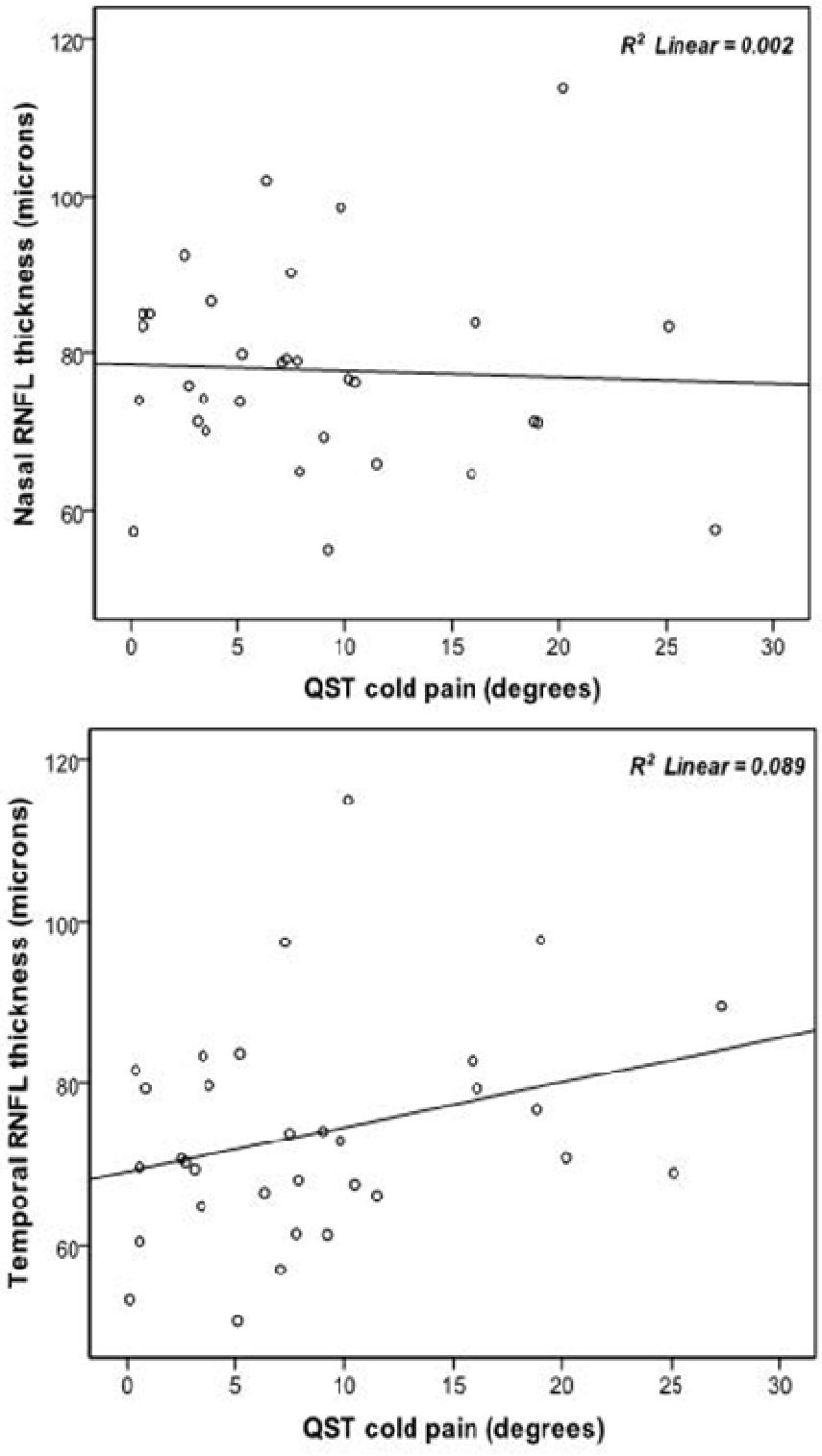


Figure35.Association between nasal and temporal RNFL measurements and cold-induced pain sensation after exclusion of the floor values. Sensitivity decreases to the left on the x-axis for each panel. Scale for y-axis is different for each graph.

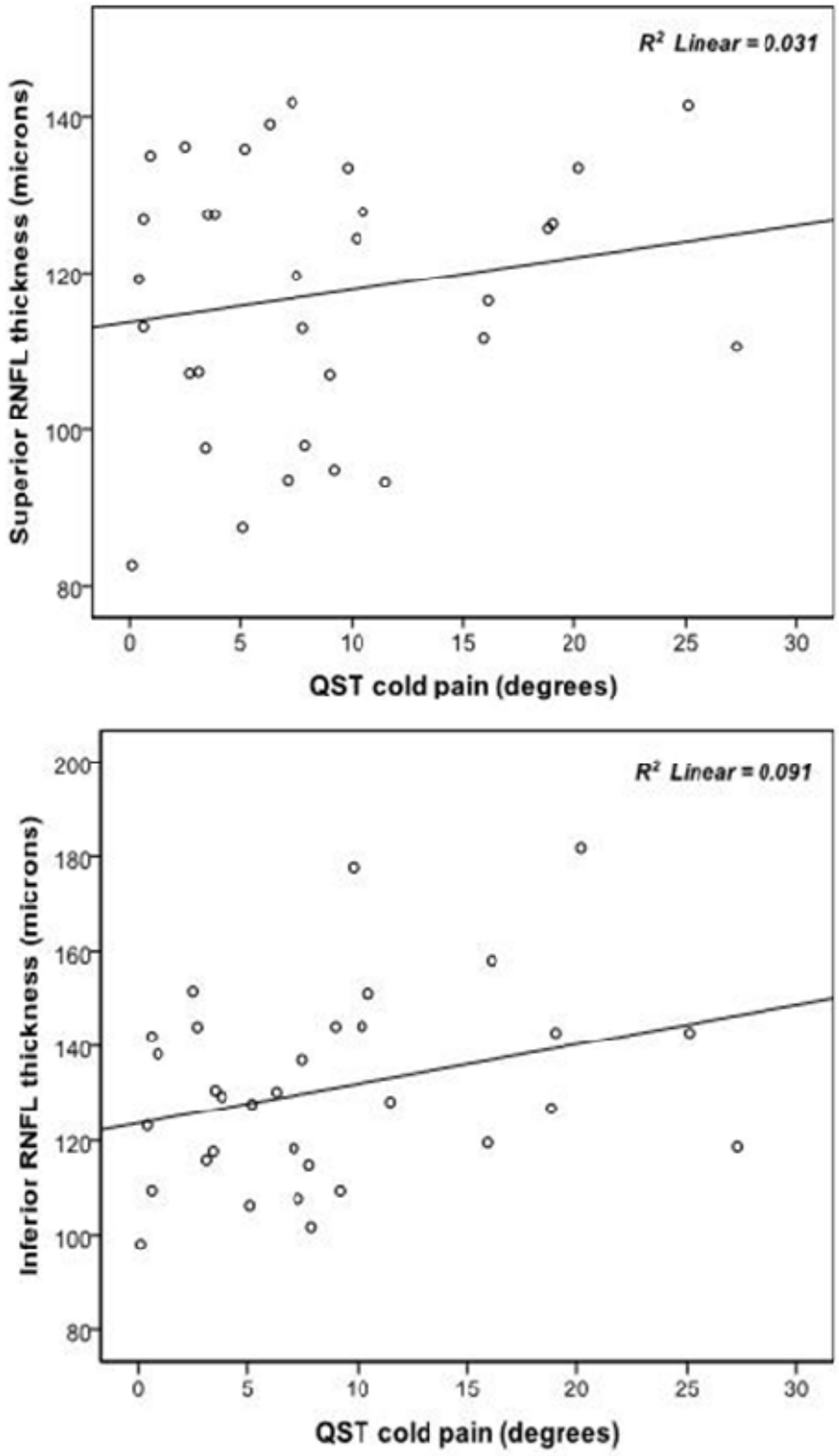


Figure36.Association between superior and inferior RNFL measurements and cold-induced pain sensation after elimination of the floor values. Sensitivity decreases to the left on the x-axis for each panel. Scale for y-axis is different for each graph.

6.5.2 Heat-induced pain

When including the ceiling values, it was observed that more than half of the participants (54% of total cohort) achieved a value of 50°C as their warm-induced pain threshold. Medianwarm induced pain for participants was 50 degrees ranging from 42.6-50 degrees. As outlined in Table 23, warm pain threshold was neither significantly associated with global nor with quadrant RNFL thicknesses. Table 23 summarizes the statistical results for all regression models. A scatter plot of global RNFL thickness and warm pain threshold is shown in Figure 37 to Figure39.

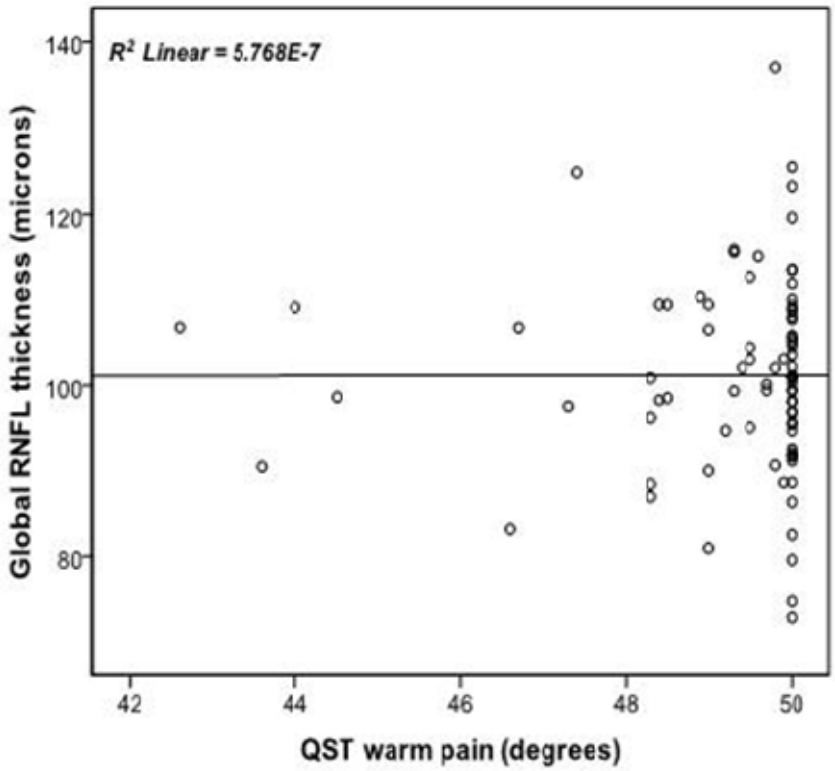


Figure 37.Association between global RNFL thickness and heat-induced pain sensation.Note the y-axis scale differs for each plot. Decrease in sensitivity is to the right on the x-axis for this panel.

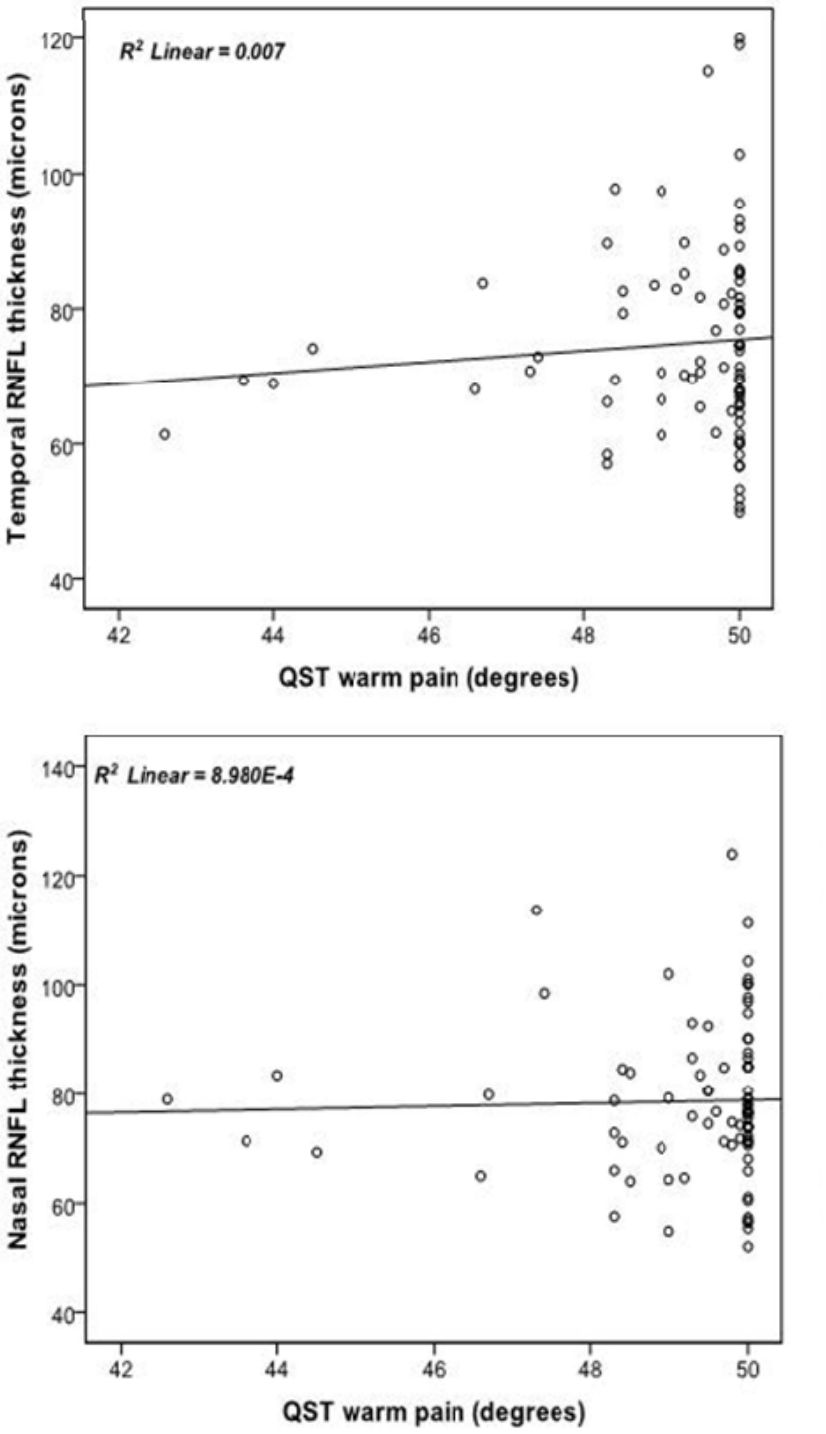


Figure38.Association between temporal and nasal RNFL measurements and heat-induced pain sensation.Note the y-axis scale differs for each plot. Decrease in sensitivity is to the right on the x-axis for each panel.

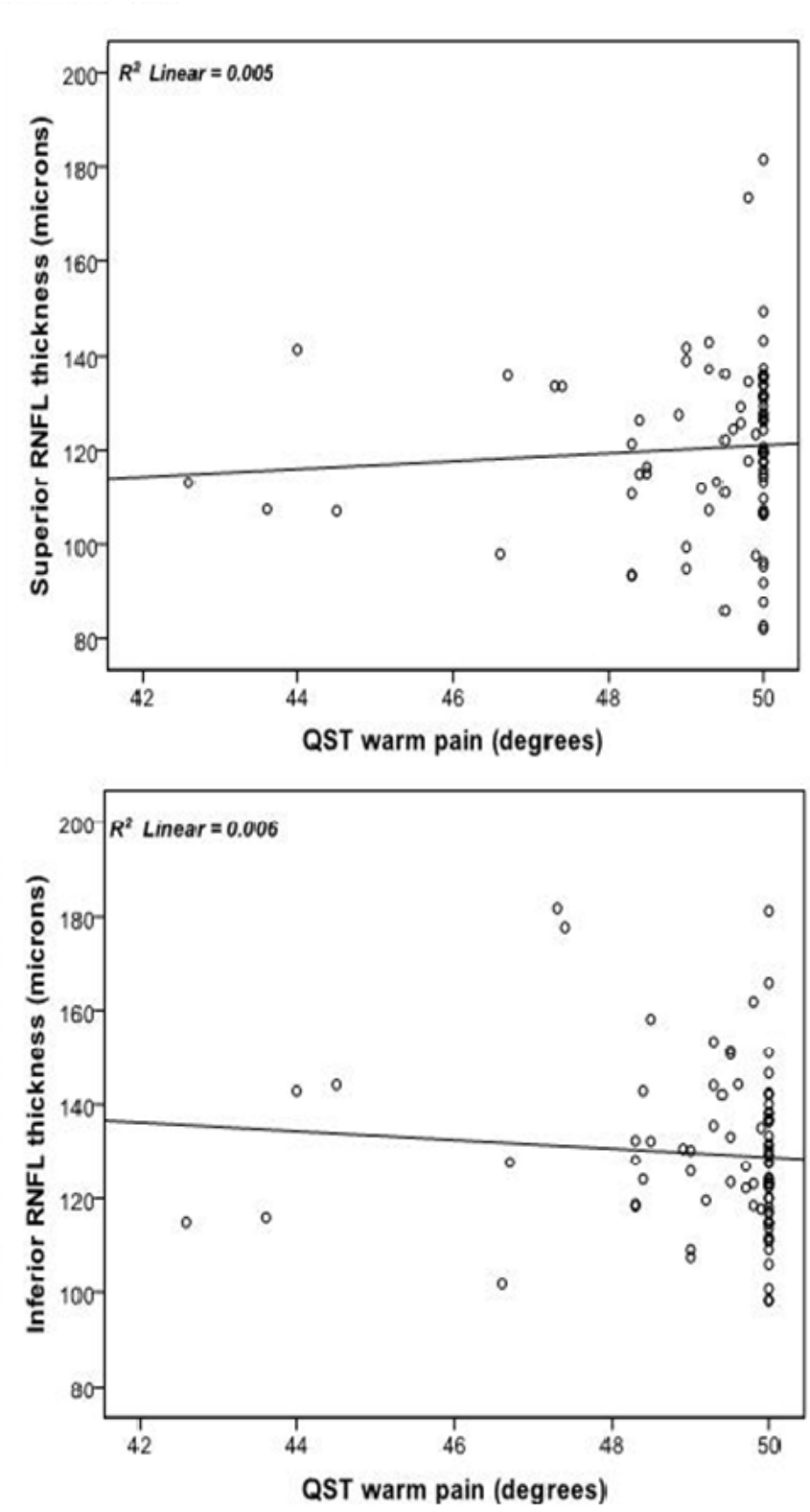


Figure39.Association between superior and inferior RNFL measurements and heat-induced pain sensation. Note the y-axis scale differs for each plot. Decrease in sensitivity is to the right on the x-axis for each panel.

The second approach involved elimination of the ceiling values. Thirty eight participants (45.6%; 21 males) remained after ceiling values of fifty degrees were excluded. Median age for these participants was 61.5 years ranging from 45 to 77 years. According to the NDS grouping reported in the previous chapter (*section 5.3.1*), of 44 eliminated participants, 24 were classified as having moderate or severe neuropathy. The final number of participants per assigned groups was as follows: no neuropathy (n=15), mild neuropathy (n = 20), moderate neuropathy (n=2), severe neuropathy (n=1). Please note that the explanatory variable (i.e. QST heat-induced pain) is a continuous variable and the group numbers demonstrated above are for understanding the severity of DPN based on NDS groups only.

Median warm pain threshold was 49 degrees and the range was between 42.6 - 49.9 degrees. Using a regression models, no significant relationship between global or quadrant RNFL thicknesses with warm-induced pain thresholds was observed (statistical reports are outlined in Table 23. Temporal RNFL quadrant showed the closest linear association with heat-induced pain ($p = 0.05$). Regression scatter plots in Figure 40-Figure42 revealed a mild tendency towards an increase in RNFL thickness in temporal and superior quadrants as heat-induced pain threshold increased (i.e. loss of sensitivity). However the associations are not statistically significant (Table 23).

Table 23. Regression coefficients for RNFL measurements and warm pain when including ceiling values (50°C)

	HIP		Age (yrs)		DD (yrs)		DR		
	B =	p =	B =	p =	B =	B =	B =	p =	Adj R^2
Global	1.10	0.29	-0.48	0.14	-0.21	0.24	-0.32	0.92	-0.03
Temporal	2.14	0.05	-0.36	0.20	-0.20	0.53	-3.54	0.17	0.12
Superior	1.40	0.41	-0.54	0.24	-0.06	0.90	-2.62	0.52	-0.05
Nasal	1.12	0.39	-0.45	0.20	-0.49	0.29	-3.30	0.30	<-.001
Inferior	1.36	0.41	-0.05	0.90	-1.11	0.03*	-4.40	0.27	0.03

*HIP: heat-induced pain, DD: duration of diabetes, DR: diabetic retinopathy, Adj R^2: adjusted R^2, b: regression coefficient, * indicates significance at 0.05 level*

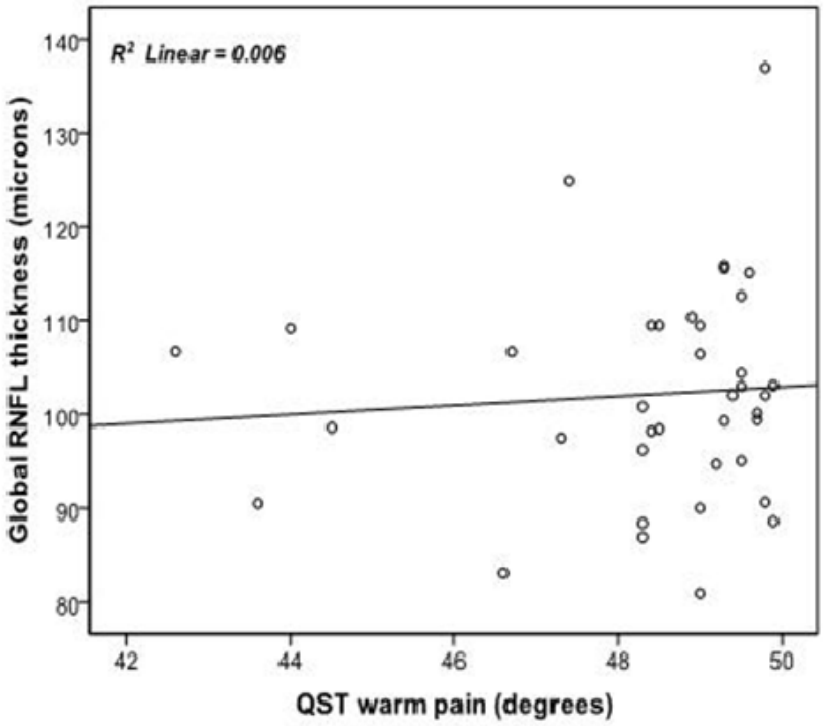


Figure 40.Association between global RNFL thickness and heat-induced pain sensation after exclusion of ceiling values. Decrease in sensitivity is to the right on the x-axis for each panel.

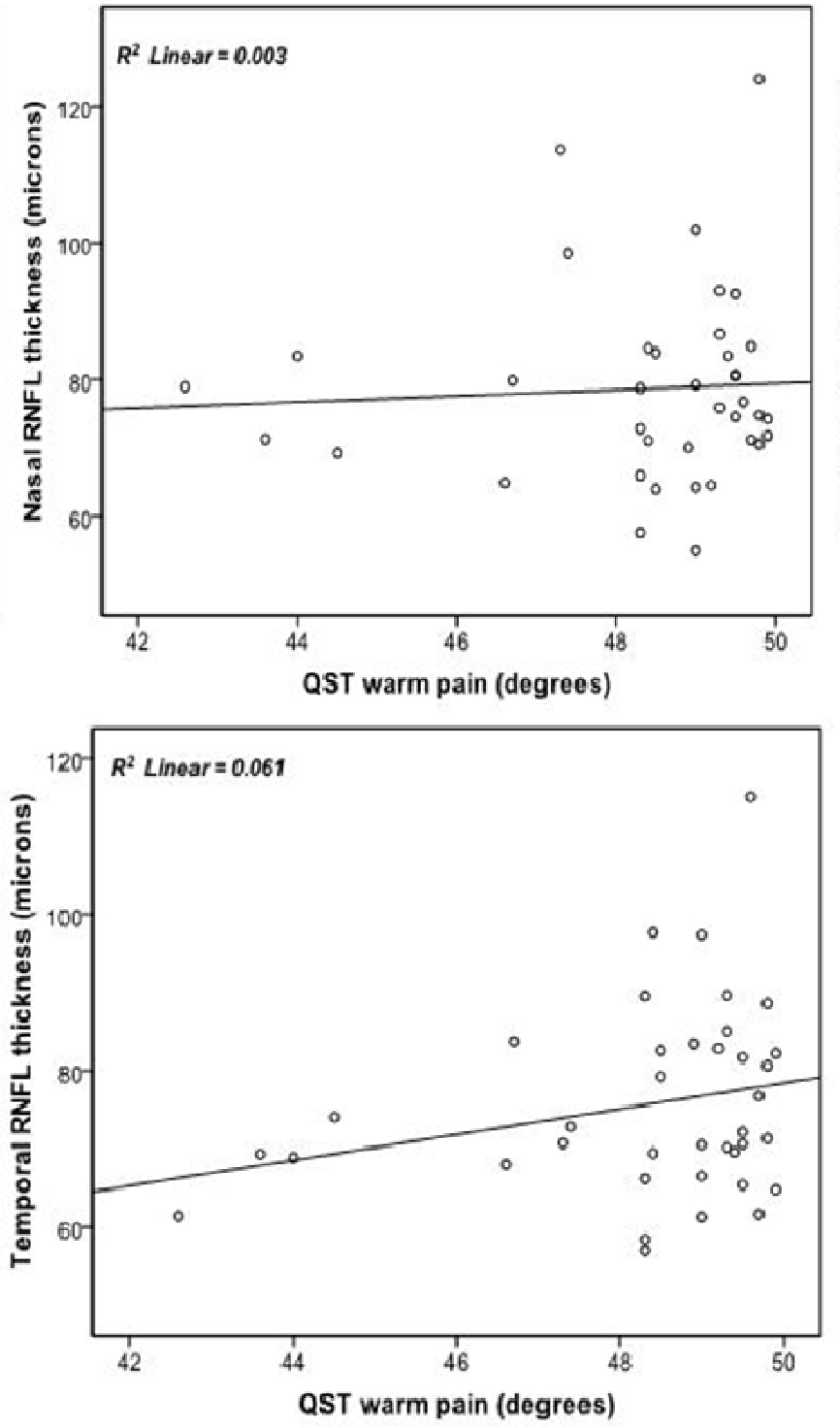


Figure41.Association between nasal and temporal RNFL measurements and heat-induced pain sensation after elimination of ceiling values. Decrease in sensitivity is to the right on the x-axis for each panel.

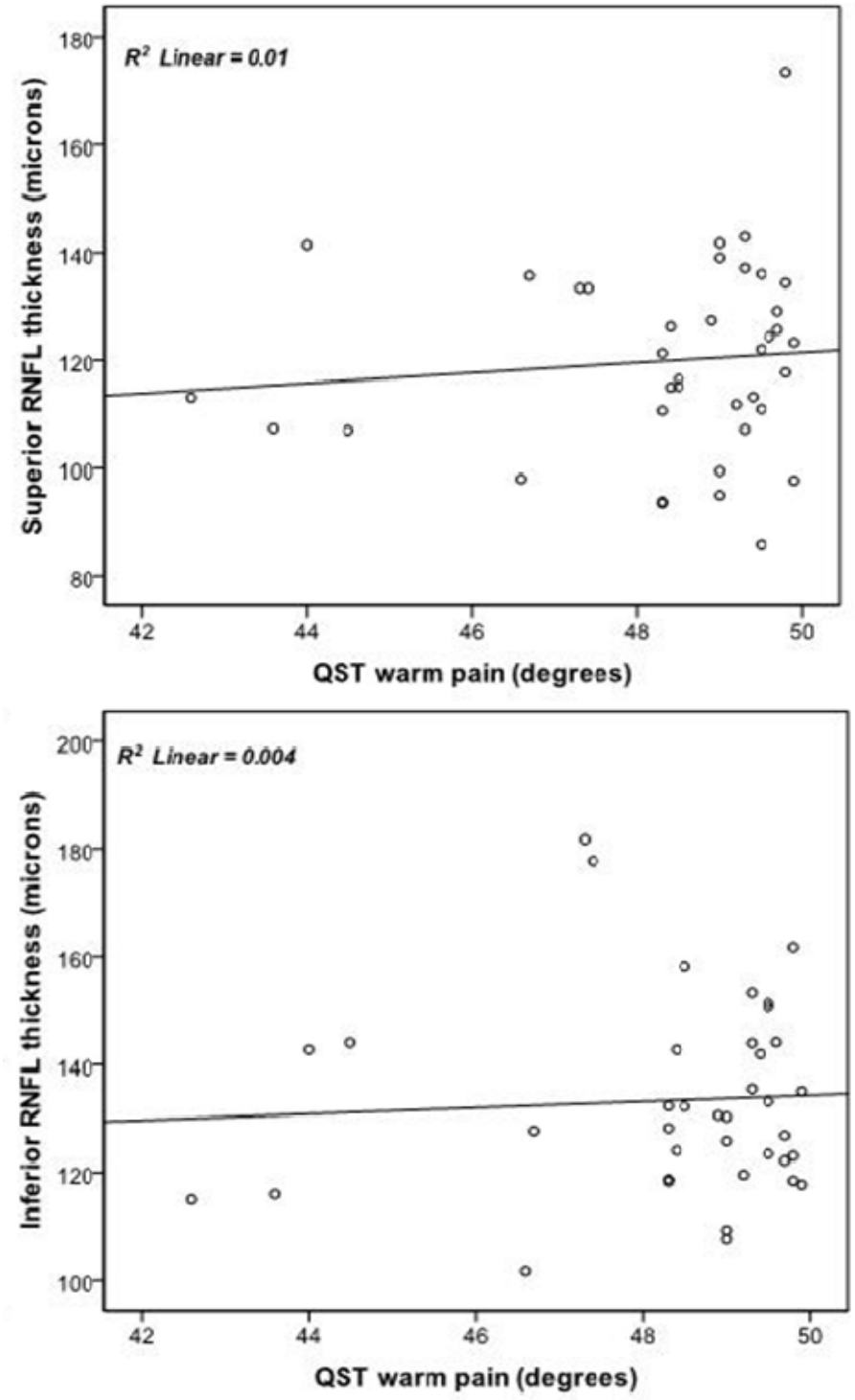


Figure42.Association between superior and inferior RNFL measurements and heat-induced pain sensation after exclusion of ceiling values.Decrease in sensitivity is to the right on the x-axis for each panel.

6.6 Discussion

The current study aimed to investigate retinal nerve fibre layer thickness in people with type 2 diabetes in association with severity of peripheral neuropathy as measured by QST. The results did not show any meaningful association between RNFL thickness and QST parameters, which indicate that RNFL thickness for this cohort is not as predictive of neuropathy status as NDS (see *Chapter 5*).

There was substantial variance in the distributions for each of the QST modalities. This could possibly be explained by the psychophysical nature of QST where cooperation of the participants plays an important role in the outcome findings. Instructions given to participants regarding test requirements can greatly influence the outcomes; in this study care was taken in consistency of instruction to eliminate variance attributable to this factor. It is noteworthy to mention that response time in a psychophysical test is greatly dependant on cognitive style and age of individuals [262].

The specific hypothesis of the study was that retinal nerve fibre layer is thinner with increased degree of neuropathy measured by QST. Retinal nerve fibre layer is a part of the CNS while QST parameters investigate the function of fibres in the PNS. Previous studies have shown that neuropathy in diabetes is not only a disease affecting the PNS, but also it can affect the CNS; one example is a condition referred to as diabetic encephalopathy. Functional impairment of cognition, cerebral signal conduction and underlying structural pathology associated with diabetes are known to be caused by diabetic encephalopathy [263].For instance, studies on evoked potential (electrical field potentials that are generated by particular brain structures in response to specific stimuli) in diabetic rats have shown increased visual and auditory latencies [255, 264].

Sima*etal.*[264] have shown that diabetic rats develop diabetic sensory neuropathy characterised by functional impairment of evoked potentials and dystrophy of retinal nerve fibre axons. These studies suggest that a CNS-based function, although unexpected, canpotentiallypredict anatomical changes in the PNS. As such, investigation of a PNS-based function (QST) in association with possible CNS anatomical changes (RNFL thickness) was of interest in the current experiment.

A second approach to rationalise the hypothesis of this study would be investigation of similarities between the fibre types in CNS and PNS. Müller cells are the major glial cells of the retina, located in the gaps between the neuron cell bodies, including ganglion cells, and these have a diverse function in maintaining sufficient glycogen in the retina. The small unmyelinated C-fibres in the PNS are responsible for slow pain and warm sensation perception. The spaces between these fibres are filled by Schwann cells. These are the major glial cells in the PNS and they have a key role in regeneration of fibres in this system. If there is an association between RNFL and warm sensation or heat-pain thresholds, thinning of RNFL may be expected as warm sensitivity decreased. However, only a minor non-significant tendency towards thinning of RNFL was found. This was similar for all other findings involving A-delta fibres (cold sensation and fast pain perception) and A-beta fibres (vibration perception) in this experiment, meaning that these findings are not strong enough to accept the hypothesis of a significant association.

Quantitative Sensory Testing vibration and thermal perception measurements are effective ways of documenting abnormal sensation in patients with diabetic neuropathy [235]; however it is as yet unclear whether the method is capable of detecting pre-clinical neuropathy in people with diabetes. Although the stimulus in QST is automatically controlled, the technique still relies on the concentration of the person

being tested. Additionally, it has been recommended that diagnosis of peripheral neuropathies should not be made on QST results only[24]. Furthermore, factors like room and foot temperature can influence the accuracy of the result. However these factors were controlled for in this experiment.

The association between Neuropathy Disability Score and RNFL, as described in *Chapter 5*, was found to be significant in at least one quadrant particularly at late stages of neuropathy. However in this experiment RNFL thickness showed no meaningful association with QST, indicating that RNFL is not a good predictor of neuropathy when the condition is assessed by QST.

7 Association between Standard Automated Perimetry and Diabetic Peripheral Neuropathy as measured by Neuropathy Disability Score

7.1 Introduction

Early diagnosis of diabetic peripheral neuropathy (DPN) can prevent endpoint complications including lower limb amputation[265]. This is currently possible using invasive procedures such as skin biopsy; however the nature of this technique makes it difficult to be used in routine clinical examinations. Non-invasive assessment of corneal nerves by means of corneal confocal microscopy has been shown to be a relatively sensitive method of diagnosing early neuropathy [50]. However, one limitation of the technique is direct contact with the cornea under local anesthesia. In this chapter, standard automated perimetry (SAP) or green-on-white visual function has been investigated as another potential non-invasive ophthalmic method of diagnosing DPN.

Impaired visual function has been shown in eyes with normal visual acuity and minimal evidence of diabetic retinopathy[50]. Contrast sensitivity and colour vision have been shown to be reduced at early stages of diabetic retinopathy[202, 266]. Other studies have used more sophisticated techniques such as multifocal electroretinogram to assess visual function in diabetes and have found reduced wave-latency [162]. Additionally, short-wavelength-sensitivecones have been shown to be damaged in diabetic patients

without retinopathy as determined by blue-on-yellow perimetry[181]. Very few studies have investigated the ability of SAP in detecting contrast sensitivity changes in diabetic individuals[186]. Additionally, to-date, no other groups have examined the association between diabetic peripheral neuropathy and visual function, which is the fundamental focus of this experiment.

7.2 Aims and hypotheses

The aims of this experiment were:

1. To investigate standard automated perimetry in people with type 2 diabetes with and without peripheral neuropathy and healthy controls.

2. To assess the relationship between standard automated perimetry and severity of peripheral neuropathy as measured by neuropathy disability score (NDS).

This experiment was specifically testing the following hypotheses:

1. Visual field parameters (Overall Defect, Pattern Defect and contrast sensitivity) as determined by standard automated perimetry are significantly different between :

    a. Healthy control individuals and those with type 2 diabetes without DPN.

    b. Four groups with varying levels of neuropathy (none-mild-moderate and severe).

c. Individuals at higher risk of foot ulceration (NDS ≥ 6) and those who are not.

2. The magnitude of the reduction in sensitivity of standard automated perimetryis related to the severity of diabetic peripheral neuropathy.

7.3 Methods

7.3.1 Participants

Inclusion and exclusion criteria for the participants have been described in *Chapter 3*. Specific criteria for this experiment included a visual acuity of 6/9 or better, a negative history of glaucoma or high intra-ocular pressure, no history of retinal laser photocoagulation in the study eye, no significant chronic diseases other than diabetes and no mental health problems (*see Chapter 3, inclusion/exclusion criteria).*

Twenty-four healthy control participants and 105 people with type 2 diabetes were consented to the study; however results for 73 individuals with diabetes were included in the analyses for this chapter. The reliability criteria for SAP measurements have been explained previously (*Chapter 3*, *3.7.3.6.2*). Thirty-two participants had unreliable SAP measurements (false positive error, false negative error, fixation loss error > 33%), thereby were excluded from the analysis.

Mean age of the control individuals was not significantly different from type 2 participants (Student t = 1.03, p = 0.30). There was no significant difference in age of participants between neuropathy groups - none, mild, moderate and severe neuropathy (ANOVA F = 0.73, p = 0.53). Duration of diabetes did not differ significantly between

the NDS groups either (ANOVA F = 1.54, p = 0.21). Characteristics of participants are outlined inTable 24. In the diabetes cohort, visual field overall defect was significantly different between males and females (t = 3.76, p = 0.001) but not pattern defect (t = -1.59, p = 1.21). Global and superior hemi-filed contrast sensitivities were significantly higher for male participants (t = 2.2, p = 0.03 and t = 2.5, p = 0.01; respectively) but not inferior hemi-field (t = 1.6, p = 0.11)

To address the hypothesis regarding association between contrast sensitivity (CS) and severity of neuropathy a sample of 48 will provide 90% power (α=0.05) that an association between CS and NDS where one exists. A sample of 6 per group is necessary to reveal a clinically meaningful difference of 2 dB between diabetes groups and controls for CS when standard deviation of 1.5 dB is applied (values from unpublished pilot data in our lab).

7.3.2 Assessment of neuropathy

Peripheral neuropathy was assessed using a modified neuropathy disability score (NDS) test [45]. This technique has been described comprehensively in *Chapter 3*. Participants were grouped based on their NDS scores (0-10): none, mild, moderate and severe neuropathy. NDS cut-off point of six was used to determine individual at higher risk of foot ulceration (NDS $\geq$ 6) [45].

7.3.3 Assessment of visual contrast sensitivity

Visual fields (VF) were evaluated using an automated visual field analyser (Medmont M700, Medmont International Ltd, Melbourne, Australia). Standard automated perimetry (green-on-white) was performed within the central 30 degrees eccentricity on

the eye with better visual acuity. A brief demonstration was given prior to testing to familiarize the participants with the test procedure.Central fixation was controlled using visual monitoring by the operator as well as software scheduled blind-spot presentations.The refractive errors of the participants were corrected if required. Participants were given breaks between and within tests if necessary. Visual field results with reliability indices (fixation loss, false positive error and false negative error) greater than 33% were eliminated from the analysis. Overall Defect (comparison with expected contrast sensitivity values in a normative database, dB) and Pattern Defect (deviation from a normal hill-of-vision, dB)were recorded for each participant. For more detail regarding the instrument and reliability indices refer to *Chapter 3, section3.7.3.6.3*.

Global visual field contrast sensitivity average threshold in decibels (dB) was calculated by taking the average of all test point thresholds (106 points). As stated in the chapter 5, the retinal nerve fibre layer thickness was measured for the inferior and superior quadrant around the optic nerve head. The contrast sensitivity in the superior hemi-field is known to be related to the inferior retinal nerve fibres and vice versa[267]; therefore in order to follow a similar approach, average contrast sensitivity threshold(dB) in the superior and inferior hemi-fields were calculated separately. Each hemi-field contained 53 points within the central 30° of eccentricity. The two test points above and below the blind spot (perceptual region related to ONH) were not included in the analysis to eliminate the effect of naturally larger or anatomically variant optic nerve heads.

7.3.4 Assessment of diabetic retinopathy

It has been demonstrated that diabetic retinopathy may influence visual field measurements and this has been shown to be more evident at later stages of the disease

[268, 269]. Therefore assessment of retinopathy is critical for a reliable interpretation of visual field outcomes. In the current study, fundus photographs of 45° field were captured using a non-mydriatic camera (Visucam Pro, Carl ZeissMeditec, USA). Diabetic retinopathy was graded according to the Australian National Health and Medical Research Centre (NHMRC) guidelines and was classified as either none, minimal, mild, moderate or severe. No one had moderate or severe non-proliferative or any level of proliferative retinopathy; all participants were classified as having none (n = 33), minimal (n = 17) or mild (n = 23). Overall Defect, Pattern Defect, average global contrast sensitivity and average hemi-field sensitivity were not significantly different between the three levels of retinopathy (

Table 25).

Table 24.Demographics for the study cohort.Participants are grouped according to Neuropathy Disability Score

Parameter	Control	No neuropathy	Mild neuropathy	Moderate neuropathy	Severe neuropathy
N	24	20	30	14	9
Age (yrs)	59.8 ± 7.2	58.9 ± 6.1	61.5 ± 5.6	61.3 ± 8.1	61.2 ± 5.6
Gender (M/F)	13/11	10/11	21/8	8/6	6/3
Duration of diabetes (yrs)	n/a	12.4 ± 10.5	11.7 ± 6.8	14.5 ± 9.9	18.0 ± 10.0

Table 25.Standard automated perimetry outcomes for each group of diabetic retinopathy. The data shown are median and range (min-max).Note that the numbers are derived dB values.

	Diabetic Retinopathy				
Parameter	**None**	**Minimal**	**Mild**	**Kruskal-Wallis statistics**	**P =**
Overall Defect	2.47 (-1.75 – 5.41)	1.98 (-0.22 – 5.28)	2.37 (0.22 - 4.34)	1.27	0.53
Pattern Defect	2.53 (1.62 – 5.69)	2.80 (1.82 – 10.71)	1.81 (1.47 – 5.40)	2.54	0.28
Globally	21.99 (15.95 – 24.25)	21.34 (11.57 – 24.25)	24.99 (15.48 – 23.94)	1.00	0.60
Superior hemi-field	21.84 (15.95 – 24.96)	21.16 (9.45 – 24.35)	21.55(17.07 – 23.71)	1.83	0.40
Inferior hemi-field	22.35 (15.94 – 24.71)	21.15(13.68 – 24.14)	22.53 (13.88 – 24.16)	0.99	0.61

7.3.5 Statistical analysis

The distribution of the average global contrast sensitivity for the entire study cohort was found not to be normal (Kolmogorov-Smirnov statistics = 0.15, $p < 0.001$) and the histogram is shown in Figure 43. Descriptive statistics are demonstrated as median and range (maximum-minimum). To address the first hypothesis (comparison of control participants with type 2 diabetics without DPN), Mann-Whitney U test was applied. Kruskal-Wallis test was used for comparisons between NDS group. To address the second hypothesis of the study, univariate regression analysis was used to assess the association between NDS and SAP thresholds. Each regression model comprised main effect of NDS, age, duration of diabetes and level of diabetic retinopathy. A p value less than 0.05 was considered to be statistically significant. Statistic package for social sciences (SPSS) version 18 was used to analyse the data.

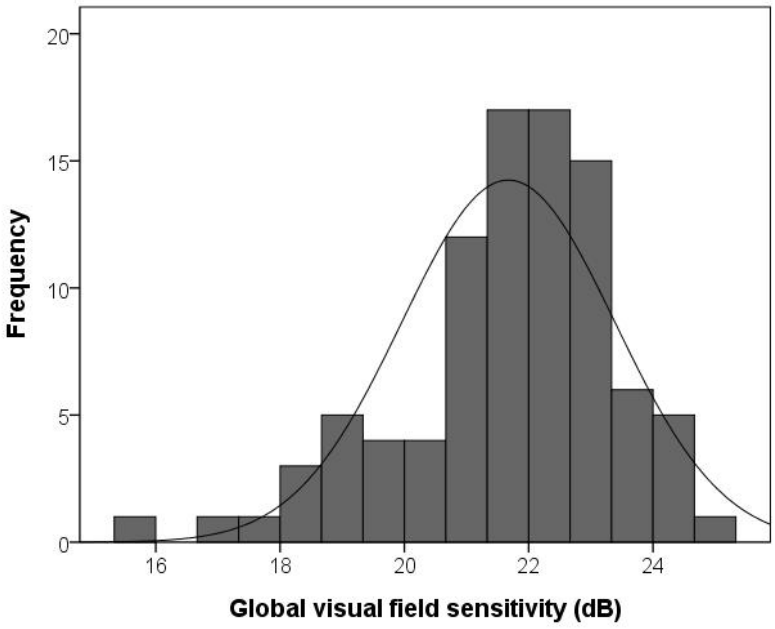


Figure 43.Histogram showing non-normal distribution of sensitivity in the global visual field.

7.4 Results

7.4.1 Comparison between control participants and individuals with type 2 diabetes without neuropathy

Overall Defect and Pattern Defect indices were found to be significantly different between controls and individuals with type 2 diabetes without DPN (Table 26). The remaining comparisons for contrast sensitivity globally and in each hemi-field were found not to be statistically different between the two groups (Table 26).

Table 26.Mann-Whittney U test comparisons of standard automated perimetry parameters between control participants and type 2 diabetes cohort.

Parameter		Median (Range Min - Max)	U =	p =
Overall Defect(dB)	**No DPN**	2.58 (1.74 – 6.15)	122	<0.01*
	Control	2.04 (1.36 – 3.60)		
Pattern Defect(dB)	**No DPN**	2.59 (-1.75 – 5.41)	151	0.03*
	Control	1.81 (0.22 – 3.93)		
Globally(dB)	**No DPN**	22.32 (19.73 – 24.37)	236	0.93
	Control	22.31 (15.95 – 24.13)		
Inferior hemi-field(dB)	**No DPN**	22.66 (15.94 – 24.71)	184	0.19
	Control	22.24 (19.58 – 24.68)		
Superior hemi-field(dB)	**No DPN**	21.99 (15.95 – 24.96)	199	0.33
	Control	22.39 (19.88 – 24.85)		

** indicates statistical significance, DPN: diabetic peripheral neuropathy*

7.4.2 Visual field analyses for type 2 cohort

7.4.2.1 Overall and Pattern Defect

The NDS groups had neither statistically significantly different OD values nor PD outcomes. Results for both variables are shown inTable 27.

Overall Defect was significantly associated with NDS score, showing a reduction of 0.13 units with each increase in unit of NDS. The regression model was not a strong fit (R^2_{adj} = 0.7%), however the outcome was statistically significant ($p < 0.05$). Association between age and OD also reached statistical significance ($p = 0.04$). Duration of diabetes and level of retinopathy had no significant effect on OD outcomes ($p = 0.35$ and $p = 0.48$; respectively). Table 28 outlines the regression model outcomes. Pattern Defect was not associated with NDS score, age, duration of diabetes and level of retinopathy ($p > 0.45$ for all variables, Table 28). Figure 44represents the relationship between overall and PD with NDS score.

Mean age ± standard deviation and gender distribution of participants at lower risk of foot ulceration (n = 50) and higher risk of foot ulceration (n = 23) were as follows respectively: 60 ± 6 years (32 males) and 61 ± 7 (14 males).There was no significant differences between the age of these two groups ($t = -0.5$, $p = 0.62$). Comparison of OD between individuals at risk of foot ulceration (NDS $\geq$ 6) and those who were not, marginally failed to reach statistical significance ($U = 410$, $p = 0.05$).Pattern Defect also did not differentiate those who were at higher risk of foot ulceration from the remaining type 2 participants ($U = 461$, $p = 0.17$). These findings are summarized in Table 29.

Table 27. Comparisons of standard automated perimetry parameters between NDS groups.

Parameter	NDS Groups	Median (Range Min - Max)	Kruskal-Wallis statistics	p =
Overall Defect(dB)	**None**	2.58 (1.74 – 6.15)	2.48	0.47
	Mild	2.53 (1.47 – 4.71)		
	Moderate	2.69 (1.69 – 10.71)		
	Severe	2.78 (1.70 – 7.40)		
Pattern Defect(dB)	**None**	2.59 (-1.75 – 5.41)	5.47	0.14
	Mild	2.48 (0.01 – 4.52)		
	Moderate	2.37 (- 0.22 – 5.28)		
	Severe	0.77 (0.28 – 2.87)		
Globally(dB)	**None**	22.32 (19.73 – 24.37)	6.43	0.09
	Mild	21.88 (18.35 – 24.57)		
	Moderate	21.61 (11.57 – 24.25)		
	Severe	21.33 (15.48 – 22.09)		
Inferior hemi-field(dB)	**None**	22.71 (15.94 – 24.71)	6.97	0.07
	Mild	22.41 (18.30 – 24.67)		
	Moderate	22.07 (13.68 – 24.14)		
	Severe	21.54 (13.88 – 22.82)		
Superior hemi-field(dB)	**None**	22.00 (15.95 – 24.96)	5.41	0.14
	Mild	221.56 (17.81 – 24.46)		
	Moderate	20.90 (9.45 – 24.35)		
	Severe	20.69 (17.07 – 21.69)		

NDS: neuropathy disability score test

Table 28. Regression models showing associations between standard automated perimetry parameters (dB) and predicting variables.

Parameter	NDS		Age (yrs)		DD (yrs)		DR		Adj R^2
	B =	p =	B =	p =	B =	p =	B =	p =	
Overall Defect	-0.13	0.02*	-0.03	0.04*	-0.01	0.35	-0.14	0.92	-0.48
Pattern Defect	0.04	0.45	<-0.01	0.90	<-0.01	0.95	0.13	0.58	-0.04
Globally	-0.18	0.04*	-0.02	0.53	-0.01	0.72	0.08	0.81	0.03
Inferior hemi-field	-0.19	0.02*	-0.01	0.50	-0.02	0.74	0.16	0.62	0.04
Superior hemi-field	-0.17	0.07	-0.02	0.75	-0.01	0.52	<-0.01	1.00	0.01

NDS: neuropathy disability score, DD: duration of diabetes, DR: diabetic retinopathy, Adj R^2: adjusted R^2, B: regression coefficient

Table 29. Comparisons of standard automated perimetry parameter between participants at higher risk of foot ulceration (NDS≥6) and those at lower risk (NDS< 6). Group statics have been described in section 7.4.2.1.

Parameter		Median (Range Min - Max)	U =	p =
Overall Defect (dB)	**NDS < 6**	2.48 (-1.54–5.71)	410	0.05
	NDS ≥ 6	1.54 (-0.22–5.28)		
Pattern Defect (dB)	**NDS < 6**	2.55 (1.47–6.15)	461	0.18
	NDS ≥ 6	2.72 (1.69–10.71)		
Globally (dB)	**NDS < 6**	22.04 (15.95–24.57)	401	0.04*
	NDS ≥ 6	21.33 (11.57 – 24.25)		
Inferior hemi-field(dB)	**NDS < 6**	22.51 (15.94–24.71)	395	0.03*
	NDS ≥ 6	21.88 (13.68–24.14)		
Superior hemi-field(dB)	**NDS < 6**	21.83 (15.95–24.96)	400	0.04*
	NDS ≥ 6	20.77 (9.45–24.35)		

*NDS: neuropathy disability score, U: Mann-Whitney U test, * indicates significance*

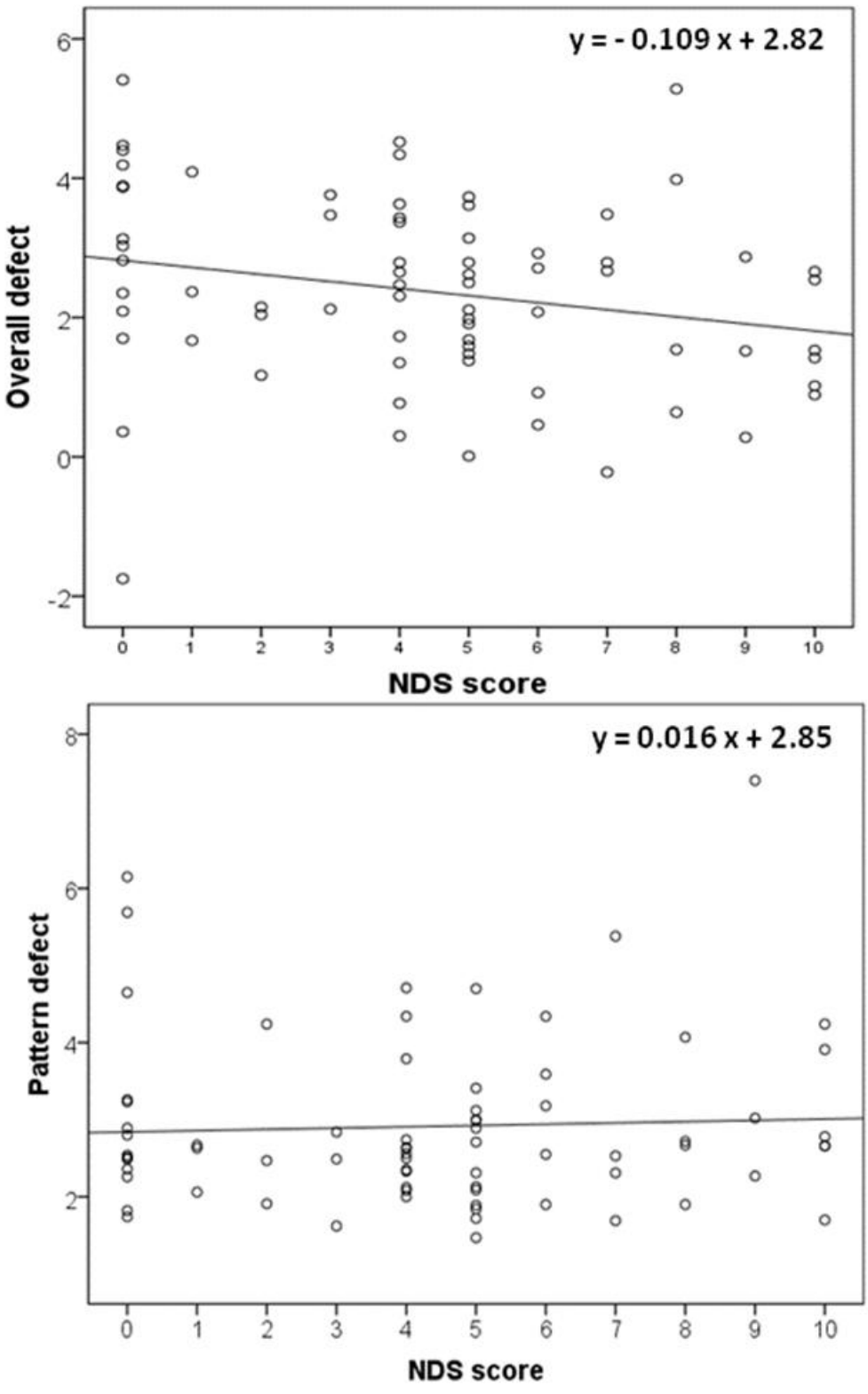


Figure 44.Scatter plot of Pattern Defect and Overall Defect against neuropathy disability score.

7.4.2.2 Contrast sensitivity threshold outcomes globally and in the superior and inferior hemi-fields

Superior hemi-field

Contrast sensitivity in the superior hemi-field was not different between NDS groups (p = 0.14, Table 27); however, a progressive reduction in sensitivity was observed along with increasing neuropathy (Figure 45). Association between NDS score and contrast sensitivity in the superior hemi-field did not quite reach statistical significance (b = -0.17, p = 0.07). Age, duration of diabetes and level of diabetic retinopathy did not have significant effects on contrast sensitivity in the superior hemi-field (Table 28).

Participants who were at lower risk of foot ulceration (NDS < 6, n = 23) had higher contrast sensitivity levels in this hemi-field than those at higher risk (n = 50) (p = 0.04, Table 29). Figure 45 a shows the comparison of contrast sensitivity in the superior hemi-field between NDS groups.

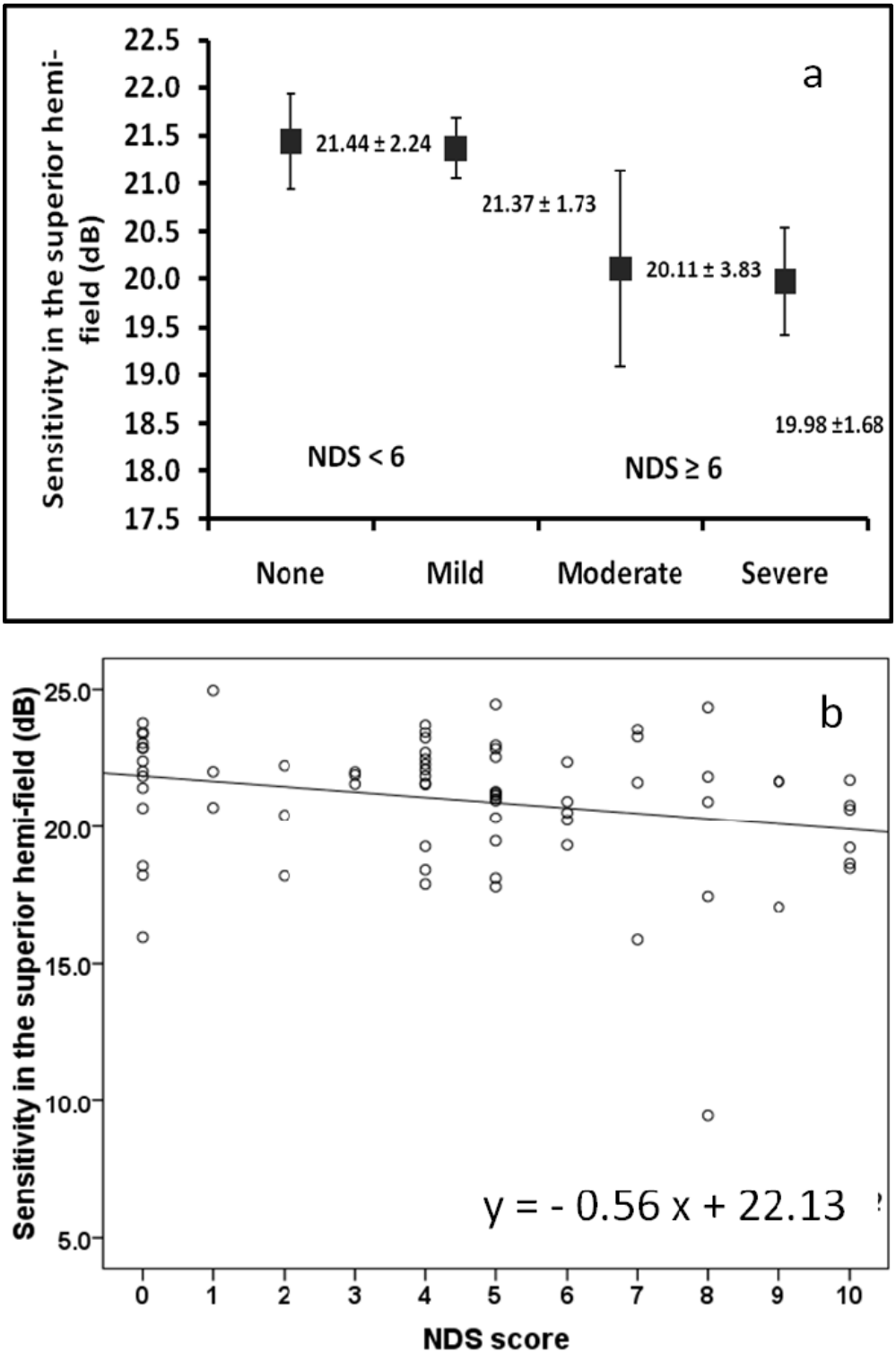


Figure 45. Mean ± standard error of the average contrast sensitivity in the superior hemi-field. Note that the data labels contain mean ± standard deviation (a), scatter plot for association between NDS score and contrast sensitivity in the superior hemi-field (b)

Inferior hemi-field

The differences for the mean contrast sensitivity levels in the inferior hemi-field between the NDS groups did not quite reach statistical significance ($p = 0.07$, Table 27, Figure 46 a). A significant association was found between NDS score and contrast sensitivity in the inferior hemi-field ($p = 0.02$). Contrast sensitivity in this hemi-field was reduced by 0.19 dB for each unit increase in NDS score (Table 28, Figure 46 b). Main effect of age, duration of diabetes and level of diabetic retinopathy were not associated with contrast sensitivity in the inferior hemi-field (Table 28).

Inferior hemi-field contrast sensitivity for individuals at higher risk of foot ulceration (NDS $\geq$ 6, n = 23) was significantly different from those who were not at risk of ulceration ($p = 0.03$, Table 29). Figure 46a demonstrates mean sensitivity in the inferior hemi-field for each NDS group.

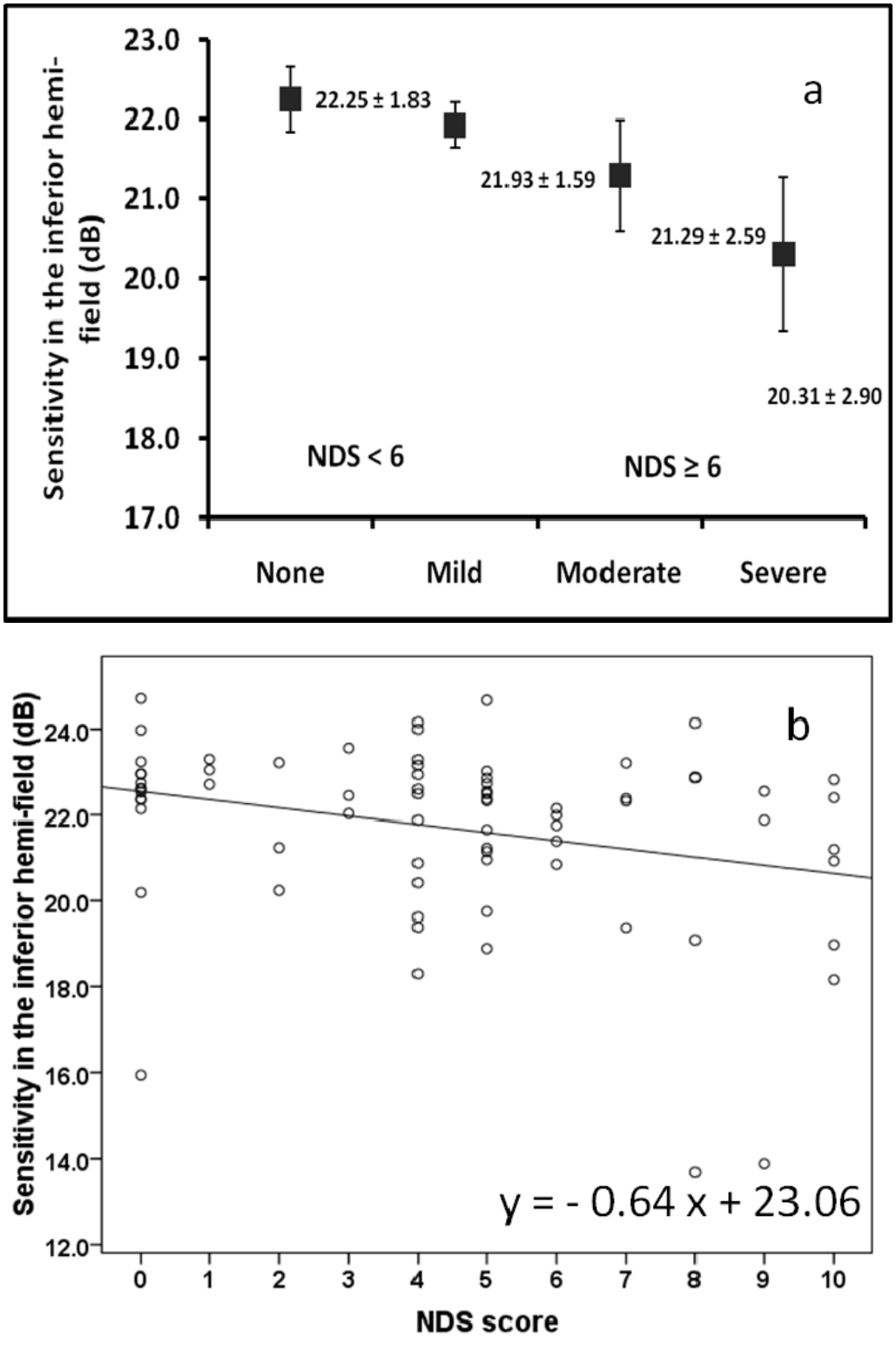


Figure 46.Mean ± standard error of the mean contrast sensitivity in the inferior hemi-field (dB). Note that the data labels show mean ± standard deviation (a), Scatter plot showing association between NDS scores and contrast sensitivity in the inferior hemi-field (b)

Global contrast sensitivity

Global average contrast sensitivity was not significantly different between the NDS groups ($p = 0.09$, Table 27, Figure47 a). Additionally, NDS score was significantly associated with global contrast sensitivity ($b = -0.14$, $p = 0.04$, Figure47 b). Age, duration of diabetes and level of diabetic retinopathy had no significant effect on global contrast sensitivity thresholds (Table 28).

Individuals at higher risk of foot ulceration (NDS ≥ 6) had a significantly different global contrast sensitivity compared to those at lower risk ($p = 0.03$, Table 29, Figure47 a).

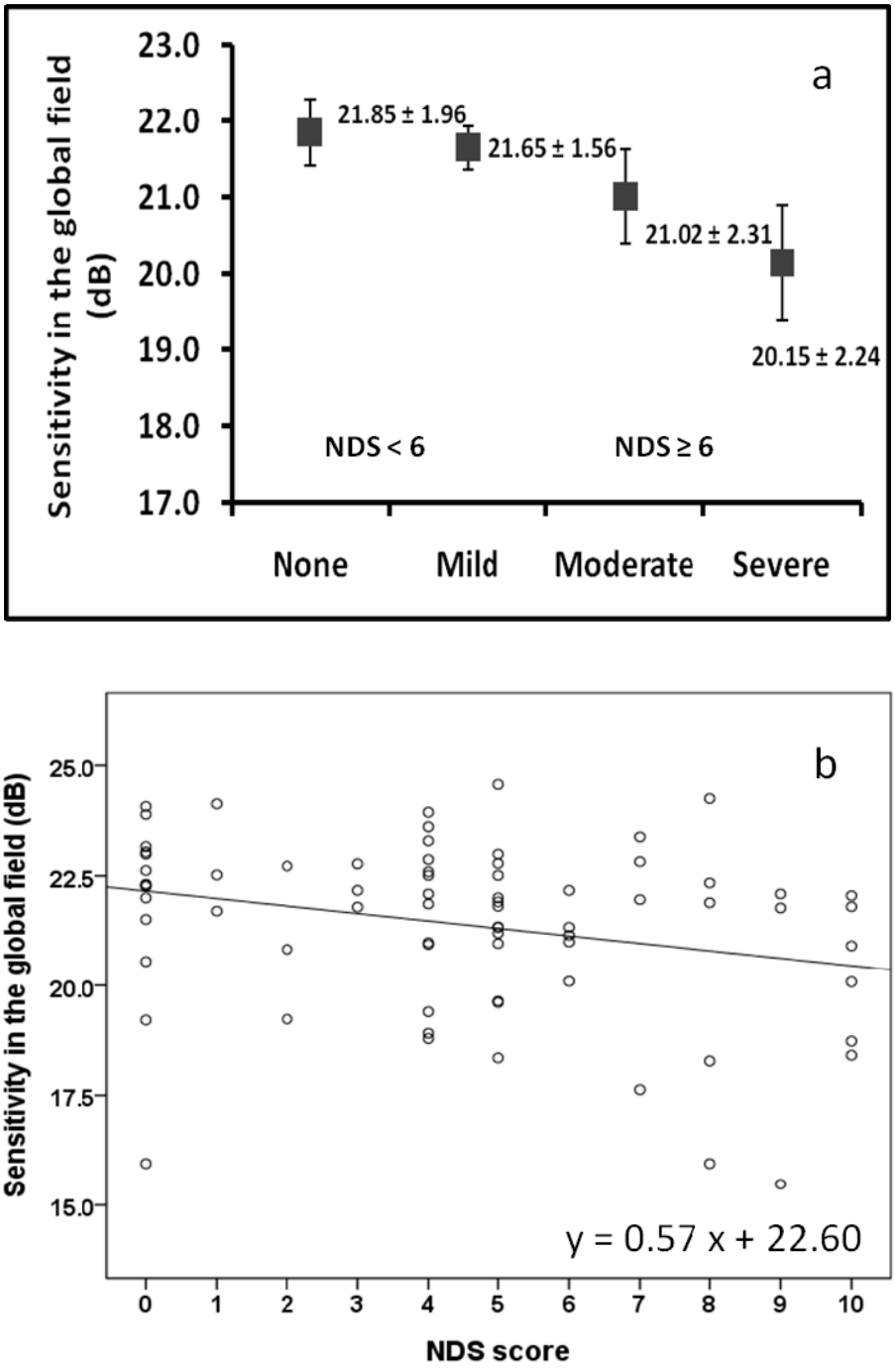


Figure47.Average ± standard error of the mean global contrast sensitivity (dB). Note that the data labels show mean ± standard deviation (a), Scatter plot showing association between NDS scores and global contrast sensitivity (b).

7.5 Discussion

This study investigated standard automated perimetry (SAP) outcomes in healthy control participants and individuals with type 2 diabetes who had different levels of diabetic peripheral neuropathy. It was postulated that SAP derived visual function in these people would be significantly reduced with increasing severity of peripheral neuropathy.

As previous explained, OD is a measure of non-spatially defined differences between the measured sensitivity and age-adjusted normative-based sensitivities, therefore describing a general depression or elevation of the visual field[270].In the current study, Overall Defect was found to be progressively reduced with increasing severity of neuropathy. Visual function in people with diabetes can be influenced by factors like level of diabetic retinopathy, duration of diabetes as well as laser photocoagulation treatment for retinopathy [228]. Given that none of these factors were influential in this experiment, it can be presumed that the found reduced OD is related to neuropathic status of the cohort. A derivative mean contrast sensitivity global value for the 106 points is not referenced to age-related normative data. As such it may not necessarily give the same outcomes as a comparison based on OD. However in this case, it also showed not to be a useful indicator of diabetic neuropathy or risk of ulceration.

High PD values indicate irregularity in contrast sensitivity levels between different regions across the field of vision. This measure rules out the effect of diffuse loss that might have been caused by cataract and therefore, shows abnormalities that are mostly other-disease-related [271].Pattern Defect has been largely designed with glaucomatous visual field loss in mind. The current experiment showed that participants with diabetes

but without peripheral neuropathy have different PD values from the healthy control individuals indicatingPD index may have an association with the effect of diabetes per se -on visual field outcomes; howeverit may not be useful in predicting DPN.

Previous work on contrast sensitivity in people with diabetes has focused on analysis of different eccentricities. Roth [182] reported visual field defects in the central 20 degrees of fixation in people with very mild diabetic retinopathy. Henricsson and Heijl[268] investigated contrast sensitivity within different eccentricity zones (0 - 10°, 10 - 20° and 20 - 30°) in a cohort who had various levels of diabetic retinopathy. Their results showed similar sensitivity loss for all eccentricities and reduced pattern and OD in more advanced retinopathy.

In the current experiment, sensitivity in the superior and inferior hemi-fields were analysed separately. This approach was taken in order to link the results with outcomes from the previous chapters. Association between structural and functional damage has been investigated previously in other retinal pathologies and it has been demonstrated that sensitivity in the superior hemi-field is related to the inferior retinal nerve fibre thickness, and vice versa [272]. In addition, analyses of clustered regions of visual field have been suggested to be a better determinant of pathology progression in other disease models [273].

Mean contrast sensitivity levels globally and in both hemi-fields failed to differentiate those who had no neuropathy from the control participants as well as the remaining type 2 participants with DPN. When considering the risk of ulceration (NDS cut-off point of six), contrast sensitivity globally and in both hemi-fields showed moderately reduced thresholds for groups who were at a higher risk of ulceration independent from the effect

of duration of diabetes, age or level of diabetic retinopathy. This finding is partially in agreement with localised loss of sensitivity in the superior hemi-field that has been previously reported for a group of people with type 2 diabetes and mild retinopathy [186]. This may indicate that, similar to retinal nerve fibre layer thickness results in Chapter 5, SAP derived visual function may be a potential marker for late stage neuropathy. A combination of these two outcomes may prove to be an even more useful composite indicator of foot ulceration risk. However, these cross sectional results independently are unable to demonstrate this conclusively; a larger cohort longitudinal study would be required.

The regression analysis for contrast sensitivity in the inferior hemi-field showed an association with severity of peripheral neuropathy if structure and function were to spatially match long with predictive models..As such, thinning of RNFL in the superior quadrant may be anticipated with increasing neuropathy. The regression model for RNFL in the superior quadrant (as demonstrated in *Chapter 5*) did not; however show a significant association with NDS. Therefore, unlike the outcomes for contrast sensitivity in the superior hemi-field, superior structural findings reported in Chapter 5 do not show a corresponding deficit with a functional loss in the inferior hemi-field. A possible explanation for such discrepancy in these structure-function associations could be that RNFL thickness was analysed in quadrants while visual field outcomes were analysed in hemi-fields. Hence, there is no comparable correspondence between the inferior hemi-field and superior RNFL indicating that a structure-function association must be interpreted cautiously.

This experiment was primarily designed to investigate the association between SAP outcomes and severity of diabetic peripheral neuropathy; however, the visual

implications of these findings should also be considered. It was demonstrated that participants with type 2 diabetes and with increasing levels of peripheral neuropathy have mildly reduced contrast sensitivity, globally and in both hemi-fields. In comparison with a person without neuropathy, an NDS score of 10 would relate to a maximum reduction in contrast sensitivity of 2 dB inferiorly and even less superiorly. This is unlikely to create practically significant repercussions for people who are affected.

In conclusion, contrast sensitivity in the central 30 degrees of visual field reduces in association with severity of peripheral neuropathy; however this occurs more prominently in the late stages of the disease. Standard automated perimetry is currently the 'gold standard' clinical technique for detecting other retinal pathologies including glaucoma [267].This is the first study to investigate the potential association between standard automated perimetry outcomes and peripheral neuropathy in diabetes.

8 Relationship between Standard Automated Perimetry and Diabetic Peripheral Neuropathy as determined by Quantitative Sensory Testing

8.1 Introduction

Diabetes-related impairment of visual function has been shown in eyes with normal visual acuity and minimal evidence of diabetic retinopathy[162, 202, 266, 274]. Additionally, short-wavelength-sensitive cones have been shown to be damaged in diabetic patients without retinopathy as determined by blue-on-yellow perimetry (which isolates and measures the function of these short wavelength sensitive cells) [181]. Very few studies have investigated the ability of standard automated perimetry (SAP) to detect contrast sensitivity changes in individuals with diabetes [186].

Quantitative sensory testing (QST) has been shown to be an arguably reliable method of detecting diabetic peripheral neuropathy [235]. The method can be used to assess damage to the small nerve endings, which detect changes in temperature, and the large nerve endings, which detect vibration. Additionally, it can be applied at a number of anatomical sites but is most commonly used on the lower limbs in people with suspected diabetic peripheral neuropathy.

Previously in *Chapter 6*, the association between retinal nerve fibre layer thickness and QST was investigated and it was demonstrated that retinal nerve fibre layer, structurally

as a part of central nervous system, has no meaningful association with QST measurements of the peripheral nervous system function. However, it is a part of central nervous system, has no meaningful association with QST measurements of peripheral nervous system sensory function. However, it is nonetheless of interest to assess the relationship between central nervous system mediated visual function as measured by SAP and QST as a measure of peripheral diabetic neuropathy. This is the primary focus of the current experiment.

8.2 Aim and hypotheses

The chief aim of this experiment was:

1. To investigate the relationship between contrast sensitivity (assessed using SAP) and peripheral sensory function (assessed using QST) in people with type 2 diabetes, with and without DPN.

This experiment was testing the following hypotheses:

1. Contrast sensitivity in the global visual field and the superior and inferior hemi-fields are significantly related to reduced sensory thresholds as measured by QST

2. Reduced visual field "overall" and "pattern" defect indices are significantly associated with reduced peripheral sensory thresholds as determined by QST

8.3 Methods

8.3.1 Participants

Characteristics of participants have been described in *Chapter 7, section 7.3.1.*Of 105 individuals who consented to the study, 73 volunteers with type 2 diabetes formed the study group for the experiment described in this chapter. Thirty two individuals were excluded from the study as the outcome measurements for visual field testing did not meet relevant reliability criteria (false positive, false negative or fixation loss error rate was > 33%) and/or had received retinal laser treatment for diabetic retinopathy. Gender-related differences have been previously demonstrated in *section 7.3.1.* Additionally, no control participants were included in this chapter as the no-neuropathy group can readily be compared with the normative data-base which acts as a de facto control group.

8.3.2 Assessment of diabetic peripheral neuropathy

Assessment of peripheral neuropathy was performed on the foot of the hand-dominant side of each participant using quantitative sensory testing (QST) with the Medoc instrument. A comprehensive description of the technique has been provided in *Chapter 3*.

8.3.3 Assessment of contrastsensitivity across the visual field

Static, standard automated perimetry was performed monocularly using an automated field analyser (Medmont M700, Medmont International Ltd, Victoria, Australia).

Perimetry was performed on the eye with better visual acuity within central 30 degrees eccentricity. The test and reliability indices (i.e. fixation loss, false positive and false negative errors) have been explained previously (*section 3.7.3.6.3*). Overall Defect(OD) is an index of global differences between measured sensitivity for an individual observer and age-related normative-based sensitivities, therefore describing a general depression or elevation of the visual field [270].The PD is the measurement of how much the shape of the hill of vision deviates from the shape of the "normal" hill of vision for the age of the patient, after being corrected for intra-test variability. Overall Defect and Pattern Defectare derived by the instrument's own software package and both were recorded for each participant. Global contrast sensitivity (average threshold of 106 test points), superior and inferior hemi-fields' mean contrast sensitivity (average threshold for 53 test points for each hemi-field) were also calculated.

8.3.4 Statistical analysis

The distribution of global average contrast sensitivity was found to be non-normal ($W = 0.95$, $p = 0.007$). Figure 48shows the distribution histograms for visual field data. Both hypotheses were addressed using univariate regression analyses. Median and range (min-max) global contrast sensitivity and the corresponding values for each hemi-field are reported in Table 30. QST variables (cold sensation, warm sensation, cold-induced pain, heat-induced pain and vibration perception), age, duration of diabetes and grade of diabetic retinopathy were fitted as main variables in all regression models. Analyses of contrast sensitivity in association with cold and heat-induced pain were performed using two approaches. This has been addressed more comprehensively in *section 6.5*. In brief, the Medoc equipment is limited to zero and fifty degrees for determination of cold-

induced and heat-induced pain threshold; respectively. These floor and ceiling values may not necessarily reflect the true temperature-induced pain threshold. Hence, one analysis was performed including these values and another was performed after elimination of these values. A p-value < 0.05 was considered to be statistically significant. Statistical Package for Social Sciences (SPSS) v.18 was used to analyse the collected data.

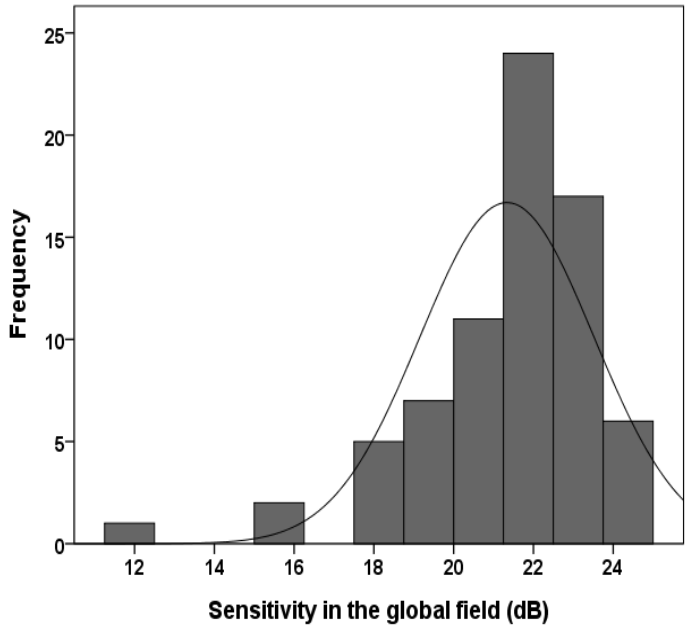


Figure 48.Distribution histogram for global average contrast sensitivity.

Table 30.Median (range: min - max) (dB) for contrast sensitivity globally and in superior and inferior hemi-fields.

	Contrast sensitivity (dB)		
	Global	**Superior hemi-field**	**Inferior hemi-field**
Median (min-max)	21.89 (15.48-24.57)	21.55 (15.87-24.96)	2.37 (13.88-24.71)

8.4 Results

8.4.1 Contrast sensitivity globally and in superior and inferior hemi-fields

Reduced global contrast sensitivity was found in association with increased QST cold sensation thresholds (b = 0.06, p = 0.01). This equates to a loss of cold sensitivity. Similarly, contrast sensitivity thresholds in the superior hemi-field were increased by 0.07 dB for every degree increase in cold sensation threshold (p < 0.01). Contrast sensitivity threshold in the inferior hemi-field was also increased by 0.05 dB with increasing cold sensation threshold (p = 0.02). These associations are shown inFigure 49. Statistical details for the regression models are reported in Table 31. Additionally, warm sensation had no significant associationwithglobal contrast sensitivity (p = 0.13). Similar non-signifcant results were found for sensitivity in the superior and inferior hemi-fields (Table 31 and Figure50).

There was no significant relationship between cold-induced pain and contrast sensitivity globally or in superior and inferior hemi-fields (p > 0.20 for all regression models). Similarly, results for heat-induced pain showed no significant association with contrast sensitivity in any of the models (p > 0.23 for all regression models,Table 31, Figure51and Figure52).

Vibration perception threshold also did not have significant associations with contrast sensitivity measurements globally (p = 0.43) and in each hemi-field (p = 0.44 and p = 0.29 for superior and inferior models; respectively, Table 31 and Figure53). Associations with age, duration of diabetes and level of diabetic retinopathy in all regression models are summarized inTable 31.

Table 31.Associations between standard automated perimetry parameters andQST, age, duration of diabetes and diabetic retinopathy.

		Visual field parameter														
		Global			Superior hemi-field			Inferior hemi-field			Overall Defect			Pattern Defect		
QST	Covariate	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2
CS	Main€	0.06	0.01*	0.06	0.07	< 0.01*	0.07	0.05	0.02*	0.04	0.04	0.02*	0.07	-0.02	0.12	-0.01
	Age	<-0.01	0.82		<-0.01	0.95		-0.01	0.70		0.06	0.02*		-0.01	0.66	
	DR	-0.12	0.69		0.20	0.57		-0.05	0.87		<0.01	0.98		0.18	0.41	
	DD	<-0.01	0.91		< 0.01	0.89		<0.01	0.95		-0.01	0.60		<-0.01	0.77	
WS	Main	-0.08	0.13	<0.01	-0.10	0.11	<0.01	-0.07	0.20	- 0.01	-0.07	0.03*	0.05	0.01	0.66	-0.04
	Age	-0.02	0.51		-0.02	0.61		-0.03	0.44		0.05	0.04*		<- 0.01	0.94	
	DR	0.12	0.73		-0.19	0.60		-0.07	0.94		0.01	0.96		0.17	0.43	
	DD	<-0.01	0.98		< 0.01	0.98		<-0.01	0.90		<-0.01	0.67		<-0.01	0.94	
CIP	Main	0.05	0.25	-0.01	-0.05	0.31	-0.02	0.05	0.23	-0.01	0.03	0.21	0.01	-0.01	0.72	-0.05
	Age	-0.04	0.33		-0.04	0.40		-0.04	0.30		0.04	0.11		<- 0.01	0.99	
	DR	-0.14	0.67		-0.21	0.56		-0.06	0.83		<-0.01	0.97		0.18	0.42	
	DD	<-0.01	0.77		-0.01	0.77		<-0.01	0.80		- 0.01	0.40		<0.01	0.99	
HIP	Main	-0.20	0.26	-0.01	-0.19	0.33	-0.02	-0.07	0.51	<- 0.01	-0.07	0.51	<-0.01	-0.07	0.51	<- 0.01
	Age	-0.03	0.40		-0.03	0.47		0.04	0.10		0.04	0.10		-0.04	0.10	
	DR	-0.12	0.70		-0.20	0.59		< 0.01	0.98		< 0.01	0.98		<0.01	0.98	
	DD	<-0.01	0.83		<-0.01	0.81		-0.01	0.39		- 0.01	0.39		-0.01	0.39	
VP	Main	<-0.01	0.42	-0.02	<-0.01	0.52	-0.02	<- 0.01	0.35	-0.02	<-0.01	0.20	0.01	<0.01	0.89	-0.05
	Age	-0.03	0.52		-0.03	0.57		-0.03	0.50		0.05	0.05		<-0.01	0.97	
	DR	-0.07	0.83		-0.15	0.69		0.01	0.97		0.05	0.79		0.17	0.45	
	DD	<-0.01	0.81		<-0.01	0.79		<-0.01	0.86		-0.01	0.51		<0.01	0.98	

*€: refers to main effect of the QST parameter, * indicates significance, CS: cold sensation, WS: warm sensation, CIP: cold-induced pain, HIP: heat-induced pain, VP: vibration perception, DD: duration of diabetes, DR: diabetic retinopathy, b: regression coefficient, Adj R^2: adjusted R^2*

8.4.2 Overall Defect and Pattern Defect

Overall Defect was significantly associated with cold sensation and warm sensation but not with the remaining QST subtests ($p = 0.02$ and $p = 0.03$; respectively). Statistical outcomes are summarized inTable 31. Main effect of age on OD in association with cold and warm sensation was also statistically significant ($p = 0.02$ and $p = 0.04$; respectively). However age, duration of the disease and level of diabetic retinopathy were found not to be associated with OD in the remaining regression models ($p > 0.05$ for all models,Table 31).

Association between PD and cold sensation was close to significant ($p = 0.07$); however none of the associations between PD and QST parameters reached statistical significance (Table 31). Age, duration of diabetes and severity of diabetic retinopathy did not have significant outcomes in any of the regression models (Table 31).

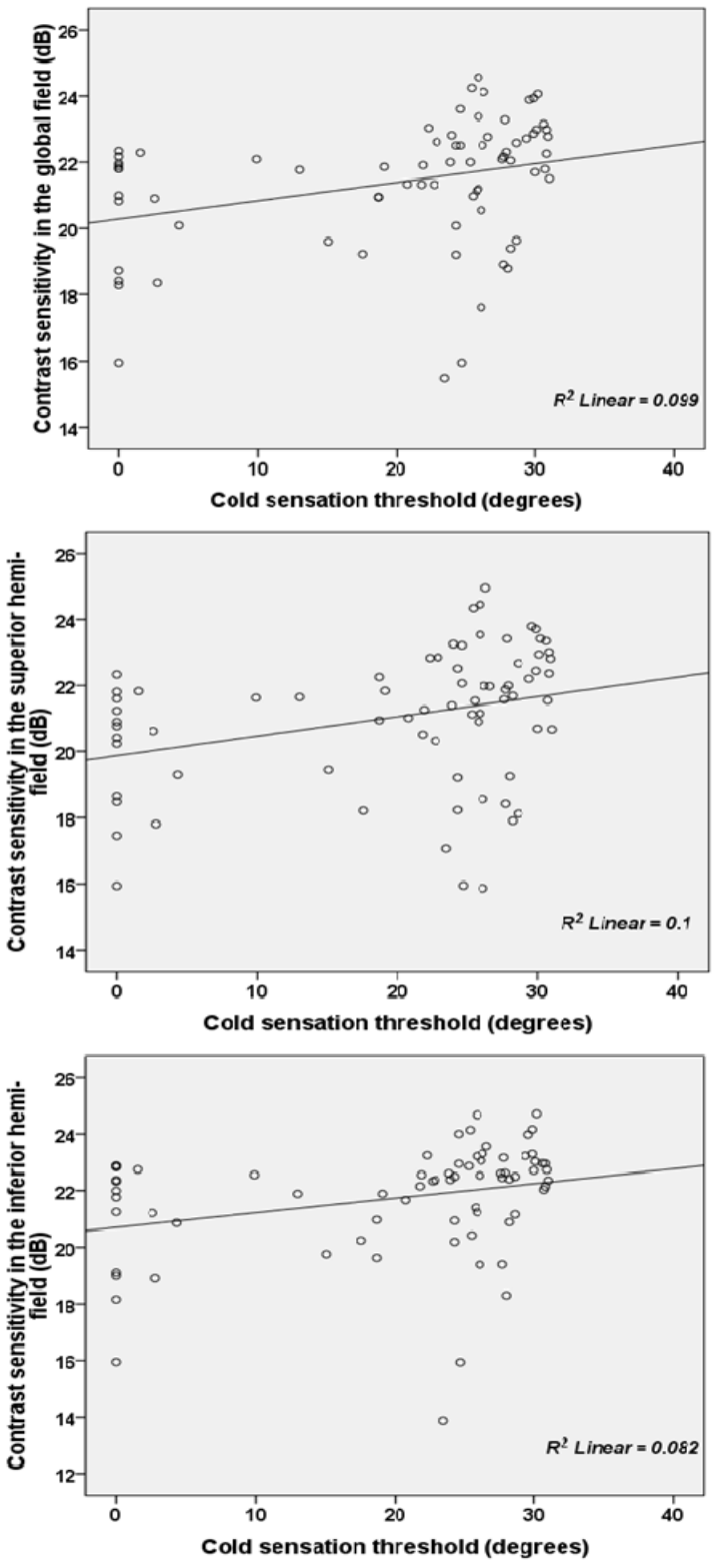


Figure 49. Scatter plots showing significant linear associations between QST cold sensation threshold and contrast sensitivity thresholds globally and in superior and inferior hemi-fields.Y axis scale differs for each plot. Decrease in cold sensitivity is to the left on the X axis

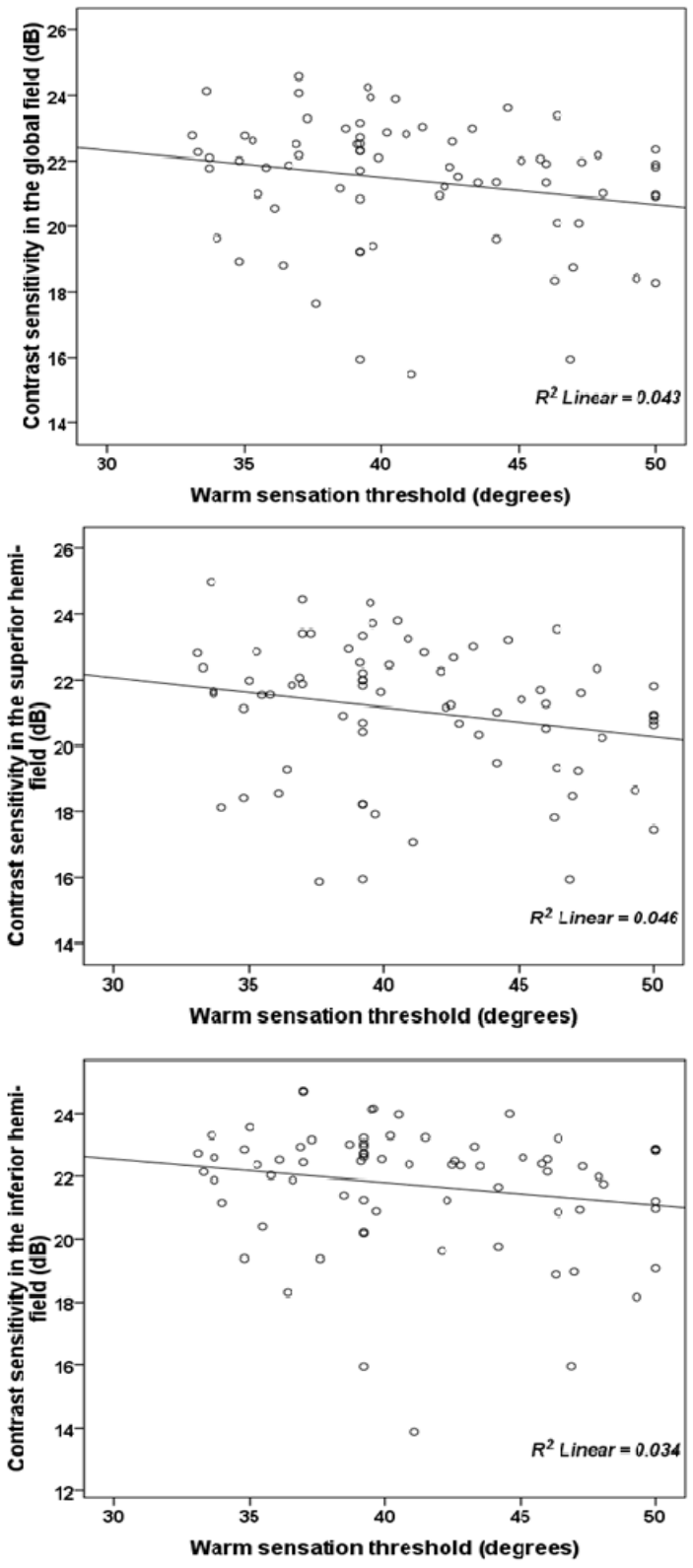


Figure50. Associations for QST warm sensation threshold and contrast sensitivity thresholds globally and in the superior and inferior hemi-fields. Y axis scales are different for each plot. Decrease in warm sensitivity is to the right on X axis.

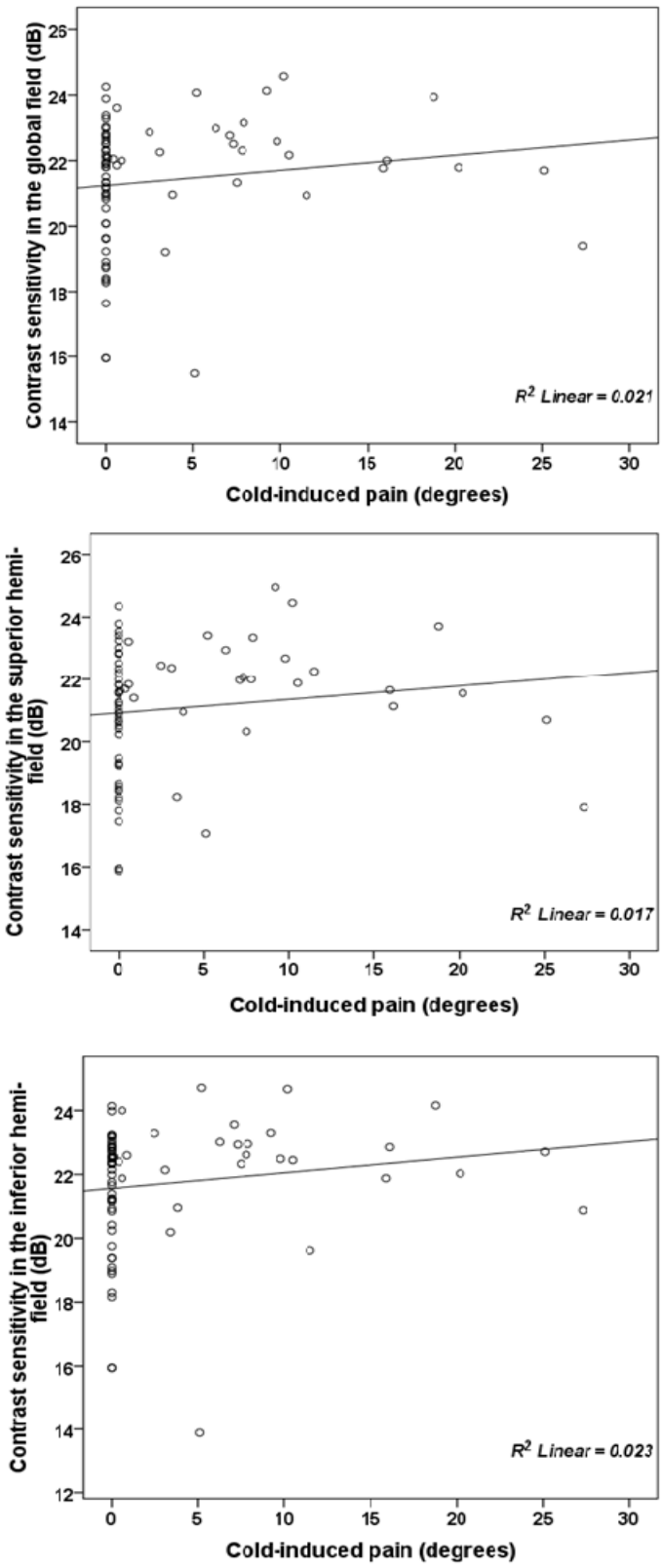


Figure51. Scatter plots for QST cold-induced pain threshold and contrast sensitivity thresholds globally and in each hemi-field. Y axis scale is different for each plot.

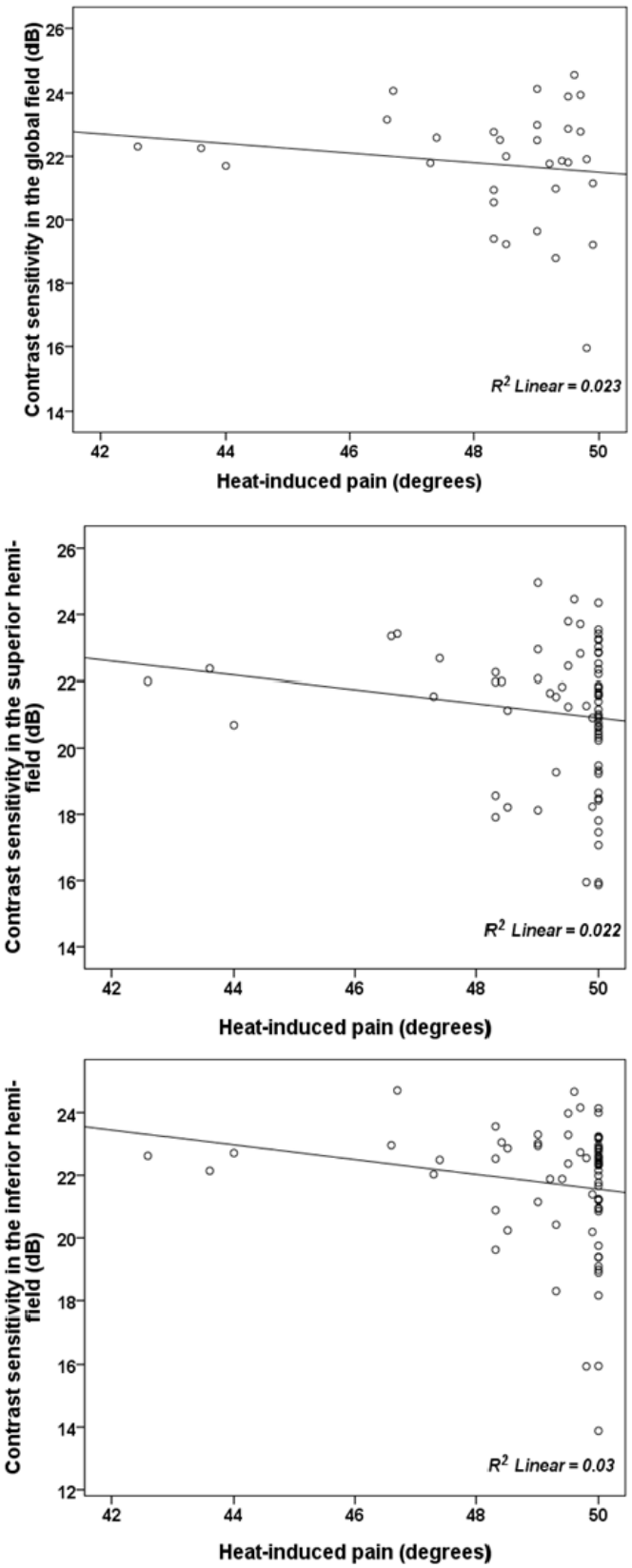


Figure52. Scatter plots indicating linear association for QST heat-induced pain threshold and contrast sensitivity thresholds. Y axes are not similar in each plot.

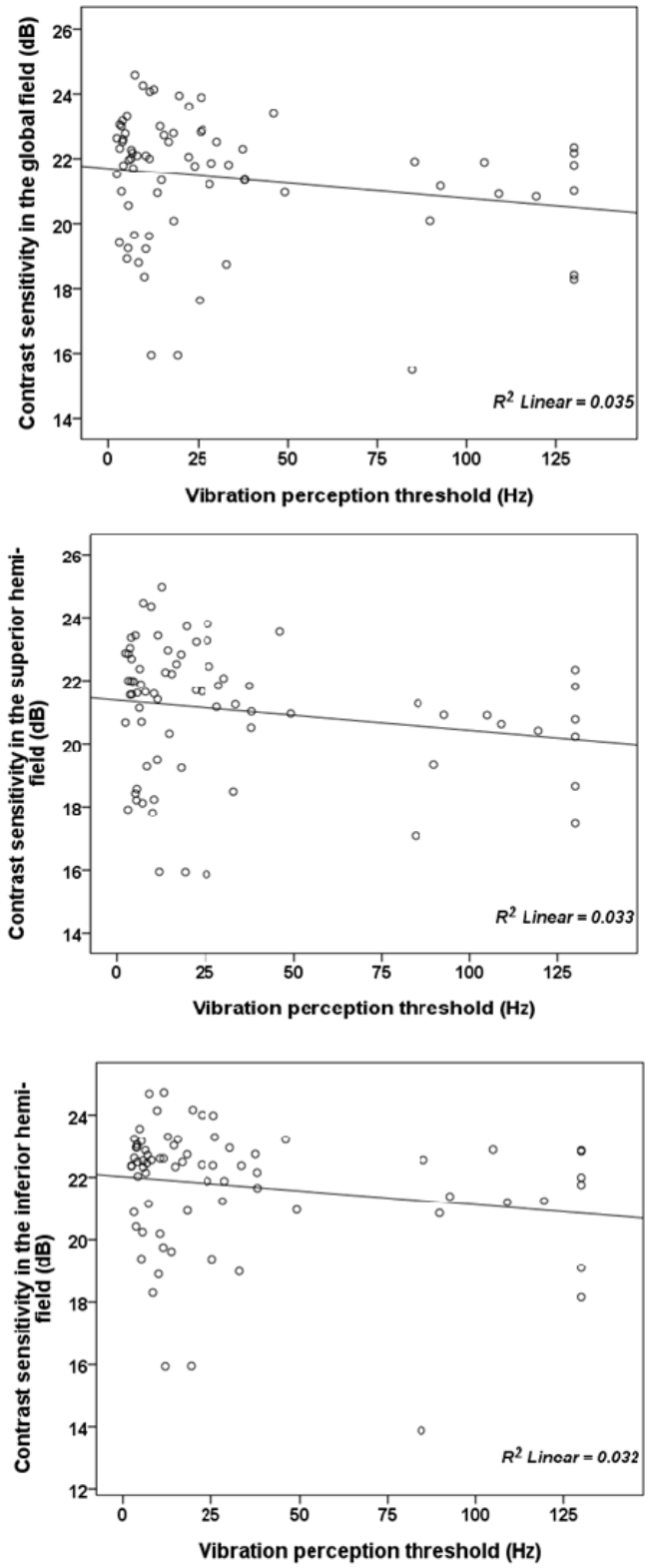


Figure53. Scatter plots showing associations between QST vibration perception threshold and contrast sensitivity thresholds globally and in both hemi-fields. The Y axis scale is different in each graph. Decrease in vibration perception is to the right on X axis.

8.4.3 Excluding floor values from cold-induced pain threshold analysis

The rationale for eliminating the floor values has been explained in *Chapter 6*. Sixty one precent of the participants (n = 45) had a cold pain threshold of zero degrees, that indicates the limit of the test (zero degrees) was reached in 45 individuals due to extremely poor cold sensitivity in the foot. As such, only 28 individuals were included in the analysis (age ± SD: 60 ± 6 years, 18 males).

No significant association was found between cold-induced pain threshold and average contrast sensitivity globally ($p = 0.84$), in the superior hemi-field ($p = 0.51$) or inferior hemi-field ($p = 0.81$). Similar non-significant associations were found for OD and PD indices ($p = 0.88$, $p = 0.95$; respectively). Detailed statistics for the regression models are presented in Table 32. Figure54shows the association between these variables. Age, duration of diabetes and level of diabetic retinopathy also had no significant associations with the outcome variables ($p > 0.23$ for all regression models, Table 32

Table 32. Regression analyses for association between QST cold and heat induced pain and visual field parameters after excluding floor and ceiling values.).

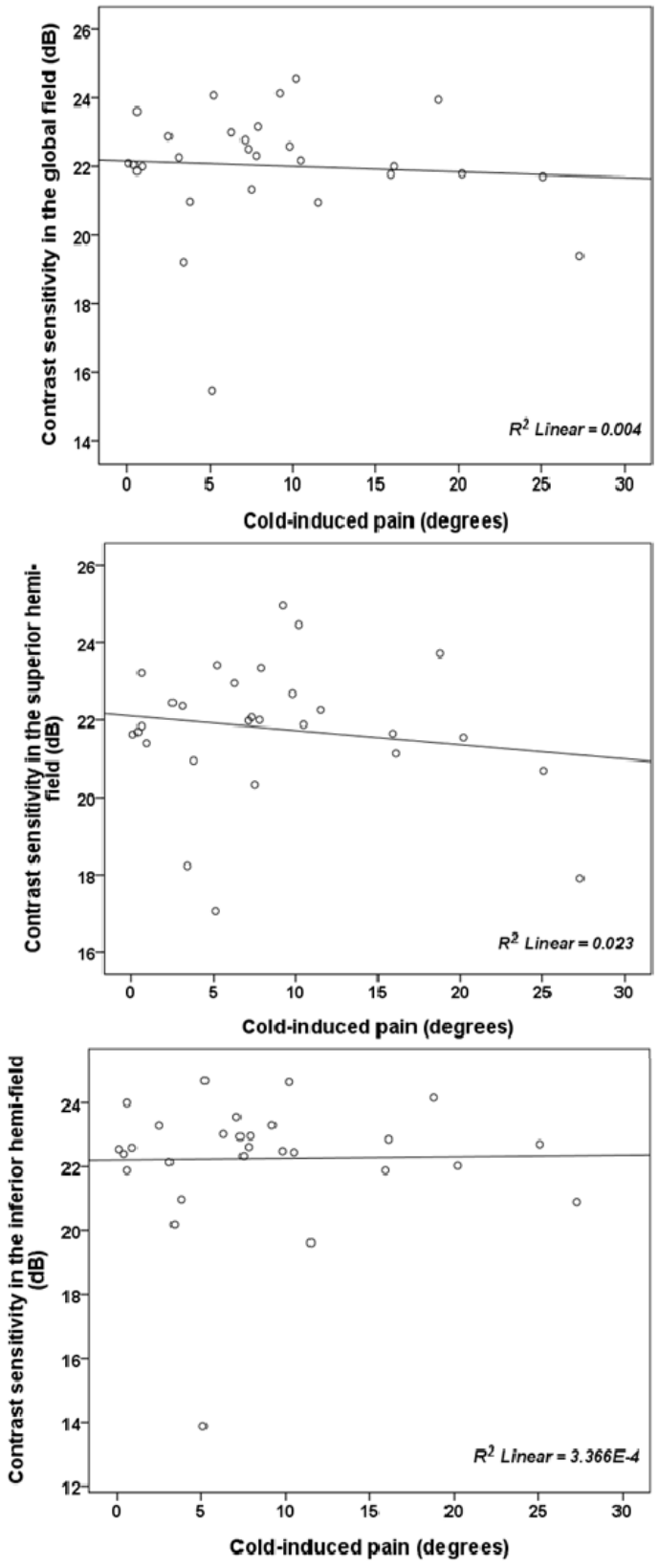


Figure54. Scatter plots showing linear associations between contrast sensitivity thresholds and cold-induced pain threshold after eliminating zero values (n = 28).

8.4.4 Excluding ceiling values from heat-induced pain threshold analysis

Forty one participants (56%) had a heat-induced pain threshold of 50 degrees. These participants were eliminated based on the rationale explained in *Chapter 5*. As a result, 32 individuals were included in this part of the analysis (mean age ± SD: 60 ± 6 years, 18 males).

The average global contrast sensitivity was not associated with heat-induced pain threshold ($p = 0.33$). Similar non-significant findings were observed for contrast sensitivity in the superior hemi-field ($p = 0.58$), inferior hemi-field ($p = 0.26$), OD ($p = 0.78$) and PD ($p = 0.78$). Details for the regression models are outlined in Table 32. Figure55represents the association between contrast sensitivity globally and in each hemi-field with QST heat-induced pain. Additionally, age, duration of diabetes and level of diabetic retinopathy did not have significant associations with any of the outcomes variables ($p > 0.23$ for all associations, Table 32).

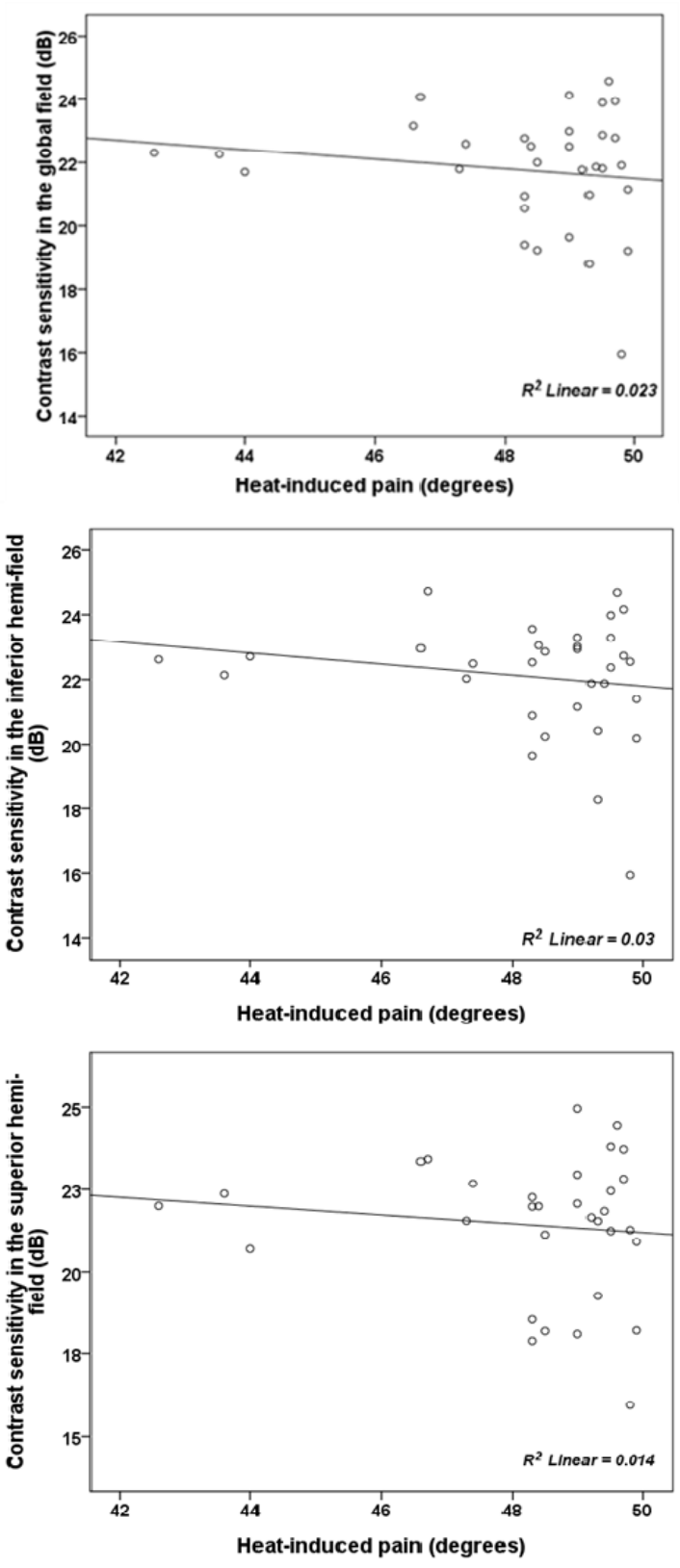


Figure55.Scatter plot showing linear associations between heat-induced pain thresholds and contrast sensitivity threshold globally and in the superior and inferior hemi-fields after eliminating the ceiling values.

Table 32. Regression analyses for association between QST cold and heat induced pain and visual field parameters after excluding floor and ceiling values.

		Visual field parameter														
		Global			Superior hemi-field			Inferior hemi-field			Overall Defect			Pattern Defect		
QST	Covariate	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2	B=	p=	Adj R^2
CIP	Main€	-0.01	0.84	-0.01	-0.03	0.51	< -0.01	0.01	0.81	-0.03	< -0.01	0.88	-0.06	<0.01	0.95	-0.11
	Age	-0.05	0.34		-0.05	0.41		-0.06	0.35		0.04	0.27		0.01	0.70	
	DR	-0.04	0.91		-0.02	0.95		-0.06	0.89		-0.06	0.81		-0.03	0.89	
	DD	-0.05	0.23		-0.05	0.23		-0.05	0.29		-0.03	0.31		0.03	0.35	
HIP	Main	-0.15	0.43	-0.15	-0.11	0.62	-0.12	-0.20	0.30	- 0.07	0.04	0.78	-0.03	0.03	0.78	-0.11
	Age	-0.01	0.85		-0.02	0.70		<0.01	0.92		0.04	0.23		- 0.02	0.58	
	DR	0.03	0.94		-0.09	0.86		-0.16	0.82		0.20	0.55		0.03	0.90	
	DD	0.01	0.78		<-0.01	0.92		0.04	0.49		0.01	0.69		0.02	0.62	

€: refer to main effect of the QST parameter, CIP: cold-induced pain, HIP: heat-induced pain, DD: duration of diabetes, DR: diabetic retinopathy, B: regression coefficient, Adj R^2: adjusted R^2

8.5 Discussion

This study aimed to investigate the association between various quantitative sensory testing (QST) parameters and contrast sensitivity, as measured by standard automated perimetry, in volunteers with type 2diabetes. It was hypothesized that the magnitude of contrast sensitivity loss can be predicted by QST measurements that are indicative of loss of peripheral sensory function.

Contrast sensitivity was assessed globally (single average for 106 individual points) as well as for hemi-fields (superior and inferior, 53 points each). The sub-analysis of hemi-fields was performed on the basis of findings from previous glaucoma studies, where loss of retinal nerve fibre layer thickness matched the loss of contrast sensitivity in the corresponding visual field [275, 276].

In the current study, it was demonstrated that contrast sensitivity globally, and superior and inferior hemi-fields were all significantly but weakly associated with cold sensation thresholds. Contrast sensitivity reduction with decreased cold sensation in the foot was marginally more evident for the global visual field than for either of the hemi-fields. While trends towards reduced sensitivity with decreased warm sensation were observed, each of these warm sensation trends, as well as the remaining findings with QST sub-tests, did not show any meaningful association.

Overall Defect, a software-derived parameter from standard automated perimetry, was found to decrease mildly in association with warm sensation while a slight increase in OD was found in association with cold sensation thresholds. These findings may indicate that the ‘height’ of the hill of vision in individuals with diabetic neuropathy is

changing with loss of warm and cold sensitivity (that is, thresholds closer to extremes). However it should be acknowledged that ODcan be influenced by other factors such as media opacity and refractive error. These factors were controlled for in this study by exclusion or compensation. Visual field PD, another software-derived metric, was not meaningfully associated with any of the QST measurements.

The variance in the distributions for each of the QST modalities, as described previously in *Chapter 5*, could possibly be explained by the highly subjective nature of both QST and SAP tests. In these techniques, test familiarity, fatigue, subject cooperation and reaction time can each influence the outcome findings. As shown in previous chapters, Neuropathy Disability Score was a more useful predictor than QST of visual function loss, suggesting that performance on the two highly subjective variables themselves may contribute to a significant discrepancy in the outcomes.

Significant visual field loss has been reported in people with more severe diabetic retinopathy [184]. However, many studies have reported visual dysfunction in people with diabetes with minimal levels of retinopathy [186, 207]. In the current study, (as previously discussed in *Chapter 5*), retinopathy level was no greater than moderate non-proliferative, with a majority of participants having minimal or no retinopathy at all. Even though many of the findings of this section of the study proved not to be significant, regression models showed that retinopathy did not have a significant independent effect on those outcome variables that did show a significant association with QST.

It has been shown that diabetes-induced pathology of the inner retina can lead to reduced oxygen diffusion and hence dysfunction of the retinal neural components [181]. Given

that the retina is a highly demanding metabolic tissue [277], the combination of high metabolic demand and minimal vascular supply may limit the ability of the inner retina to adapt to the metabolic stress of diabetes, leading to mildly reduced visual function – in this case, contrast sensitivity. Hence, it is important to investigate the prospect of reduced visual function in people with diabetes. The study cohort in this chapterhad general reductions in average contrast sensitivity that were not explained by retinopathy. However, these reductions did not prove to have a strong relationship with peripheral neuropathy, as measured by QST.

It should be acknowledged that visual field responses are a function of the central nervous system and the outcomes reflect the performance of the entire visual system, including lateral geniculate body and visual cortex – not just the retina. It has also been shown that patients with diabetic neuropathy elsewhere in the body can additionally suffer from disturbances of the central nervous system [278]. Therefore any microvascular changes that happens in accordance with peripheral neuropathy in diabetes, could also potentially affect the visual system in a generalised manner. Increased latency of the P100 wave in visually evoked potentials – thought to be generated by visual cortex – has been shown to be correlated with diabetic peripheral neuropathy [175]. This supports the prospect of diabetic peripheral neuropathy demonstrating an association with visual function.

In conclusion, the current study did not show any strong and clinically meaningful associations between contrast sensitivity thresholds, overall and PD indices and quantitative sensory testing. This indicates that visual field results are not likely to prove useful in predicting neuropathy status when this is defined by any of the QST sub-tests.

9 Assessment of Flicker Sensitivity in association with Diabetic Peripheral Neuropathy

9.1 Introduction

Flickersensitivity or temporal visual processing is the ability to detect intermittent light and dark alternations of a visual stimulus. Assessment of this specific visual function is feasible by means of flicker perimetry where light and dark alternation contrast sensitivity of an observer is examined at various locations of the visual field. The technique can be used to provide earlier detection of neuro-sensory disease than other conventional methods of assessing visual function [279]. The technique has the capability to characterize the nature of a sensory deficit and to provide information regarding disease-affected retinalmechanisms [280].

The efficacy of flicker perimetry in detecting reduced visual sensitivity in individuals with minimal diabetic retinopathy has been previously demonstrated [191, 192]. Given that such changes precede clinically evident micro-vascular changes of the retina, it can be speculated that they can be mediated by a neuropathic source. Flicker perimetry may be a sensitive measure of early subtle functional changes that might be related to peripheral neuropathy in diabetes and this will the prominent focus of this chapter.

9.2 Aims and hypotheses

The two major aims of the study were:

1. To investigate flicker sensitivity in people with type 2 diabetes with and without diabetic peripheral neuropathy

2. To assess the relationship between flicker sensitivity and severity of peripheral neuropathy as measured by Neuropathy Disability Score

The experiment was specifically testing the following hypotheses:

1. Flicker sensitivity parameters, including sensitivity globally and in hemi-fields, Overall and Pattern Defects, are significantly different between:

    a. People with type 2 diabetes and DPN compared to those without peripheral neuropathy

    b. NDS groups (none, mild, moderate and severe)

    c. Individuals at higher risk of foot ulceration (NDS $\geq$ 6) and lower risk of foot ulceration (NDS <6).

2. Reduction in flicker sensitivity parameters is related to the severity of diabetic peripheral neuropathy

9.3 Methods

9.3.1 Participants

Participants who were recruited for the standard automated perimetry experiment were also included for this study (*see Chapter 8*). Participants were 37 years of age or older (mean ± standard deviation: 59.2± 8.1, ANOVA $F = 0.31$, $p = 0.81$). Characteristics of the eligible participants are outlined in Table 33. Overall defect was significantly higher for female participants than males ($t = -2.52$, $p = 0.01$). There were no gender-related significant differences between other measures of flicker sensitivity: $t \geq 18$, $p \geq 0.49$ for pattern defect, flicker sensitivity globally and in hemi-fields. Furthermore, as the no-neuropathy group can readily be compared with the normative data-base which acts as a de facto control group, no healthy control participants were included in this study.

Table 33.Characteristics of the study cohort classified to fourNDS groups

Parameter	No neuropathy	Mild neuropathy	Moderate neuropathy	Severe neuropathy	Total
N	13	13	11	6	43
Age (yrs)	58.77 ± 9.65	57.62 ± 9.15	60.91 ± 9.03	60.33 ± 9.34	59.19 ± 8.75
Gender (M/F)	8/5	12/1	7/4	4/2	31/12
Duration of diabetes (yrs)	10.15 ± 10.86	13.15 ± 10.43	16.36 ± 9.68	17.16 ± 6.30	13.62 ± 9.97

9.3.2 Assessment of flicker sensitivity

Flicker sensitivity was evaluated on 73 participants with type 2 diabetes using Medmont field analyser M700 after assessment for eligibility criteria (*see Chapter 3*). The test was performed on the eye with better visual acuity, within central 30 degrees eccentricity. Refractive error was corrected were appropriate by inserting 38 mm compensating lenses in a lens holder in front of the eye. Participants were all inexperienced with the procedure and they were instructed to press the response button only when a flickering stimulus was perceived. Duration for each stimulus was approximately 0.2 sec. The test was executed in a dark and quiet room in order to avoid any distractions and participants were given enough breaks to avoid fatigue. Only visual field results with reliability indices (fixation loss, false positive error and false negative error < %33) were only included in the analysis. Overall Defect and PD indices were recorded for each participant. Definition and importance of these indices have been comprehensively explained in *Chapter 7*. Of 73 flicker perimetry results, only 43 met the reliability criteria. Thirty participants were eliminated due to high percentage of false positive errors and/or fixation losses (> 33%).

9.4 Assessment of diabetic retinopathy

The rationale and importance of assessment of diabetic retinopathy has been explained in *Chapter 7, section 7.3.4*. Outcomes for flicker sensitivity globally and in superior and inferior hemi-fields as well as OD and PD for groups of diabetic retinopathy (none, minimal, mild, moderate and severe) are shown in Table 34. No significant differences were found when the abovementioned variables were compared between the retinopathy groups ($p = 0.49$, $p = 0.46$, $p = 0.54$, $p = 0.10$, $p = 0.94$; respectively).

Table 34.Flicker sensitivity values (mean ± standard deviation) for each level of diabetic retinopathy

	Level of Diabetic Retinopathy					
Parameter	**None**	**Minimal**	**Mild**	**Moderate**	**F =**	**P =**
N	20	8	14	1		
Overall Defect	1.1 ± 1.1	2.2 ± 0.7	1.5 ± 1.5	- 0.4 ± 0.0	2.16	0.10
Pattern Defect	2.7 ± 2.1	3.5 ± 2.0	3.0 ± 2.1	4.4 ± 0.0	0.13	0.94
Global	19.18 ± 2.72	18.50 ± 2.72	18.05 ± 3.65	15.97 ± 0.0	0.81	0.49
Superior hemi-field	18.90 ± 2.81	18.43 ± 2.99	17.75 ± 2.11	15.19 ± 0.0	0.87	0.46
Inferior hemi-field	19.46 ± 2.71	18.57 ± 2.71	18.34 ± 2.71	16.54 ± 0.0	0.72	0.54

F: Analysis of Variance statistics

9.4.1 Assessment of diabetic peripheral neuropathy

The level of neuropathy was classified using the Neuropathy Disability Score (NDS). The technique has been explained comprehensively in *Chapter 3*. An NDS ≥ 6 was considered as cut off score for higher risk of foot ulceration [45].

9.4.2 Statistical analysis

Global average flicker sensitivity, as shown inFigure 56, was found to be normally distributed using Kolmogorov-Smirnov test of normality (statistics: 0.10, p = 0.20). Frequency and descriptive statistics were calculated and the results are shown as mean ± standard deviation (SD). Student t-test and analysis of variance (ANOVA) were used for between group comparisons in order to address the first hypothesis. The groups without neuropathy (NDS score 0-2) was compared with the remaining participants in order to

assess the efficacy of flicker perimetry for early detection of neuropathy in diabetes as a part of the first hypothesis. To address the second hypothesis, linear and quadratic regression models were both investigated to evaluate the mathematical nature of the associations between the variables. Given that the outcomes for both models were not significantly different; the linear regression outcomes were only reported. A p value > 0.05 was considered to be significant. Statistical Package for Social Sciences (SPSS) version 18.0 was used for all analyses.

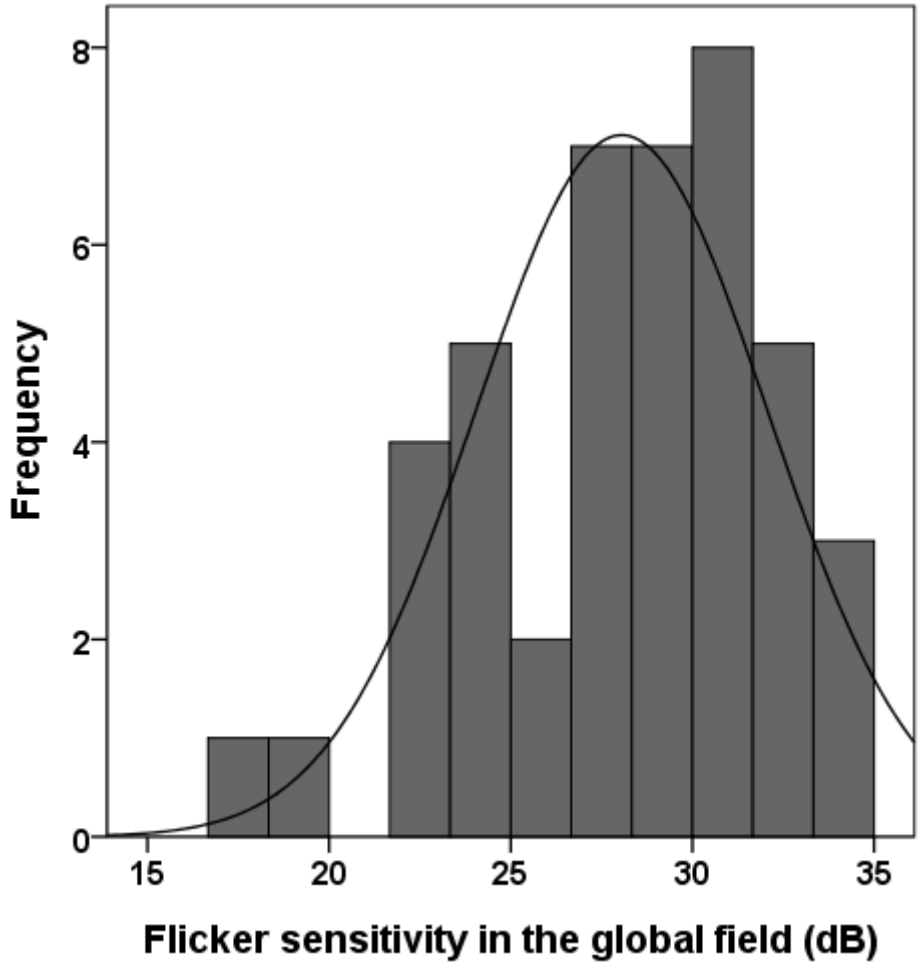


Figure 56.Histogram plot showing normal distribution for global flicker sensitivity (dB) in the study cohort.

9.5 Results

9.5.1 Comparison of Overall and Pattern Defects

Comparisons across the NDS groups (none, mild, moderate and severe)

Differences in OD between the four NDS groups was marginally statistically significant (p=0.05); however, there was a trend towards reduced OD as the severity of neuropathy increased (Figure 57 a). Pattern Defect did not differ significantly between the NDS groups (p=0.09) and the value was the highest for the group with moderate neuropathy (Figure 57 b). Statistical details are outlined in Table 35.

Comparison betweengroups with and without neuropathy

Overall Defect outcomes were found to be statistically significantly different between individuals without neuropathy (NDS score 0 - 2) and the remaining type 2 participants (p = 0.04). Results for PD, however, did not reach statistical significance (p = 0.25). Comparisons of OD and PD for groups with and without neuropathy are summarized in Table 36.

Assessment of Overall Defect and Pattern Defect in detecting risk of foot ulceration

Twenty-six individuals had NDS ≤ 6 (lower risk of foot ulcers) of which 20 were males. Overall Defect was found to be statistically different between individuals who were at higher risk of foot ulcers (n = 17, 11 males) compared with those who were at lower risk

(p = 0.04). However the results for PD was not statistically significantly different (p = 0.84). Detailed statistics are shown in Table 37.

Table 35. Comparison of flicker perimetry parameters between Neuropathy Disability Score test groups.

Parameter	NDS	Mean ± SD	ANOVA F =	P =
Global flicker sensitivity (dB)	**None**	19.14 ± 2.64	1.31	0.28
	Mild	19.33 ± 2.33		
	Moderate	17.40 ± 2.62		
	Severe	18.17 ± 3.56		
Flicker sensitivity in the superior hemi-field (dB)	**None**	18.93 ± 2.72	1.08	0.36
	Mild	18.99 ± 2.39		
	Moderate	17.20 ± 3.07		
	Severe	17.91 ± 3.26		
Flicker sensitivity in the inferior hemi-field (dB)	**None**	19.34 ± 2.78	1.41	0.25
	Mild	19.68 ± 2.20		
	Moderate	17.60 ± 2.33		
	Severe	18.44 ± 3.88		
Overall Defect (dB)	**None**	2.09 ± 1.23	2.69	0.05
	Mild	1.67 ± 1.29		
	Moderate	1.03 ± 1.21		
	Severe	0.60 ± 1.09		
Pattern Defect (dB)	**None**	4.28 ± 1.81	2.31	0.09
	Mild	5.21 ± 2.17		
	Moderate	5.79 ± 2.08		
	Severe	3.48 ± 1.52		

NDS: neuropathy disability score, SD: standard deviation

Table 36. Comparison of Overall Defect, Pattern Defect, flicker sensitivity values globally and in hemi-fields in participants with neuropathy (n= 30) and without (n=13).

Parameter	Status	Mean ± SD	t =	P =
Overall Defect (dB)	No neuropathy	2.09 ± 1.23	2.10	0.04*
	Rest	1.22 ± 1.25		
Pattern Defect (dB)	No neuropathy	4.28 ± 1.80	- 1.16	0.25
	Rest	5.07 ± 2.13		
Global flicker sensitivity (dB)	No neuropathy	28.80 ± 3.98	0.80	0.42
	Rest	27.72 ± 4.05		
Superior hemi-field (dB)	No neuropathy	18.93 ± 2.71	0.87	0.38
	Rest	18.11 ± 2.84		
Inferior hemi-field (dB)	No neuropathy	19.34 ± 2.77	0.74	0.46
	Rest	18.66 ± 2.71		

** indicates significance, SD: standard deviation, t: student t-test statistics*

Table 37. Comparison of flicker perimetry parameters between group at higher (n = 18) and lower risk of ulceration (n = 25).

Parameter	Risk of ulceration	Mean± SD	t =	p =
Overall Defect (dB)	**NDS < 6**	1.82 ± 1.24	2.04	0.04*
	NDS ≥ 6	1.03 ± 1.27		
Pattern Defect (dB)	**NDS < 6**	4.79 ± 2.04	- 0.19	0.84
	NDS ≥ 6	4.91 ± 2.13		
Global flicker sensitivity(dB)	**NDS < 6**	19.30 ± 2.42	2.02	0.04*
	NDS ≥ 6	17.67 ± 2.81		
Flicker sensitivity in the superior hemi-field (dB)	**NDS < 6**	19.03 ± 2.53	1.89	0.06
	NDS ≥ 6	17.43 ± 2.97		
Flicker sensitivity in the inferior hemi-field (dB)	**NDS < 6**	19.56 ± 2.50	2.04	0.04*
	NDS ≥ 6	17.91 ± 2.79		

**indicates significance, SD: standard deviation, t: student t-test statistics*

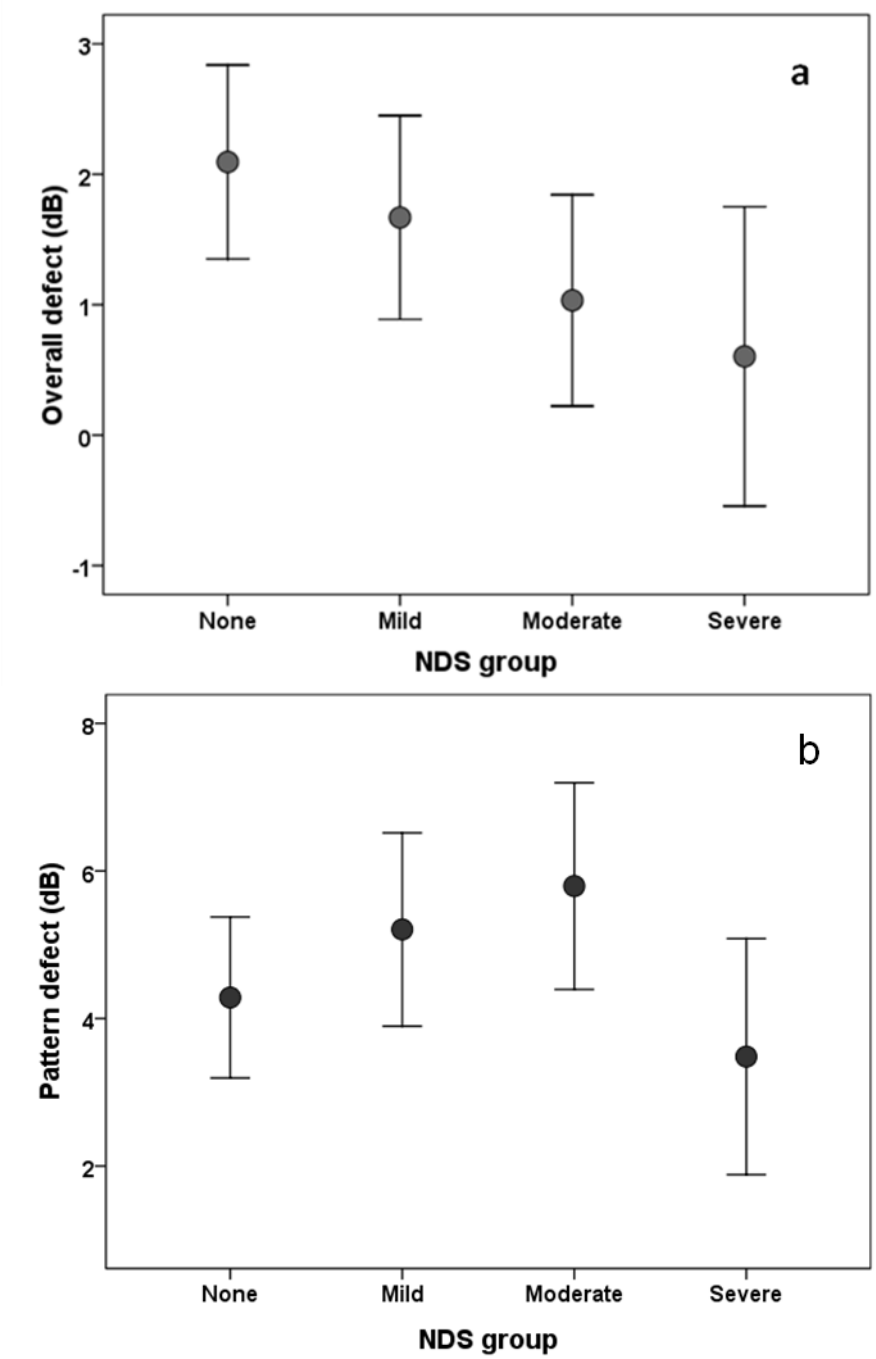


Figure 57.Overall (a) and Pattern Defect (b) for NDS groups (mean ± standard error of the mean). Note that the scales on Y axes are not identical.

Association with NDS score

NDS score was significantly associated with OD outcomes, showing a 0.21 unit decrease with each unit increase in NDS score (p = 0.002), but not with PD (p = 0.53). Age, duration of diabetes and level of diabetic retinopathy did not show significant associations with either OD or PD values (p > 0.16 for all models).Details of the regression models are presented in Table 38.

Table 38. Regression models for association between flicker perimetry parameters (dB) and explanatory variables.

	NDS		Age (yrs)		DD (yrs)		DR		Adj R^2
Parameter	**B =**	**p =**	**B =**	**p =**	**B =**	**p =**	**B =**	**p =**	
Overall Defect	-0.21	<0.01*	0.01	0.65	0.03	0.16	0.21	0.37	0.17
Pattern Defect	0.07	0.53	0.05	0.17	-0.03	0.36	-0.20	0.61	-0.02
Global	-0.03	0.77	-0.10	0.01*	-0.08	0.06	0.06	0.88	0.22
Inferior hemi-field	-0.02	0.83	-0.10	0.02*	-0.10	0.02*	0.04	0.93	0.26
Superior hemi-field	-0.06	0.73	-0.11	0.02*	-0.06	0.21	0.17	0.72	0.17

NDS: neuropathy disability score, DD: duration of diabetes, DR: diabetic retinopathy, Adj R^2: adjusted R^2, B: regression coefficient

9.5.2 Flicker sensitivity globally and in superior and inferior hemi-fields

Comparison across the NDS groups (none, mild, moderate and severe)

The between group comparisons for flicker sensitivity globally (average of 106 test points) and in each hemi-fields were found not to be statistically significantly different ($p > 0.25$ for all comparisons). Table 35presents these comparisons.

Comparison between individuals with no neuropathy vs the remaining participants

Average global flicker sensitivity as well as flicker sensitivity in both hemi-fields was found not to be statistically significantly different between participants who did not have neuropathy from the remaining type 2 participants who had neuropathy ($p > 0.36$ for all comparisons, Table 36).

Assessment of flicker sensitivity and risk of foot ulceration

Participants who were at lower risk of foot ulceration (NDS < 6, n = 28) demonstrated higher sensitivity in the superior hemi-field but the result was marginally statistically significant ($p = 0.06$). Flicker sensitivity globally and in the inferior hemi-field was statistically significantly higher for those who were at lower risk of foot ulcerations than those who were at higher risk ($p = 0.04$ for both). Detailed statistics are provided in Table 37.

Association with NDS score

Average global flicker sensitivity, although showed a mild trend towards reduction, was found not to be significantly associated with increased neuropathy ($p = 0.77$, Table 38, Figure 58). Age was statistically significantly associated with global flicker sensitivity ($p = 0.01$). Results for duration of diabetes was close to significant ($p = 0.06$); however, non-significant outcome was found for diabetic retinopathy ($p = 0.88$).

Neuropathy Disability Score, duration of diabetes and level of diabetic retinopathy had no significant associations with flicker sensitivity in the superior hemi-field (Table 38, $p > 0.21$ for all regression models). However age proved to have a significant association with flicker sensitivity in this hemi-field ($p = 0.02$). Figure 59represents the association between NDS scores and flicker sensitivity in the superior hemi-field.

Flicker sensitivity in the inferior hemi-field was found not to be significantly associated with NDS scores ($p = 0.83$) (Table 38, Figure 60). Age and duration of diabetes were both found to be significantly associated with flicker sensitivity in this hemi-field ($p = 0.02$ for both) but not so for level of diabetic retinopathy ($p = 0.93$).

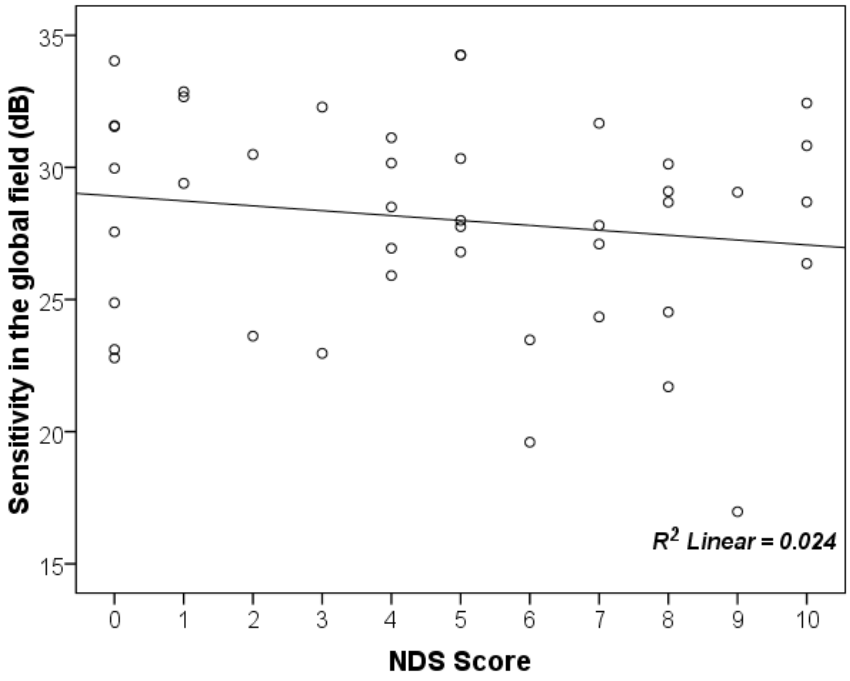


Figure 58. Scatterplot showing a non-significant association between global flicker sensitivity and NDS score.

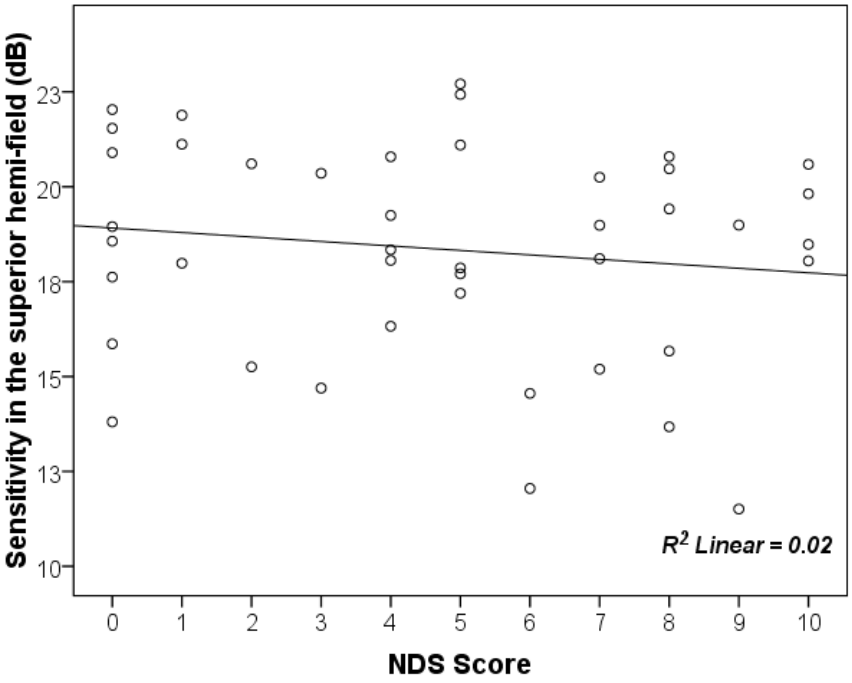


Figure 59. Association between flicker sensitivity in the superior hemi-field with NDS score.

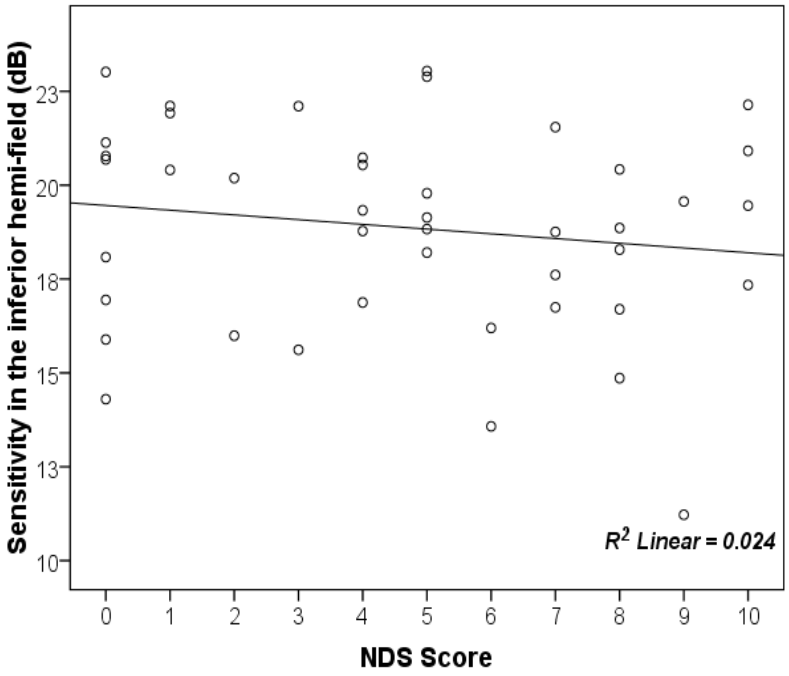


Figure 60.Association between flicker sensitivity in the inferior hemi-field with NDS score.

9.6 Discussion

The current study aimed to assess the association between flicker sensitivity and diabetic peripheral neuropathy, based upon the hypothesis that there would be a significant reduction in flicker sensitivity with increasing severity of neuropathy.

Findings of this study showed that OD was different between those who did not have peripheral neuropathy and the remaining individuals, indicating that reduced flicker sensitivity has the potential to predict diabetic peripheral neuropathy. Additionally, OD was found to be capable of differentiating individuals at higher risk of foot ulceration. Moreover, OD was negatively associated with increasing severity of neuropathy.

Findings for PD in the current study, however, proved not to be as good indicator for presence of neuropathy or high risk of ulceration as OD.

Overall Defect, as mentioned previously in *Chapter 6*, is a measure of non-spatially defined differences between the measured sensitivity and age-adjusted normative-based sensitivities, therefore describing a general depression or elevation of the visual field [270]. Age, duration of diabetes as well as retinal laser photocoagulation treatment for retinopathy as factors that are known to affect the overall pattern outcomes [228];these proved not be influential in any of the models in this study. In particular, the level of diabetic retinopathy did not account for these results. Therefore, it is most likely that reduced OD can be explained by the neuropathy status of the participants. It should also be acknowledged that ODcan be influenced by other factors such as media opacity and refractive error. These factors were controlled for in this study by exclusion or compensation.

Flicker sensitivity averages for the entire visual field (determined by global analysis) as well as inferior hemi-field were both found to be reduced in individuals who were at higher risk of foot ulceration. Previously in *Chapter 7*, it was demonstrated that contrast sensitivity in the superior hemi-field as determined by standard automated perimetry, can identify individuals who are at higher risk of foot ulceration. As determined by regression models, age seemed to have the highest effect on flicker sensitivity in the entire visual field and hemi-fields, signifying that these outcomes may not be purely caused by neuropathy status in this cohort.

Flicker sensitivity has been shown to have better capability of detecting field defects than static perimetry by isolating certain types of retinal ganglion cells [281]. It is also

known to be an effective method for diagnosing early retinal pathologies as it has improved robustness to factors like retinal image blur compared with conventional visual field assessment techniques [282, 283]. Previous work on flicker perception in people with diabetes has also shown that flicker perimetry can be a sensitive measure of retinal changes prior to micro-vascular abnormalities. Lobefalo*et al*.[191] found reduced flicker sensitivity in a group of young individuals with diabetes who had minimal diabetic retinopathy. Stavrou and Wood [192] also reported reduced flicker thresholds in presence of minimal retinopathy. However, these studies have used different analysis techniques including various eccentricities analysis, or hill-of-vision cluster analysis, to what has been used in the current experiment. Additionally, none of these studies have addressed the neuropathy condition of their study cohorts.

Reduced or impaired flicker sensitivity in individuals with diabetes can be explained by a number of potential mechanisms. Flicker sensitivity is perceived by larger retinal M ganglion cells and conducted via their axons through magnocellular pathway (M-pathway) in the lateral geniculate nucleus (LGN) for perception [284]. The M-pathway is known to have a fast response to neural activity, hence contributing to transient visual motion perception [285]. Incidence of pathology in any of the aforementioned divisions can result in flicker sensitivity loss. However, there is no reported association of pathology of LGN with peripheral neuropathy.

Another potential explanation can be the combination of high metabolic demand for flicker perception and minimal vascular supply in the retina which may limit the ability of the retina to adapt to the metabolic stress of diabetes [260]. It has been demonstrated that diabetes-induced insulin impairment can affect the retina, just as this affects all tissues in the body including the brain and peripheral nerves; however, given that insulin

penetration rate is much slower for the eye compared with the peripheral nerves [286], the retina shows a slower response to such impairment. Hence, techniques like flicker perimetry that are more sensitive to subtle changes of visual function, are theoretically more likely to detect retinal or other visual pathways abnormalities. However, the findings of this study, similarly to the outcomes from the RNFL study (*Chapter 5*), showed that flicker sensitivity is a more useful predictor of late-staged disease rather than an early indicator of DPN.

OD results in both SAP and flicker chapters suggested that there's a general reduction in contrast sensitivity in the group with DPN compared to those without. However PD and the effect size between the two hemispheres were not good predictors of neuropathy. This suggests a non-spatially specific, generalized loss of visual function in association with diabetic neuropathy. This finding is important since it can be readily distinguished from the pattern of loss that occurs in glaucoma or vascular lesions of the visual pathways.

Visual field techniques, including flicker perimetry, are based on psychophysical procedures where the outcomes can be highly influenced by participants' fatigue, concentration and learning effects. Given that the test failed to serve as an early marker for neuropathy, this suggests that flicker perimetry may not be a sensitive method of assessing visual function changes in relation with peripheral neuropathy compared with other means of assessing peripheral neuropathy.

10 Summary and conclusion

10.1 Overview

Diabetic peripheral neuropathy is one of the most common complications of diabetes, affecting between 50 and 60 precent of people with diabetes. The condition involves progressive loss of nerve fibres, in a range of nervous system divisions centrally and peripherally (somatic or autonomic). In the past, the lack of awareness and early diagnosis of this condition, led to complications including foot ulceration and lower limb amputation. Hence, the use of early diagnosis methods to help avoid such endpoints has been a research priority in the past few years.

The invasive nature of most accurate early diagnostic methods, such as skin biopsy, limits the ability of the techniques to be employed in routine clinical management of diabetic neuropathy. Other techniques aimed at early diagnosis, such as corneal confocal microscopy (CCM), introduced the possibility of considering anatomical sites elsewhere in the body as markers of diabetic neuropathy. The technique of CCM still requires contact with the cornea under local anaesthesia. Accordingly, this body of work set out to investigate whether there are measurable changes to retinal anatomy or visual function that exhibit a relationship with peripheral neuropathy in diabetes. It also aimed to see whether these ocular parameters could contribute usefully to the diagnostic process for DPN

The associations between peripheral neuropathy in diabetes and retinal structure and visual function have not been investigated previously. The majority of published studies

on diabetes have focused on the retinal vascular complications of diabetes and their effect on visual function [132]. Although a potential relationshipbetween the presence of retinal microvascular abnormality in diabetes and the severity ofdiabetic peripheral neuropathy has beensuggested[26], a clear link between retinopathyand neuropathy has not been established andonly a limited number of studies have investigatedthis relationship[26, 139].

Nerve damage in diabetes is caused by a combination of mechanisms with an increasing body of evidence suggesting oxidative stress as having a key role in the pathogenesis of diabetic peripheral neuropathy [287-289]. Furthermore, diabetes-induced functional and structural changes have been identified before major retinal vascular pathology develops, suggesting that diabetes can directly affect the neural retina, rather than necessarily being secondary to breakdown of the blood–retinal barrier [290].

The principal aim of the work described in this thesis was to investigate the association between retinal neural structure (focusing on retinal nerve fibre layer) and diabetic peripheral neuropathy as well as assessing the relationship between visual function (focusing on standard automated perimetry and flicker sensitivity) and diabetic peripheral neuropathy in people with type 2 diabetes. *Chapter 2* outlined what is known about this topic from previous literature and *Chapter 3* detailed methodology common to all subsequently-described experiments. The first experimental section (*Chapter 4*) investigated the association between two established methods of diagnosing and assessing peripheral neuropathy: neuropathy disability score test and quantitative sensory testing. *Chapters 5* and *6* examined the relationship between retinal nerve fibre layer thickness and these two neuropathy assessment methods. *Chapters 7* and *8*investigated the association between visual function, as determined by standard

automated perimetry, with diabetic peripheral neuropathy as measured by both NDS and QST. The last section of this thesis focused on the relationship between flicker sensitivity and diabetic peripheral neuropathy as measured by NDS (*Chapter 9*).

10.2 Summary of Individual Chapter Findings

Is there an association between neuropathy disability score test and quantitative sensory testing?

In*Chapter 4* significant associations were found for quantitative sensory testing sub-tests (cold sensation, warm sensation, cold-induced pain, heat-induced pain and vibration perception) with Neuropathy Disability Score test outcomes. It was found that QST sensation thresholds decrease with increasing score of neuropathy. In other words, higher severity of neuropathy as determined by NDS scores (0 - 10) was associated with decreased temperature and vibration sensation in feet, as might broadly be expected. Additionally, it was demonstrated that QST outcomes were significantly different between healthy volunteers and people with type 2 diabetes. Outcomes for QST and NDS in the healthy control group were found not to be well correlated, indicating that both of these techniques may be capable of demonstrating presence of nerve damage related to diabetes but do not give identical information. Furthermore, groups of neuropathy (none, mild, moderate and severe) were found to have significantly different QST outcomes. These findings were in agreement with previous work on validating NDS results against QST measurements in adults [237, 291]. The outcomes of this experiment, albeit demonstrating a relatively strong association between these two approaches, confirmed that NDS and QST nonetheless provide different measures of peripheral neuropathy in diabetes. Understanding the association between these techniques was crucial as it indicated that retinal structure outcomes as well as visual

function measurements should be assessed analysed against each of these methods separately.

Is retinal nerve fibre layer thickness associated with diabetic peripheral neuropathy?

The main focus of *Chapter 5* was investigating the association between peripheral neuropathy and retinal nerve fibre layer thickness. Mild trends towards thinning of global, inferior and temporal RNFLwere observedwith an increase in the level of DPN; however the mean differences between the four NDS groups with diabetes were not statistically significant. Additionally, no significant differences were observed between RNFL measurements for healthy controls and those with diabetes but no DPN. Furthermore, it was found that those who were at higher risk of foot ulceration had significantly thinner inferior RNFL compared with those who were at lower risk of ulceration. Importantly, diabetic retinopathy was found not to have a significant effect on the outcome measurements for retinal nerve fibre layer thickness.

These findings could not be directly compared with any of the previously published work on RNFL thickness in diabetes since no other studies have investigated retinal structure in association with severity of neuropathy in diabetic individuals. However, previous established work has shown retinal nerve fibre layer thinning in individuals with diabetes who did not have clinically detectable diabetic retinopathy [135, 140, 141]. Additionally, unlike findings from the previous studies, this experiment showed no RNFL thickness differences between healthy controls and participants with type 2 diabetes without DPN. However, none of these studies have classified their cohort with diabetes based on their neuropathy status. Additionally, reduced inferior RNFL thickness was a more prominent finding in this experiment, while other studies have

reported decreased RNFL in the superior quadrant [135]. However, the cohort for this experiment was stratified based on their level of neuropathy, which was a new approach in assessment of retinal structure in people with diabetes.

Results from this study showed that retinal nerve fibre layer thickness could not be considered as a marker for early diagnosis of diabetic peripheral neuropathy; however it was effective in detecting individuals who were at higher risk of foot ulceration and as such could feasibly play a role in detecting progression to later stages of the disease.

Is there an association between retinal nerve fibre layer thickness and quantitative sensory testing outcomes?

This component of the broader cross-sectional study was undertaken in order to investigate the relationship between retinal nerve fibre layer thickness and quantitative sensory testing measurements in individuals with type 2 diabetes. All linear regression analyses showed non-significant associations, indicating that RNFL was not a good predictor of neuropathy when the condition was assessed by QST. Additionally substantial variance was observed in QST outcomes; this was potentially caused by the highly subjective nature of this psychophysical test. Although QST has been shown to be a relatively reliable method of assessing neuropathy in diabetes [48]; it is not generally considered clinically to be a sole diagnostic technique for diabetic peripheral neuropathy.

Are the outcomes for standard automated perimetry associated with severity of diabetic peripheral neuropathy?

In *Chapter 7*, the association between severity of diabetic peripheral neuropathy as determined by neuropathy disability score (NDS) and contrast sensitivity thresholds as measured by standard automated perimetry (SAP) was investigated. Visual field Overall Defect (OD) and Pattern Defect indices were not significantly different between the NDS groups. However, it was shown that there is an association between NDS score and OD (1.3% reduction with each increasing unit of NDS score) and these results were independent from the effect of age and duration of diabetes as well as level of diabetic retinopathy.

This experiment was also aiming to discover whether contrast sensitivity thresholds, as determined by SAP, could be a potential surrogate marker for diabetic peripheral neuropathy; the results did not meaningfully differentiate between contrast sensitivity levels for individuals without neuropathy and participants who did have neuropathy. As such, it appears that standard automated perimetry results as analysed in this body of work, are not useful cross-sectional predictors of neuropathy outcomes as defined by NDS.

When participants were grouped according to risk of foot ulceration, it was found that those who were at higher risk however, had lower mean contrast sensitivity in the superior hemi-field. Additionally, contrast sensitivity in the inferior hemi-field and mean global values for the visual field also discriminated between the two groups indicating that a structure-function model cannot be concluded from these findings.

Assessment of visual field in diabetes in previous published work has shown reduced contrast sensitivity levels at different eccentricities, but focused on the relationship with diabetic retinopathy [268] – these findings do not provide highly relevant information related to the current topic – the primary association with neuropathy. Despite the outcomes not being particularly strong, this cross-sectional study did manage to demonstrate an association between the severity of diabetic peripheral neuropathy and contrast sensitivity thresholds from perimetry.

Is there an association between standard automated perimetry results and Quantitative Sensory Testing outcomes?

The association between various Quantitative Sensory Testing (QST) parameters and contrast sensitivity, as measured by standard automated perimetry, in volunteers with type 2diabetes was investigated in Chapter 8. QST cold sensation was found to be the only sub-test associated with contrast sensitivity levels globally and in superior and inferior hemi-fields – showing a 5 - 7 % increase in contrast thresholds with every degree reduction in cold sensation. These outcomes were independent of the effect of diabetic retinopathy and age.

Is flicker sensitivity associated with diabetic peripheral neuropathy?

Association between flicker sensitivity and severity of diabetic peripheral neuropathy was assessed in *Chapter 9.* Overall Defect (OD) was found to be significantly different between controls and individuals with type 2 diabetes without neuropathy as well as between those at higher risk of foot ulceration and those at lower risk. Comparison of

OD between the four NDS groups was very close to statistically significant. Additionally OD was found to be reduced progressively with increasing severity of neuropathy (reduction of 2.1% per increasing unit of NDS). Pattern defect, however, was not found to be different between any of the groups (Control versus people with diabetes but no DPN, stratified NDS groups, and risk of ulceration groups); nor was it associated with NDS results. These findings were again independent from the effect of age, disease duration and level of retinopathy.

Flicker sensitivity globally and in the inferior hemi-field was found to be significantly different between individuals at higher and lower risk of foot ulceration. Comparisons for flicker sensitivity in the superior hem-field, however, did not show any significant differences between these two groups. Using regression models, global and each hemi-fields' flicker sensitivities did not seem to be associated with severity of neuropathy; age however did had a significant effect in these models.

Evaluation of visual function by means of flicker perimetry in people with diabetes has shown abnormalities prior to clinically visible retinal vascular changes [191]. Presence of such abnormalities has been shown to be accompanied by poor quality of metabolic control or increased duration of diabetes in the studied population. Additionally, *microalbuminuria* (leakage of albumin into urine) has been suggested to be associated with impaired flicker sensation in people with diabetes [281]. However, the neuropathy status of the study cohort has not been evaluated; results in the current study suggest that impaired flicker perception in people with diabetes before clinically manifested vascular complications of the retina may be related to neuropathy.

The findings of this study, with the exception of OD index, did not support the hypothesis that neuropathy is associated with flicker sensitivity. Hence, this specific visual function test cannot be considered to be an indicator for early diagnosis of diabetic neuropathy. Age of the participants was found to be more associated with reduced flicker sensitivity globally and in both hemi-fields. When considering risk of foot ulceration, however, most of the measured flicker perimetry parameters proved to be capable of differentiating individuals at higher risk of foot ulcers from those who were at lower risk. These findings were similar to outcomes from *Chapter 5* and *Chapter 7* where thickness of retinal nerve fibre layer and contrast sensitivity were also shown to be good indicators of end-stagedneuropathy.

10.3 Implications for Clinical Practice

There are a number of real world implications arising from this research. This work has investigated both retinal anatomy and visual function in people who had type 2 diabetes and different levels of diabetic neuropathy. Eye-care practitioners are in contact with a considerable portion of people with diabetes and the care provided for the patients is generally focused on diagnosis or monitoring of vascular complications of the retina only.

Retinal nerve fibre layer thickness was found not to be an early marker for diabetic peripheral neuropathy; however the reduced nerve thickness, more prominently in the inferior region, may be a promising indicator of risk of foot ulceration in patients with type 2 diabetes. The findings of this study also demonstrated that individuals with type 2 diabetes and more severe peripheral neuropathy also have reduced contrast sensitivity broadly in the visual field. This also indicates that measurements of contrast sensitivity

as well as flicker sensitivity may not be useful as novel markers for diagnosing early neuropathy in people with diabetes, but can potentially be considered as markers for late-stage disease.

Eye-care practitioners should be mindful when interpreting retinal nerve fibre layer thinning in individuals with diabetes. It is possible that RNFL thickness in people with advanced diabetic peripheral neuropathy may fall outside the 95% reference limit for age. If these changes are due to yet-to-be diagnosed neuropathy, the RNFL thinning may be mis-diagnosed as glaucoma. Alternately, if individuals with diabetes already have an established diagnosis of glaucoma, further RNFL thinning that is actually related to neuropathy may be misinterpreted as glaucoma progression. This could argue a case for eye-care practitioners to actively enquire regarding neuropathy status for individuals with diabetes. Despite this, the loss associated with diabetic neuropathy appears, from this study's findings to have a distinctly different spatial profile to other neuro-ocular disease known to affect visual fields.

10.4 Overall Research Strengths and Limitations

There are a number of strengths and limitations that should be considered in relation to the experimental approach of the research described in this thesis. The first strength involves the application of two non-invasive methods for assessment of retinal structure and visual function. Optical coherence tomography and visual field analysis are relatively inexpensive, readily clinically available techniques. The findings of this work cautiously suggest the role of OCT and perimetry could be expanded for patients with diabetes to assist with predicting the risk of foot ulceration related to peripheral neuropathy.

Another important strength of this research is the use of two separate and non-identical measures of diabetic peripheral neuropathy. Neuropathy disability score, further to being an easy and fast method of assessing peripheral neuropathy has been proposed as the 'gold standard' for assessing diabetic neuropathy [45]. Quantitative sensory testing is known to be a safe and arguably more accurate diagnostic method as it has the capacity to measure peripheral nerve function over a range of sub-tests. The results in chapter 4 demonstrated that NDS and QST do not provide identical information regarding neuropathy status and therefore it was important to use them separately against the ocular markers of interest. The use of these techniques for extensive neuropathy assessment is preferred over only relying on completely subjective neuropathy symptom questionnaires. It should be also acknowledged that none of NDS, QST or questionnaires are recommended as sole criteria for diagnosing peripheral neuropathy due to their individual limitations [36].

Selection of participants was performed after a comprehensive investigation of medical background for each individual. This is particularly important as certain conditions or diseases including alcoholism and psychiatric disorders can affect the outcome measurements. Therefore, careful selection of the study cohort as well as strong medical and ocular inclusion/exclusion criteria add to the strength of this work.

The cross-sectional design of this research limits the conclusions that can be derived; nonetheless, these findings open the door to further longitudinal studies with better predictive and diagnostic capability. The study aimed to recruit a higher number of individuals with severe neuropathy; however as these individuals typically have a longer disease duration, they also suffer from more severe levels of complications and incapacitating illness. As such a higher number declined involvement in the study or

were excluded due to having received laser treatment for retinopathy or due to poor visual acuity. The relatively low numbers of severe neuropathy cases limited the options for analysis across all of the NDS groups.

Assessment of glycated haemoglobin (HbA1c) is of importance in studies on diabetes and its complications. All tests for this work were performed in a clinical setting where there were no facilities for collecting blood samples. Ideally the effect of HbA1c levels could have been considered in the statistical models; however this was not logistically feasible in this study.

As previously discussed in *Chapter 3*, there are a number of factors influencing accurateassessment of retinal nerve fibre layer thickness. Age, duration of diabetes and level of diabetic retinopathy were the factors that were included in the regression models in the current thesis. For a more precise quantification of the layer, it is also important to consider other factors such as optic nerve head size. It should be acknowledged that, the RTVue instrument automatically takes into account the effect of age and ONH size on outcomes measurements. Additionally, the effect of ONH size "on the blind spot" was controlled for in the visual function studies by eliminating two data points above and below the optic nerve head.

Contrast sensitivity thresholds as determined by automated standard perimetry and flicker sensitivity were only measured once due to already substantial demands on the time and energy of the participants. An ideal and perhaps more accurate approach would be performing the test twice in order to confirm the repeatability of any findings and avoid misinterpretation. Nonetheless, the effect of a single measure only of visual fields was identical for each comparison group; learning and fatigues effects were balanced by

randomizing test orders. Additionally the tests were performed on one eye only and the outcomes can not necessarily be extrapolated for the fellow eye.Assessment of visual function (both SAP and flicker) in this thesis was performed within the central 30 degrees eccentricity. A comprehensive evaluation of hill of vision should ideally exceed the central 30 degrees (up to 80 degrees) as some visual field deficits may occur beyond this eccentricity.

10.5 Recommendation for future work

The findings of this thesis need to be validated in a larger sample size specifically in a larger number of individuals with diabetes and with severe peripheral neuropathy. Future research should examine the effect of metabolic factors such as HbA1c and cholesterol levels on retinal nerve fibre layer measures in association with peripheral neuropathy. Application of more sophisticated methods of diagnosing diabetic peripheral neuropathy such as Nerve Conduction Studies, may prove a better association between Retinal nerve fibre layer thickness and DPN.

Further longitudinal study is essential to provide a clearer picture of the effect of DPN on changes in RNFL thickness overtime. Future research should also attempt to build a retinal structure- visual function model in association with diabetic peripheral neuropathy.

10.6 Conclusion

This work is important and novel as it addressed the potential effect of diabetic peripheral neuropathy on retinal structure and visual function for the first time. The findings have shown retinal anatomy and visual function cannot readily be used as

markers for early neuropathy, however they can be useful in predicting neuropathy progression and risk of foot ulceration. Both of these ocular measures in isolation may have limits to their practical diagnostic value; however a combination of structural and functional tests may yet prove to be useful in assessment of diabetic peripheral neuropathy. Additional work would be required before the true efficacy of these potential markers and their clinical utility is effectively understood.

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Appendices

Appendix A: Letter of invitation and study brochure

Princess Alexandra Hospital
Health Service District
DEPARTMENT OF DIABETES & ENDOCRINOLOGY
Phone: (07) 3240 2890
Clinic: (07) 3240 2834
Fax: (07) 3240 2973
TTY: (07) 3240 7737
AR:ci

Queensland Government
Queensland Health

Date

[title first name surname]
[street address]
[suburb state postcode]

Dear [title surname],

I was wondering if you might consider being involved with a study assessing damage to nerves in patients with diabetes.

This is a novel idea trying to determine if changes seen on photos of the nerves in the front and back of the eye correlate with changes in feeling in the feet of patients with diabetes. To pursue this we need to assess people with and without evidence of damage to the nerves of the feet.

An information sheet is enclosed and if you are interested could you contact our PhD scholar Ayda Moaven Shahidi on (07) 3138 6156. Alternatively you can send an e-mail to landmark@qut.edu.au. Also feel free to contact me to discuss further on #. If we haven't heard from you within one to two weeks a research team member will give you a call.

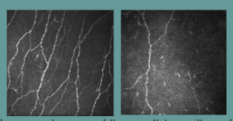
With kind regards,

DR ANTHONY RUSSELL
Endocrinologist
Department Director

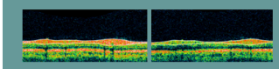
Office	Postal	Phone	Fax
Princess Alexandra Hospital Health Service District	Ipswich Road WOOLLOONGABBA Q 4102	(07) 3240 2111	(07) 3240 5677

Nerves and the Eye in Diabetes

Our preliminary studies have shown that individuals with diabetes who have nerve damage in the limbs, or diabetic neuropathy, also show similar damage and thinning to the nerves in their eyes. These nerves are in the cornea (the clear window at the front of the eye) and in the retina (the light sensitive inner lining of the eye).



The corneal nerves of the eye (taken with confocal microscopy at 700x magnification) of a healthy, non-diabetic individual (left) and of an individual with severe diabetic neuropathy (right).



The retinal nerves of the eye (taken with optical coherence tomography) of a non-diabetic individual (left) and of an individual with severe diabetic neuropathy (right); the thinning of the retinal nerve fibre layer is observed in the right image.

For further information, or to enquire about participation, please contact:

LANDMARK Study
landmark@qut.edu.au

Ayda Moavenshahidi
(07 3138 6156
* ayda.moavenshahidi@qut.edu.au

LANDMARK Study

Postal address:
LANDMARK Study
Anterior Eye Lab
IHBI QUT
60 Musk Ave
Kelvin Grove Qld 4059

Refreshments and transport provided

QUT ihbi Institute of Health and Biomedical Innovation

LANDMARK STUDY

Longitudinal Analysis of Novel Diabetic Markers

A Queensland University of Technology and Princess Alexandra Hospital Study

Now recruiting:

Individuals with Type 2 diabetes

Queensland Government Queensland Health

Princess Alexandra Hospital Health Service District

WHAT IS THE PROJECT ABOUT?

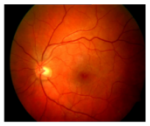
You are invited to participate in a study to investigate how features of the eye relate to nerve damage due to diabetes (diabetic neuropathy), and how this changes over time.

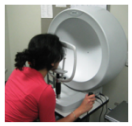
The nerves in the eye will be examined in people with and without diabetic neuropathy.

What will participation mean for me?

You will be asked to reveal eye and medical history and undergo several eye-related tests similar to having an eye exam, including assessment of the appearance and function of the eye.

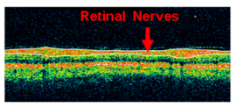
Eye examination (above), retinal image (bottom left) and flicker field test (bottom right).



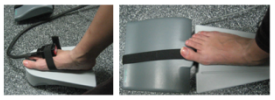


The eye tests involve having a drop of anesthetic in the eye, after which you shouldn't rub your eyes for 40 minutes.

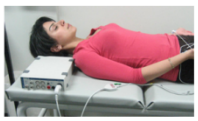
The ocular coherence tomographer (above), and an image of the retinal nerve fibre layer, produced by coherence tomography (below)



Nerve assessment will also be performed using tests of pain/touch, vibration and temperature as well as measurements of nerve signal conduction and tests of heart function.



Sensory tests of temperature and vibration (above) and heart rate testing (below).



You may also be asked to submit blood and urine samples.

Simple tests of neuropathy.

The total time commitment will be approximately two to three hours each year for 5 years. Transport or parking costs will be provided at no cost to you.

What are the risks and benefits of participation?

- There are no risks to you beyond a regular eye examination or endocrinologist's assessment.
- There is no direct benefit to you from participation in this research, however, the study may benefit patients with diabetes, as well as people with other diseases where the nerves of the body are affected.
- All data will remain confidential and if presented or published, anonymous.
- This study has received ethical clearance from the QUT University Human Research Ethics Committee and the Princess Alexandra Hospital Human Research Ethics Committee.

Appendix B: Participant Information and Consent Form

Princess Alexandra Hospital
Health Service District

Participant Information and Consent Form

for a joint project by Princess Alexandra Hospital and Queensland University of Technology

Project Title: Ophthalmic Marker of Diabetic Neuropathy

Principal Researcher: Prof Nathan Efron[1]

Associates: Prof Andrew Boulton[2], Dr Rayaz Malik[2], Prof John Prins[3], Dr Anthony Russell[3], Nicola Pritchard[1] , Katie Edwards[1]

1 Queensland University of Technology, 2 University of Manchester, 3 Princess Alexandra Hospital

1. Introduction

You (or your child or the person your are responsible for) are invited to take part in this research project. This is because you (or your child or the person your are responsible for) are in the age range of 14-75 years and either have a history of diabetes, or have no history of disease that might affect the nerves of the eye or the body. People who have had eye injury or surgery, other eye diseases (e.g. glaucoma), other systemic diseases which may affect the cornea e.g. keratoconus) or body (e.g. carcinoma, leukemia), large fibre neuropathy, congestive heart failure, major psychosis, HIV-AIDS or diabetic foot ulcer or infection, or those participating in any other research trial will not be eligible.

The research project is aiming to investigate relationship between the nerves of the eye and a condition which involves the peripheral nerves of the body in people with and without diabetes. We hope to determine if some of the measures of the nerves in the eye and the sensitivity of the eye are reduced in people with peripheral neuropathy due to diabetes.

This Participant Information and Consent Form tells you (and your child or the person your are responsible for) about the research project. It explains the procedures involved. Knowing what is involved will help you (or your child or the person your are responsible for) decide if you (or they) want to take part in the research.

Please read this information carefully. Ask questions about anything that you (or your child or the person your are responsible for) don't understand or want to know more about. Before deciding whether or not to take part, you (or they) might want to talk about it with a relative, friend or healthcare worker.

Participation in this research is voluntary. If you (or your child or the person your are responsible for) don't wish to take part, you (or they) don't have to. You (or your child or the person your are responsible for) will receive the best possible care whether you (or they) take part or not.

If you (or your child or the person your are responsible for) decide you (or they) want to take part in the research project, you (or they) will be asked to sign the consent section. By signing it you (or they) are telling us that you (or they):

- understand what you (or they) have read;
- consent to take part in the research project;
- consent to participate in the research processes that are described;
- consent to the use of your (or their) personal and health information as described

You (or your child or the person your are responsible for) will be given a copy of this Participant Information and Consent Form to keep.

If you are the parent or guardian of a child or young person, as the 'person responsible' for the patient, you are invited to consider the patient's participation in this research project. Both the child/young person and the 'person responsible' must consent to participation in the study.

2. What is the purpose of this research project?

This research project focuses on patients with different types of diabetes. As you (or your child or the person your are responsible for) may know, diabetes is associated with high sugar levels in the blood due to the body not producing enough insulin to convert this sugar into energy. We think there might be some differences in the nerves of the eyes of people who have different types of diabetes and we can measure this by using new, simple methods that measure the actual nerves and nerve function. These are the eye tests: corneal confocal microscopy (CCM) can be used to look at the nerves in the front of the eye; corneal non-contact aesthesiometry (NCCA) is used to measure the sensitivity of the front of the eye; ocular coherence tomography (OCT) is used to assess the nerves and tissues at the back of the eye and flicker perimetry (FP) measures how well you can see dim lights in your field of view. The measures of nerves and nerve function made by these techniques are thought to be related to diabetic neuropathy, the damage of nerves in the peripheral limbs associated with diabetes. In the research project we aim to investigate the following:

- Changes in corneal (front of eye) nerve counts and corneal sensitivity over time.
- Changes in retinal (back of eye) nerve layer thickness and sensitivity to light over time.
- The relationship between the progression of neuropathy with the results of other traditional nerve tests such as electrophysiology, measuring vibration and temperature sensitivity and assessment of level of pain and discomfort in people with different types of diabetes.
- The ability of these eye tests to detect neuropathy earlier than traditional means.
- Identify risk factors associated with changes in nerves and nerve function in people with different types of diabetes; these may include age, height, body mass index, duration of diabetes, blood pressure, smoking, and poor blood-sugar control.

Understanding these aspects of the nerves may provide healthcare professionals with a quick, simple, cost-effective and repeatable means to identify patients at risk, anticipate and monitor deterioration, and assess new treatments.

Diabetic neuropathy is a significant clinical problem that currently has no effective therapy, and in advanced cases, it is a major cause of ill-health and death worldwide. If left unmanaged, diabetic neuropathy can lead to foot ulceration and ultimately, in some cases, foot amputation. It is therefore important to have the capacity to detect this condition early, monitor its progression and assess the benefits of any treatments.

The results of this study will develop a better understanding of small fiber peripheral nerves in patients suffering from diabetic neuropathy, and will determine the extent to which these changes are associated with clinical signs and symptoms. The significance of this study is that it will reveal the potential for these eye tests to serve as sensitive, rapid, repeatable, 'patient-friendly' eye tests for the detection, diagnosis and monitoring of the progression of diabetic neuropathy. This information will provide a sound basis for the design of trials of treatments for diabetic neuropathy. Data will also be generated which will reveal the importance (or otherwise) of blood sugar control and other metabolic abnormalities and lifestyle factors which may impact on the progression of neuropathy in diabetic patients.

A total of 220 participants will take part in this study at the Princess Alexandra Hospital in Brisbane.

Fours groups of people will be recruited:

Group 1: Patients with Type 1 diabetes and without neuropathy

Group 2: Patients with Type 1 diabetes with neuropathy

Group 3: Patients with Type 2 with neuropathy

Group 4: Non-diabetic participants without neuropathy.

Some of the results of this research will be used by the researcher Ayda Moavenshahididi to obtain a Doctor or Philosophy degree.

This research is a collaborative project between researchers at QUT, PAH and University of Manchester. It has been initiated by the investigators Professors Nathan Efron (QUT), Andrew Boulton (UM), and John Prins (PAH); Drs Rayaz Malik (UM) and Anthony Russell (PAH) and optometrists Nicola Pritchard and Katie Edwards (QUT).

This research has been funded in part by the National Health & Medical Research Council and the George Weaber Foundation (to support Ms Moavenshahidi).

3. What does participation in this research project involve?

Your participation (or that of your child or the person you are responsible for) will involve asking you (or they) to reveal eye and medical history, and undergo an examination of the front part of the eye using a slit lamp biomicroscope, read letters on an eye chart, and have the pressure of the eyes measured. We will ask you (or your child or the person your are responsible for) to complete a questionnaire about pain in your (or their) lower limbs, and undergo simple tests of your (or their) sensations of pain/touch, vibration and temperature. The tests are quick and involve use of a pointed tip, a tuning fork and warm and cool metal rods to test these three sensations. The presence of absence of the Achilles tendon reflex using a tendon reflex hammer will be tested. Your (or their) height, weight and blood pressure will also be measured.

Corneal confocal microscopy (CCM) will be performed to examine the number of nerves at the front part of the eye, the cornea. A drop of anaesthetic is applied to the eye and you (or they) will be asked to sit at an instrument and look a target while several images are captured. Initially the drop may sting for 1 or 2 seconds. Because the drop numbs the eye it is possible to scratch the eye without noticing it. Therefore please do not rub the eyes for at least 45 minutes after the drop has been placed in the eye.

Another test of your (or their) sensory stimuli will be done using an instrument that can measure when you (or they) just notice sensations of cool, warm and vibration on the foot. For example, for the coolness test you (or they) may feel like “a pulse of cooling” has touched the foot. It is important that before these tests no sedatives, tranquillisers, opiates, or stimulants have been taken in the preceding 12 hours, and not more than one hot drink has been consumed prior to the test.

Another test that can reveal alterations to the nerves is a test of heart rate variability. A measure of heart rate variability will also be conducted to show how the heart responds to deep breathing and to changes in blood pressure and posture.

Corneal non-contact aesthesiometry (NCCA) will be conducted to measure the sensitivity of the cornea. The smallest noticeable air pressure is determined by directing gentle, almost imperceptible puffs of air to the eye, and you (or they) indicate whether the air on the eye can be felt or not.

The speed the nerves conduct messages will also be tested as a measure of nerve damage. Nerve conduction velocity will be measured by putting electrodes on the ankle, wrist and elbow. The limb will be kept warm with a heat lamp if necessary. A small electrical current will be applied to the electrode which may feel like a tingling sensation and it may be uncomfortable for you (or them). You (or they) should feel no discomfort once the test is finished.

Ocular coherence tomography (OCT) involves having a drop inserted into one eye to dilate the pupil. Then you (or they) will be asked to fixate a target while seated at the instrument, and at least two OCT images are captured. A photograph of the back of the eye will also be taken using a specialised digital camera. Due to the increased size of the pupil, your (or

their) sensitivity to glare may be increased for 4 to 6 hours, so you (or they) may wish to wear dark glasses when outside and/or have someone drive or escort you (or them) home.

Flicker perimetry (FP) involves viewing a light stimulus of varying intensity, and sometimes flickering, which appears in different parts of the visual field. You (or your child or the person your are responsible for) will be required to click a button if you (or they) see the light while looking at a central spot.

At the end of the study procedures the eye will be examined again; follow-up appointments will be made if the investigator believes it is in your (or their) best interests. This study will be carried out at the Princess Alexandra Hospital, Woolloongabba.

We expect the visit will be approximately 2 to 3 hours at the PAH. You (or they) will not be paid for participation in this research, but will be provided transport to and from PAH (e.g. cab vouchers will be provided up to approximately $40) and will receive light refreshments during the visit (approximate value $10).

4. What will happen to my test samples?

You (or your child or the person your are responsible for) will be asked to provide consent for the collection of your (or their) blood during the research project. The levels of glucose, lipid and a test for antibodies for glutamic acid decardoxylase (GADAb) and antibodies to islet cells (ICAb) will be recorded. This will help investigators decide which group to assign you (or them) to. All samples will be individually identifiable at the time of collection, analysis and report. These results will only be used for research purposes, and will be stored separately from the main body of study data to protect your (or their) privacy/confidentiality and anonymity, and a re-identifiable code will be assigned your (or their) blood results. All blood samples will be assessed through the Queensland Health Pathology Service and samples are usually destroyed 7 days after collection.

5. What are the possible benefits?

There will be no direct benefit to you (or your child or the person your are responsible for) from your (or their) participation in this research. However, it may benefit the many people who have problems with diabetic neuropathy, because with these instruments and techniques we are able to look at the tissues of the eye under very high magnification. Also these new technologies may reveal features that have not, to date, been discovered but which might serve as sensitive, rapid and non-invasive techniques for the detection, quantification and monitoring of the progression of neuropathy in patients with diabetes as well as other diseases where the nerves of the body are affected. Some people find the opportunity to learn and be a part of something new an interesting experience.

We can provide you (or your child or the person your are responsible for) with state-of-the-art images of your (or their) eye if you (or they) would like them.

6. What are the possible risks?

The risks associated with participation in this study are minimal, and similar to routine diabetic and primary eye care. Minimal corneal abrasion can occur with corneal confocal microscopy, similar to that which might occur if you (or they) rub the eyes too hard; however, in our experience it is like that noted with normal daily wear of contact wearers. This type of abrasion heals quickly, without intervention, typically within 12 hours.

Having a blood taken may cause some discomfort or bruising. Sometimes, the blood vessel may swell, or blood may clot in the blood vessel, or the spot from which tissue is taken could become inflamed. Rarely, there could be a minor infection or bleeding. If this happens, it can be easily treated.

If you (or your child or the person your are responsible for) become upset or distressed as a result of your (or their) participation in the research, the researcher is able to arrange for counselling or other appropriate support. Any counselling or support will be provided by staff who are not members of the research team. In addition, you (or they) may prefer to suspend or end participation in the research if distress occurs.

There may be additional risks that the researchers do not expect or do not know about. Tell a member of the research team immediately about any new or unusual symptoms that you (ir they) get.

7. What if new information arises during this research project?

During the research project, new information about the risks and benefits of the project may become known to the researchers. If this occurs, you (or your child or the person your are responsible for) will be told about this new information and the researcher will discuss whether this new information affects you (or them).

8. Can I have other treatments during this research project?

It is important to tell your (or their) doctor and the research staff about any treatments or medications you (or they) may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your (or their) doctor and the researchers about any changes to these during participation in the research.

9. Are there alternatives to participation?

Since this study does not involve any treatments, you (or your child or the person your are responsible for) will receive the best possible care whether you (or they) take part or not. Participation in the study does not replace full eye or medical care. You (or they) may also request that your (or their) general practitioner be informed of participation in the study.

10. Do I have to take part in this research project?

Participation in any research project is voluntary. If you (or they) do not wish to take part you (or they) don't have to. If you (or they) decide to take part and later change your mind, you (or they) are free to withdraw from the project at any stage.

The decision whether to take part or not to take part, or to take part and then withdraw, will not affect your (or their) routine treatment, your relationship with those treating you (or them), nor your (or their) relationship with Princess Alexandra Hospital or Queensland University of Technology.

11. What do I need to do if I decide to withdraw from this research project?

If you (or your child or the person your are responsible for) decide to withdraw, please notify a member of the research team before you (or they) withdraw.

If you (or they) decide to leave the project, the researchers would like to keep the personal and health information about you (or them) and your (or their) blood results that have been collected. This is to help them make sure that the results of the research can be measured properly. If you (or they) do not want them to do this, you (or they) must tell them before joining the research project.

12. Could this research project be stopped unexpectedly?

There are no foreseeable reasons why this research project would be terminated before completion. In the unlikely event this did occur, you (or they) will be informed in writing and asked to attend a final study visit.

13. How will I be informed of the results of this research project?

The research team will provide regular newsletters on the progress of the study. You (or your child or the person your are responsible for) will also receive a copy of any publications that are generated as a result of this study. We expect this research project to be completed in approximately 5 years and a full summary of the results will be provided to you (or them) then. If there are abnormal results from any of the tests we perform, we will send, with your (or their) permission, a report of those results directly to your (or their) general practitioner.

14. What else do I need to know?

Any information obtained in connection with this research project that can identify you (or your child or the person your are responsible for) will remain confidential and will only be used for the purpose of this research project. It will only be disclosed with your (or their) permission, except as required by law. Information about you (or them) may be obtained from your (or their) health records held at PAH (where applicable) for the purposes of this research.

Data is stored on paper records in locked filing cabinets at PAH and QUT, and the data in electronic form (i.e. entered into a computer) is only available to the research team members and is kept secure by using password-protected limited-access environment. Data is stored during the project in a re-identifiable format i.e. coded. Your (or their) name and contact details will be held separately to the study data to protect your (or their) privacy and anonymity. In any publication and/or presentation, information will be provided in such a way that you (or they) cannot be identified, except with your (and/or their) permission. This will be done by only using the code number assigned to you (or them) for the purpose of this study.

At completion of the project your (or their) data will be decoded, such that it will not be possible to determine which data belong to which participant. Data for this project will be kept for 15 years or 5 years after the last publication. Paper files will be shredded and electronic files will be carefully removed from their storage location (not just deleted).

Information about your (or their) participation in this research project may be recorded in your (or their) health records.

How can I access my information?

In accordance with relevant Australian privacy and other relevant laws, you have the right to access the information collected and stored by the researchers about you (or your child or the person your are responsible for). You also have the right to request that any information, with which you disagree, be corrected. Please contact one of the researchers named at the end of this document if you (or they) would like to access your (or their) information.

What happens if I am injured as a result of participating in this research project?

If you (or they) suffer an injury as a result of participating in this research project, hospital care and treatment will be provided by the public health service at no extra cost to you (or them) if you (or they) elect to be treated as a public patient.

Is this research project approved?

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of the Princess Alexandra Hospital and Queensland University of Technology.

This project will be carried out according to the National Statement on Ethical Conduct in Human Research (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

15. Consent

I have read, or have had read to me in a language that I understand, this document and I understand the purposes, procedures and risks of this research project as described within it.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Queensland University of Technology concerning my health and treatment that is needed for this project. I understand that such information will remain confidential.

I consent to the use of blood samples taken from me for use in this specific research project only, as described in Section 4 of this document.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described.

I understand that I will be given a signed copy of this document to keep.

Participant's name (printed) _____

Signature _____ Date _____

Declaration by parent, guardian or person responsible (where appropriate): I agree for my child/young person or the person named above who I am responsible for to participate in this research and I believe that they have understood the explanation of the study, its procedures and risks.

Name of parent/guardian to participant's (printed) _____

Signature _____ Date _____

Name of witness to participant's signature (printed) _____

Signature _____ Date _____

Declaration by researcher*: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Researcher's name (printed) _____

Signature _____ Date _____

* A senior member of the research team must provide the explanation and provision of information concerning the research project.

Note: All parties signing the consent section must date their own signature.

16. Who can I contact?

Who you (or your child or the person your are responsible for) may need to contact will depend on the nature of your (or their) query; therefore, please note the following:

For further information or appointments:

If you (or they) want any further information concerning this project or if you (or they) have any medical problems which may be related to your (or their) involvement in the project (for example, any side effects), you can contact Dr Anthony Russell Ph: 07 3240 5914 or the following people:

Nicola Pritchard Ph: 07 3138 6414 E-mail: n.pritchard@qut.edu.au	Prof.Nathan Efron Ph: 07 3138 6401 E-mail:n.efron@qut.edu.au	Katie Edwards Ph: 07 3138 6154 Email: katie.edwards@qut.edu.au

If you (or they) feel emergency medical care is required, then go to the nearest hospital Emergency Department.

For complaints:

If you (or they) have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you (or they) may contact:

Ethics Manager	QUT Research Ethics Officer
Princess Alexandra Hospital Human Research Ethics Committee	Queensland University of Technology Human Research Ethics Committee
Ph: (07) 3240 5856	Ph: (07) 3138 2340
Email: PAH_Ethics_Research@health.qld.gov.au	E-mail: ethicscontact@qut.edu.au

Researcher Ethics Officers/Managers are not connected with the research project and can facilitate a resolution to your (or their) concern in an impartial manner.

Appendix C: Case Report Form

LANDMark Study Case Report Form **Distiller ID:**

Participant Initials:	**Date:**	**Visit:** B'line 1yr 2yr 3yr 4yr	**ID:**
Medical Investigator:		**PAH ID:**	
Ophthalmic Investigator:		**(if applicable)**	
Information & Consent	Original Retained	Duplicate Provided	
Date of Birth:	**Age:**	**Gender:**	Male / Female

MEDICAL

Blood/urine collection ☐ Nil(Type 2) ☐ IHBI ☐ Collection centre

HbA1c	GADAb		
Total Cholesterol	Triglycerides	LDL Cholesterol	HDL Cholesterol
Creatinine	eGFR	Albumin	GTT

Notes

Ethnicity:

- ☐ European (eg. European Australian, English, German, Spanish)
- ☐ South East Asian (eg. Chinese, Japanese, Korean, Indonesian, Thai, Malaysian)
- ☐ Asian (eg. Indian, Pakistani, Bangladeshi, Sri Lankan)
- ☐ Middle eastern (eg. Iranian, Iraqi, Lebanese, Syrian)
- ☐ Australian Aboriginal or Torres Strait Islander
- ☐ Other; please specify;

Hand dominance

What hand do you write with? ☐ Left ☐ Right

Ophthalmic History

General ophthalmic history

	Yes	No	
History of corneal surgery?	☐	☐	
History of cataract surgery?	☐	☐	If yes, when?
History of other ocular surgery?	☐	☐	
History of corneal trauma?	☐	☐	
History of other ocular trauma?	☐	☐	

If yes to any, please describe, including year of event:

Ophthalmic History cont.

Are you a previous contac lens wearer?		Yes		No
Are you a current contact lens wearer?		Yes		No
If yes, are your lenses soft or hard?		Soft		Hard
How long have you been a CL wearer for?	_____	years		
How frequently do you wear your lenses?	_____	hrs/day		
	_____	days/wk		
Ocular condition excludes from study?		Yes		No

Medical History

General medical history

Do you have any allergies? If yes, please describe ☐ Yes ☐ No

Have you ever had an allergic reaction to eye drops? If yes, please describe
☐ Yes ☐ No

Has a doctor ever told you that you have hypertension? ☐ Yes ☐ No

If yes, what is the current status of your hypertension?

- ☐ Treated with medication and controlled
- ☐ Treated with medication, but not controlled
- ☐ Treated with lifestyle (diet and exercise) and controlled
- ☐ Treated with lifestyle (diet and exercise), but not controlled
- ☐ Untreated

Please list all medications that you currently take. Include dosage and frequency if possible;
Prescribed (for insulin, please include name, daily dosage and injections per day)

Medical History cont.

Over-the-counter (including vitamin supplements)

Prescribed by an alternate therapist (eg.homeopath, naturopath, herbalist)

Diabetes History

In what year, and how old were you when you were first diagnosed with diabetes?

Year: _____ Age: _____

In the past, has a doctor ever told you you have any of the following, directly related to your diabetes?

	Yes	No
Nephropathy (damage to the kidneys)		
Retinopathy (damage to blood vessels in the back of the eye)		
Neuropathy (damage to the nerves)		
Stroke		
Heart disease (if yes, please describe)		

Smoking

Have you ever been a smoker?

☐ Yes (smoked more than 100 cigarettes in total in your lifetime)

☐ No (either never smoked, or smoked less than 100 cigarettes in total in your lifetime)-go to next section

Do you currently smoke?

☐ Yes

☐ No; years since quitting _____ years

If you are a current or former smoker;

How many years in total have you been/were you a smoker? _____year

On average, how many cigarettes do/did you smoke per day? _____/day

Alcohol

Do you drink alcohol

☐ No, never; go to next section

☐ Yes

☐ Yes, but not currently. If so; i) did you stop following medical advice? _____

ii) how many years ago did you stop?_____years

If you are a current or former drinker;

On average, in the past year (or if a former drinker, approx), how many days per month did you drink? _____days

On each of these occasions, how many drinks would you have? _____drinks

Pregnancy

Is it likely you are pregnant? ☐ Yes ☐ No

Quantitative Sensory Testing

Foot tested: ☐ Right ☐ Left

Initial foot temp: Room temp: Humidity:

	cold sensation		warm sensation		cold induced pain		warm induced pain		vibration	
Averages										

Compared to normalised values

cold sensation	☐	Normal	☐	Abnormal
warm sensation	☐	Normal	☐	Abnormal
vibration	☐	Normal	☐	Abnormal

Blood Pressure

		Supine	Standing (after 1 min)	Sitting
BP	Systolic			
	Diastolic			
Time				

Postural hypotension?			Yes	No
Systolic: (Supine-Standing)		Over 20?	☐	☐
Diastolic: (Supine-Standing)		Over 10?	☐	☐

Height, Weight & Waist Circumference

Height	1	☐ m	Average:	☐
	2	☐ m		
Weight	1	☐ kg	Average:	☐
	2	☐ kg		
Waist	1	☐ cm	Average:	☐
	2	☐ cm		
BMI		☐		

Heart Rate Variability (PLACE NEUROPAD ON ALSO****)**

80secs DB	☐	Filename:
5min rest	☐	Comments:
80secs DB	☐	
E/I Ratio:		

Neuropad

Foot tested: ☐ Right ☐ Left

Pink (normal) ☐
Patchy ☐
Blue (abnormal) ☐

Time:

Approx % pink: ☐ % Automated analysis % pink ☐ %

Monofilament

Foot tested: ☐ Right ☐ Left

Points felt ☐ /3 Risk of foot ulceration ☐ Yes ☐ No

Short Form McGill Pain Questionnaire

Any pain: ☐ Yes ☐ No

If yes: Pain 1 ☐ Score ☐ VAS ☐ PI ☐
Pain 2 ☐ Score ☐ VAS ☐ PI ☐

Diabetic Neuropathy Symptom Score

	Yes (1)	No (0)
1. Do you suffer from unsteadiness in walking? *(ie. need for visual control, increase in the dark, walk like a drunk man, lack of contact with floor)*	☐	☐
2. Do you have a burning, aching pain or tenderness at your legs or feet? *(ie. occurring at rest or at night, not related to exercise, exclude claudicatio intermittens)*	☐	☐
3. Do you have prickling sensations at your legs and feet? *(ie occurring at rest or at night, distal>proximal, stocking glove distribution)*	☐	☐
4. Do you have places of numbness on your legs or feet? *(ie. distal>proximal, stocking glove distribution)*	☐	☐

Total score ☐ \4

Neuropathy Deficiency Score

Record √ for correct or χ for incorrect response then record 0 for normal or 1 for abnormal (≥2/3 correct responses = normal)

Pain Sensation	R1	R2	R3				Score (/1)
				L1	L2	L3	Score (/1)
Vibration Sensation	R1	R2	R3				Score (/1)
				L1	L2	L3	Score (/1)
Temperature Sensation	R1	R2	R3				Score (/1)
				L1	L2	L3	Score (/1)

Achilles Tendon Reflex	R	Present	Present w/ reinforcement	Absent	Score (/2)
	L	Present	Present w/ reinforcement	Absent	Score (/2)

(Total) NDS ☐

Final score

None (0-2) ☐ Mild (3-5) ☐ Significant (6-8) ☐ Severe (9-10) ☐

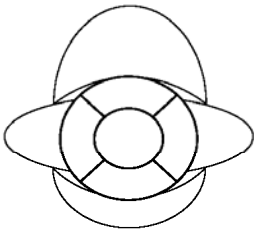
Comments

OPHTHALMIC

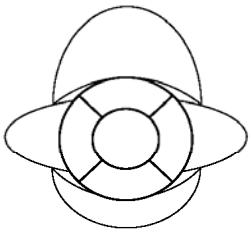
VA (logMAR) OD OS

Slit Lamp Examination

OD



OS



Corneal staining Yes No Yes No

Non-Contact Corneal Aesthesiometry

(record in millibars) Eye tested: ☐ Right ☐ Left

Stimulus	Felt?
6	

Stimulus	Felt?

Final threshold:

IOP

OD			OS		

Instrument: Time:

Spectacle Rx OD OS

Add

Fundus Image(s)

	OD	OS
Filename		
ETDRS Score		
Comments		

Perimetry

Eye tested: ☐ Right ☐ Left

	Central Perimetry	Flicker Perimetry
Filename		
	Time	Time

Notes

Ocular Coherance Tomography

	OD	OS
NHM4		
GCC		
RNFL3.45		

Corneal Confocal Microscopy

Eye tested: ☐ Right ☐ Left

Filenames:

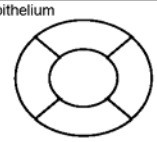
Comments

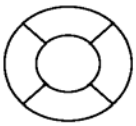
Ocular condition excludes from study ☐ Yes ☐ No

Comments

End of Visit

Corneal epithelium
NaFl





Comments

Cab Voucher ☐

Received medal ☐ If yes, number; ☐ \350

If you would like, we can send an annual report of the study findings to your GP and/or diabetes specialist

	Endocrinologist	GP
Name		
Address		

Is any immediate follow-up for this participant required? ☐ Yes ☐ No

If yes, please explain:

Medical Investigator (sign)

Ophthalmic Investigator (sign)

Participant Contact Information

Date:

Distiller ID:

Phone contacts:
Home:

Name:

Work:

DOB:

Mobile:

Address:

Email address:

Participation to continue next year? ☐ Yes ☐ No

If no, please explain;

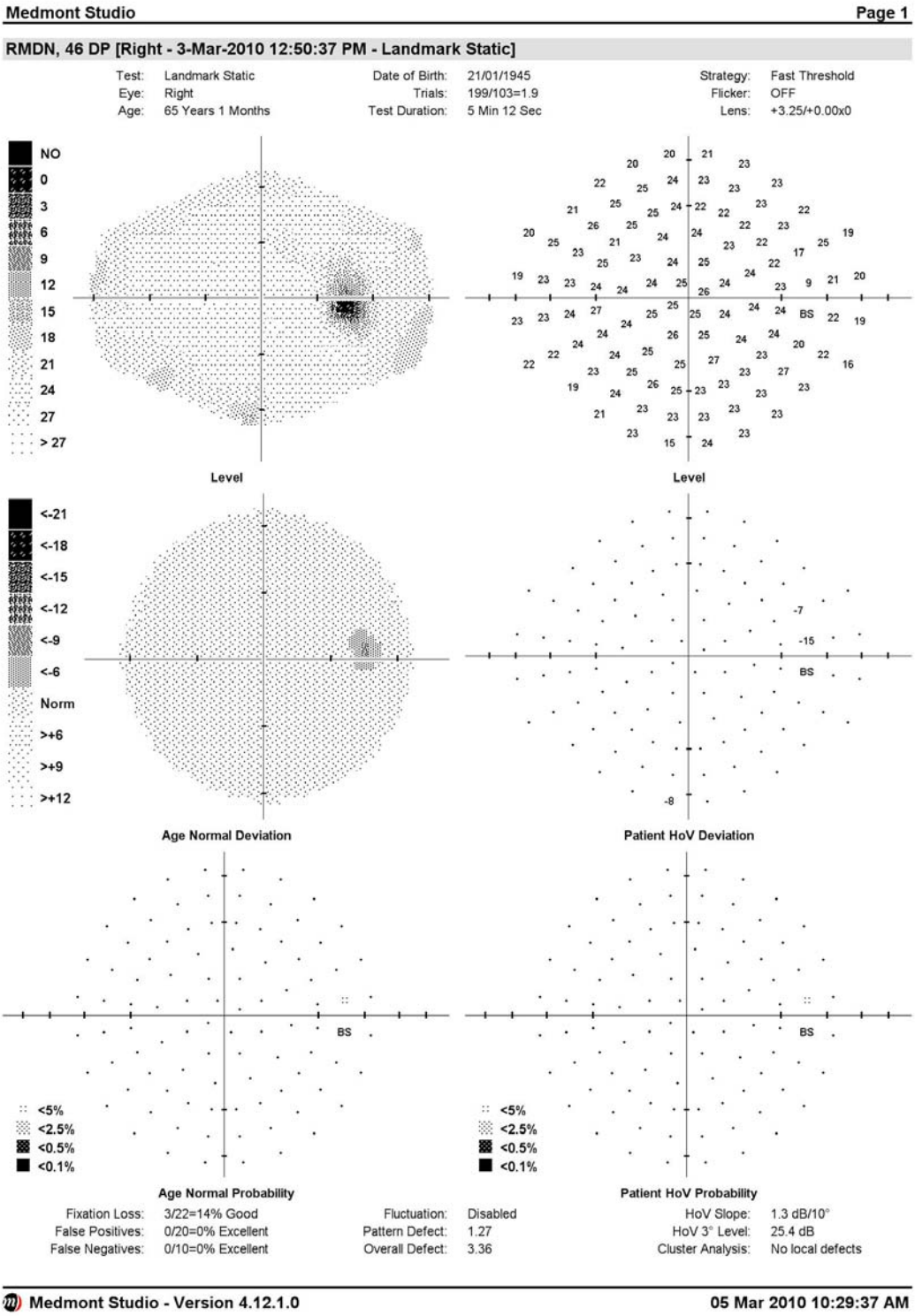
Preferred method of contact:

- ☐ Home phone
- ☐ Mobile
- ☐ Email
- ☐ Other:

Suitable for mapping ☐ Yes ☐ No

Comments

Appendix D: Sample Automated Perimetry Output



Appendix E: Sample Flicker Perimetry Output